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Abstract

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Role of HNF1β in the differential diagnosis from other germ cell tumors

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Running title: HNF1β in Yolk Sac Tumor
Abstract

Identification of the yolk sac tumor (YST) component in germ cell tumors (GCT) may prove challenging, and highly sensitive and specific immunohistochemical markers are still lacking. Preliminary data from the literature suggest that HNF1β may represent a sensitive marker of YST. The specificity of HNF1β has not been addressed in GCT. A cohort of 49 YST specimens from 45 patients was designed, occurring either as pure tumors, or as a component of a mixed GCT. Immunohistochemistry was conducted on whole tumor sections using HNF1β. SALL4, OCT4, CD30, CDX2, Cytokeratin 19, Glypican 3, and GATA3 were used for classification of the GCT components. Patients were mostly male (39/45), aged 14 months to 49 years, with primary testicular tumors (37/39), or primary mediastinal pure YSTs (2/39). All 6 primary tumors occurring in females (6/45) were pure ovarian YSTs; age range was 4 to 72 years. HNF1β nuclear reactivity was seen in the YST component in all 49 tumors, with a moderate to strong nuclear pattern of staining. Embryonal carcinoma (EC, 0/32) and seminoma (0/6) were negative. Choriocarcinoma (6/6) showed faint focal cytoplasmic reactivity to HNF1β but no nuclear staining. In teratomas, only enteric-type glands showed nuclear reactivity to HNF1β (11/16). Therefore, HNF1β sensitivity in YST component identification was 100% and specificity was 80%. Thus, in our experience, HNF1β is a sensitive and reliable marker of the YST component in GCT, and allows distinction of YST from intricately admixed EC, especially in the diffuse embryoma pattern.

Abstract Word Count: 243

Keywords: yolk sac tumor; germ cell tumor; embryonal carcinoma; immunohistochemistry; hepatocyte nuclear factor 1
1. Introduction

Gonadal germ cell tumors (GCTs) still give rise to challenging diagnostic issues. Yolk sac tumor (YST) may be underdiagnosed, particularly when closely admixed with embryonal carcinoma (EC), when present as a minor component, or when occurring as particular variants. For instance, glandular ('endometrioid-like') YSTs may be confused with endometrioid carcinomas, whereas hepatoid YST may mimic metastatic hepatocellular carcinoma. Correct identification of YST is essential for adequate treatment, since this tumor usually pursues an aggressive clinical course. Use of panels of immunohistochemical stains is of help, but sensitive and specific markers of YST are still lacking.

Preliminary data from the literature suggest that hepatocyte nuclear factor 1 beta (HNF1β) may represent a sensitive marker of YST [1]. Identified as a potent immunohistochemical marker of clear cell carcinoma of the ovary [2], HNF1β reactivity was also displayed in the 7 YSTs assessed from a large and recent series of 279 ovarian tumors [3]. The only further GCTs of this series were 8 dysgerminomas that showed no HNF1β reactivity. In a recent study on YST arising in association with clear cell carcinomas of the ovary and endometrium, Nogales et al. observed heterogeneous reactivity for HNF1β in only 50% of glandular YSTs (6/12) [4]. Therefore, the sensitivity and specificity of HNF1β still need to be addressed in GCT.

HNF1β, also known as vHNF1 and LFB3, is a transcriptional regulator involved in early embryonal development and glucose metabolism. Expression of HNF1β is first detected in the visceral endoderm at the onset of gastrulation [5]. In the yolk sac, onset of HNF1β expression is concomitant with that of α-fetoprotein (AFP) [6,7]. Later in development, expression of HNF1β is observed in several polarized epithelia of tissues of endodermal and mesodermal origin, including the primitive gut and the liver diverticulum, and expression is maintained up to the adult stage [6,7]. HNF1β is necessary for visceral endoderm differentiation, as well as for adequate neural tube and gut morphogenesis [5]. These findings provide a rationale to HNF1β expression in YST.
In this study, we assess HNF1β reactivity in a series of 49 YSTs from 45 patients, occurring either in a pure form, or as a component of a mixed GCT, and provide a comparison with previously reported YST immunohistochemical stains. To further document the potential diagnostic value and specificity of HNF1β in YST, immunohistochemical reactivity to HNF1β is assessed in the other available germ cell components, in the cohort of mixed GCTs.

2. Materials and Methods

2.1 Patient selection

Over a 21-year period (from 1996 to March 2017), yolk sac tumors (YSTs) occurring in 45 patients were retrieved from the archives of the Division of clinical pathology, Geneva University Hospitals. This study is a retrospective description, and ethics committee approval is not required. The clinical and pathological findings are summarized in Table 1. Patients were mostly males (39/45), with primary tumors occurring mainly in the testis (37/39). In one male patient, analysis was performed on two consecutive post-chemotherapy lymph node metastases with YST component: the primary testicular tumor consisted of seminoma and teratoma, but the YST component was not identified. Lymph node resection was performed respectively 3 years and 7 years after initial diagnosis. Two male patients had a primary mediastinal pure YST; primary tumor and liver metastasis resection was performed in one of the two patients 4 months after chemotherapy initiation. Additionally, 4 testicular tumors were pure YSTs. One of the four testicular pure YSTs was the sole prepubertal tumor of the series, arising in a 14 month-old boy. Age range at initial diagnosis was 14 months to 49 years (mean and median 30y).

All 6 tumors occurring in females (6/45) were primary ovarian pure YSTs. In one patient, a metachronous peritoneal metastasis was further available for analysis; no chemotherapy was administered in the interval. Two patients were of pediatric age, aged 4 and 15 years at diagnosis. Age range was 4 to 72 years (mean 35y, median 24y).

Additional tumors from 30 patients aged 17 to 63 years (mean 34y, median 33y) with no morphologically identified YST component were also considered, and consisted of either pure or mixed GCTs. All but one (a lung metastasis) were primary testicular tumors.

2.2 Immunohistochemistry
Histology of all retrieved GCTs was reviewed by the two authors, and a block with an adequate yolk sac component was selected for analysis. SALL4 reactivity was used to confirm the diagnosis of a germ cell tumor. OCT4 and CD30 helped identify and confirm the embryonal carcinoma component. The other markers used to assess the YST component were Cytokeratin 19 (CK19), Glypican 3 (GPC3), CDX-2 and GATA3; AFP was not performed, due to variable sensitivity of this marker.

Immunohistochemistry was conducted on all tumors. Briefly, unstained 3 μm sections on whole charged slides were prepared from paraffin blocks. High temperature antigen retrieval was applied. Reactivity to primary antibodies was identified using the Ultravision detection system and DAB as the chromogen substrate (Thermo Fisher Scientific) for the following antibodies: CD30 (Dako mouse monoclonal, clone Ber-H2; prediluted); Cytokeratin 19 (CK19, Dako mouse monoclonal, clone RCK108, 1:100 dilution); GATA3 (Biocare, Mouse monoclonal, clone CM 405, dilution 1:100); GPC3 (Cell Marque mouse monoclonal, clone 1G12; dilution 1:20); Spalt-like protein 4, SALL4 (BioCare Medical mouse monoclonal, clone 6E3; 1:100 dilution); CDX-2 (BioGenex Mouse monoclonal, clone CDX2-88; 1:200 dilution); OCT4 (abcam mouse monoclonal, clone SEMGC; dilution 1:2); and HNF1β (Sigma-Aldrich Rabbit polyclonal, ref. HPA002083; 1:400 dilution).

HNF1β nuclear reactivity was semi-quantitatively recorded in the yolk sac component, taking into account the most intense staining pattern observed in the tumor (0 = no reactivity; 1+ = faint reactivity; 2+ = moderate reactivity; 3+ = strong reactivity). The percentage of positive cells was estimated. Immunohistochemical score was calculated as follows: percentage of positive cells x staining intensity (0 to 300), and positivity was retained if the score was superior to 5. Consensus was reached between the two authors. The pattern of staining was also documented in the other germ cell components, whenever present.

3. Results

3.1. Histological findings

Pure YSTs
Pure YSTs were identified in 12 patients (12/45, 26.7%). All 6 tumors from female patients were pure YSTs (6/6, 100%). The eldest female patient underwent subsequent peritoneal metastasis resection, and displayed similar histological findings since no intercurrent chemotherapy was administered. In males, pure YSTs were seen in 6 patients (6/39, 15.4%), and included 4 testicular tumors (4/37 primary testicular GCTs, 10.8%), among which the prepubertal tumor in a 14-month old boy (Fig. 1A-1C), and the two primary mediastinal tumors. Although the male patient in whom lymph node metastases showed only YST, the primary testicular tumor had displayed seminoma and teratoma and could not therefore be subjected to immunohistochemical analysis; this patient was however considered as having a mixed GCT.

YST as component of a mixed GCT

Other germ cell components were identified in 32 of the 44 primary tumors with YST component subjected to immunohistochemical analysis (72.7%), and only in primary testicular tumors (32/36 testicular GCTs, 88.9%). The most frequent other germ cell component was embryonal carcinoma (32/36, 88.9%). Teratoma was seen in less than half of the mixed GCTs (16/36, 44.4%). Choriocarcinoma was rare (6/36, 16.7%); one additional tumor showed scattered syncytiotrophoblastic cells, that were considered part of the embryonal carcinoma component they mingled with. Finally, seminoma was similarly infrequent (6/36, 16.7%).

YST in post-chemotherapy specimens

Post-chemotherapy specimens were available for analysis in only two male patients. In the patient with a mixed testicular GCT without identification of YST, two lymph nodes were resected 3 years and 7 years after introduction of chemotherapy. One patient with a primary mediastinal pure YST benefited from primary tumor resection and liver metastasis resection after 4 months of chemotherapy.

GCT tumors with no YST component
This group composed of 30 tumors consisting either of pure or mixed GCT allowed for further assessment of 20 EC, 14 seminoma, and 4 choriocarcinoma components. The lung metastasis, from a 40-year-old male patient, was solely composed of choriocarcinoma.

### 3.2 Immunohistochemistry findings

SALL4 reactivity and OCT4 negativity were seen in all YSTs.

**HNF1β in the YST component**

HNF1β decorated the YST component in all 49 tumors assessed (Fig. 1B). Reactivity was widespread, with staining of 70% to 100% of the tumor cells in 42 of the 45 tumors resected prior to chemotherapy (93.3%). Figures 1A to 1C illustrate characteristic findings. In the remaining 3 tumors, reactivity to HNF1β was focal, decorating between 10% and 40% of the vitelline tumor cells. Similarly, the four post-chemotherapy specimens showed focal HNF1β staining, with 30% to 40% of the tumor cells showing reactivity. Of note, although HNF1β was diffuse, in a primary mediastinal YST before chemotherapy (with reactivity in 100% of the tumor cells), staining was only focal in the post-chemotherapy specimens from the same patient, decorating respectively 40% and 30% of the tumor cells in the mediastinal tumor and in a liver metastasis.

Nuclear intensity of staining was strong (3+) in a large majority of the tumors (46/49, 93.9%), and moderate (2+) in the remaining 3 tumors. The main immunohistochemical findings, and comparison with other YST markers are summarized in Table 2.

In the ovarian tumors with major diagnostic issues, one pure endometrioid-like with similar findings in a metachronous peritoneal metastasis (Fig. 1D-1F) and two hepatoid yolk sac tumors (Fig. 1G-1I), reactivity to HNF1β was intense (3+), in 100% and 80% of the tumor cells respectively. HNF1β reactivity was seen in all the foci of reticular/microcystic, endodermal, macrocystic, and glandular/alveolar differentiation. HNF1β reactivity intensity was moderate to strong, albeit staining different percentages of the tumor cells. Spindle to stellate cells in myxomatous foci all remained negative (7/7), similar to the absence of SALL4 reactivity in all but one tumor that showed SALL4 reactivity in the stellate and spindle cells. Solid or solid/undifferentiated foci were mostly negative for HNF1β, reactivity being observed in only one of the 5 tumors with such a pattern of differentiation (1/5, 20%).
HNF1β pattern of staining according to the histological YST subtypes is summarized in Table 3.

**HNF1β in YST intricately admixed with EC**

HNF1β highlighted the intimate association of YST and EC in 32 testicular tumors, realizing a diffuse embryoma (garland or necklace-like) pattern in 17 tumors (53.1%), and a polyembryoma (early presomite embryo-like) pattern in 9 tumors (28.1%), with coexistence of the two patterns in two additional tumors (6.2%). Representative examples of the two patterns are shown in Figures 2A-C and 2D-F respectively. Finally, the 4 remaining tumors showing both YST and EC displayed an intricate but patternless architecture. No EC component (0/32) displayed reactivity to HNF1β. Results are summarized in Table 4.

**HNF1β in the other mixed CGT components**

HNF1β reactivity was also focally observed in glands showing features of intestinal differentiation intermingled with a teratoma component (11/16, 68.8%) (Fig. 3A-B).

All 6 cases with a choriocarcinoma component displayed HNF1β reactivity (6/6). However, contrary to the reactivity seen in the yolk sac component, only cytoplasmic staining of varying intensity was seen. In one tumor, additional sub-membranous and membranous reactivity decorated the syncytiotrophoblastic cells (Fig. 3C-D). In a further mixed GCT, syncytiotrophoblastic cells were seen interspersed in the EC component; these cells did not show reactivity to HNF1β. Seminoma cases were entirely negative (0/6).

The immunohistochemical findings are summarized in Table 5.

**GCT tumors with no YST component**

In accordance with the above-mentioned results, no reactivity to HNF1β was observed in the 20 EC assessed. However, HNF1β highlighted a minor tumoral component (less than 1% of the tumor burden) arranged in small cords of nests of small glandular structures in 8/20 of the cases (40%). Upon retrospective morphological re-evaluation, in only 3 of the 8 cases were these cells consistent with a minor YST component (maximum 0.2 cm) that had initially been
overlooked; in the 5 remaining cases, retrospective evaluation failed to identify morphologically a definite YST component. Therefore, this immunohistochemical marker adds value in the identification of minute foci of YST in mixed GCT.

HNF1β also identified in one further case rare isolated tumor cells, interspersed within the seminoma (1/14, 7.1%) tumor cells. Additionally, focal (<1%) and faint (1+) HNF1β nuclear staining was seen in early invasive components at the periphery of 5 additional cases of seminoma exclusively (5/14, 35%), a finding not observed in the 6 previously assessed mixed GCT with both seminoma and YST components (in total 5/20, 25%). In these foci, faint HNF1β reactivity was observed in a minority of invasive tumor cells interspersed between residual seminiferous tubules containing HNF1β positive in situ germ cell neoplasia (IGCN). This pattern of reactivity may therefore identify a marker initially retained at the most early stages of invasion. However, the low levels of expression, and the minute foci involved, render these areas unlikely to be confused with a YST component.

Finally, the 4 additional choriocarcinomas tested all showed cytoplasmic and membranous reactivity to HNF1β, but no nuclear staining.

**HNF1β in seminiferous tubules, rete testis, epididymis and vas deferens**

In a majority of the tumors with available peritumoral seminiferous tubules (42/45, 93.3%), a few isolated non-atypical basally located large cells consistent with spermatogonia showed nuclear reactivity to HNF1β (Fig. 4A). Staining intensity was faint to moderate. Only rare HNF1β positive spermatogonia were seen in the tubules from the 14-month old boy, in keeping with immature seminiferous tubules. The three cases devoid of HNF1β positive cells in seminiferous tubules consisted of one case of diffuse germ cell neoplasia in situ (GCNIS), and two cases with diffusely atrophic tubules composed solely of Sertoli cells. Therefore, in all three cases, spermatogonia had been totally replaced.

Rete testis displayed cytoplasmic HNF1β reactivity in cubic cells, in 15 of the 16 available cases (93.8%). Staining was mainly focal, being diffuse in only one case. Additionally, in four cases, intense HNF1β nuclear reactivity was seen in more polygonal cells.

Epididymis was available for evaluation in 22 cases, and vas deferens in 11 cases (Fig. 4B). These structures all showed nuclear reactivity to HNF1β, with a mainly moderate to strong
intensity of staining. Some epithelial cells further showed apical membranous staining (epididymis: 2/22, 0.9%; vas deferens: 7/11, 63.6%).

Other YST markers

Among the other markers used to assess the yolk sac component, CK19 and GPC3 represented sensitive markers, being both positive in all but 2 cases without prior chemotherapy (43/45, 95.6%); these two cases were positive for HNF1β. CDX-2, an endodermal lineage marker, decorated mostly the glandular component in 30/44 cases (68.2%), whereas GATA3 was positive in only 27 tumors (27/45, 60%).

Immunohistochemical findings, conclusion

Overall HNF1β sensitivity in the identification of the YST component was 100%, highlighting at least focal areas in all assessed tumors with a morphologically identified YST. Moreover, a minor YST component was further highlighted by this marker in close contact to an EC component in 8 of 20 GCT initially considered as devoid of YST. In our series, high sensitivity was seen in the YST component for CK19 and GPC3 (95.6%), whereas CDX-2 and GATA3 showed poor sensitivity (68.2% and 60% respectively). HNF1β specificity was 80%, due to HNF1β reactivity in glands considered part of a teratoma component.

4. Discussion

Yolk sac tumors (YSTs) display multiple histological features and may mimic other types of tumors. Known immunohistochemistry markers tend to lack sensitivity. Correct identification of YST is however essential, since this component is an independent poor prognostic indicator [8,9]. In a series of 49 YSTs from 45 patients, we show that HNF1β, a factor necessary for early visceral endoderm differentiation, represents a highly sensitive immunohistochemical diagnostic marker for YST.

Mutations in the HNF1β gene (TCF2), a homeobox gene that functions as a homodimer or heterodimer with HNF1α, cause maturity-onset diabetes of the young type 5 (MODY5), renal
cysts, genital malformations, and pancreas atrophy [10]. HNF1β has also been shown to induce oncogenesis by acting as a transforming gene in cooperation with ERBB2, inducing epithelial-to-mesenchymal transition (EMT), and invasive phenotypes [11]. Downregulation of HNF1β contributes to drug resistance in ovarian cancer; ovarian clear cell carcinoma shows poor response to chemotherapy regimen, and poor prognosis [12]. Genome-wide association studies have linked HNF1β sequence variants with increased prostate cancer risk [13], and high expression of HNF1β has also been associated with poor prognosis and worse survival in pancreatic carcinoma [14].

At least 90% of testicular tumors are germ cell tumors (GCTs). The new 2016 World Health Organization (WHO) classification for tumors of the urinary tract and male genital organs distinguishes testicular prepubertal (non-germ cell neoplasia in situ-derived, non-GCNIS) from postpubertal-type tumours (GCNIS-derived) [15]. In particular, prepubertal-type YSTs occur primarily in pure form, and show no association with GCNIS or cryptorchidism. These tumors are biologically less aggressive than their post-pubertal counterparts [15]. Accordingly, the sole prepubertal YST in our series was diagnosed at age 14 months and consisted of a pure YST. The child shows no evidence of relapse 8 years after initial diagnosis.

In mixed GCTs, HNF1β helped delineate YST from intricately admixed embryonal carcinoma (EC). The so-called diffuse embryoma (garland or necklace-like) and polyembryoma (early presomite embryo-like) patterns have been described as infrequent, being identified in respectively 3% and 6% of admixed YSTs and ECs [16]. The diffuse embryoma pattern shows elongated stretches of EC cells admixed with a parallel layer of YST cells, whereas the polyembryoma pattern recapitulates the presomatic embryo [16]. Contrariwise, we observed a "polyembryoma-like" and/or "necklace" pattern in a majority of the mixed GCTs assessed (28/32, 87.5%), the remaining 4 tumors with mixed YST and EC components showing a patternless architecture. HNF1β helped in highlighting the intimate association between the two tumor components, displaying a perfect mirror image with the OCT-4 positive, HNF1β negative EC cells. Moreover, HNF1β further delineated in a subset of tumors a minor and initially morphologically overlooked or non-identifiable YST component.
Whereas HNF1β reactivity was observed in all YSTs, mainly with a strong and diffuse pattern of staining, nuclear positivity was also observed in a subset of glands showing intestinal differentiation. Nogales et al. accordingly observed HNF1β reactivity in half of the glandular YST components, in a series of 12 mixed clear cell carcinoma with GCT elements [4]. No other GCT component showed HNF1β nuclear reactivity, choriocarcinoma showing only faint and focal cytoplasmic-restricted reactivity to HNF1β.

Recently, Xiao et al. reported a new and sensitive marker for the detection of YST, ZBTB16. Sensitivity was high in primary YST (100%), but specificity was 66% [17]. Sensitivity in identifying metastatic and extragonadal YST was lower (91.6%) [18]. Combination of HNF1β and ZBTB16 immunohistochemistry may therefore prove highly sensitive. Further, the use of these two markers in combination with OCT4 may provide a mirror image allowing for clear distinction between YST and EC, in cases showing intimate admixture of these two germ cell components. In our series, GPC3 showed high, albeit slightly lower sensitivity than HNF1β in the detection of YST. We would certainly recommend the use of an immunohistochemistry panel combining SALL4, HNF1β, GPC3, OCT4, and possibly ZBTB16 according to Xiao et al. [17], in the evaluation of a mixed germ cell tumor, in order to adequately identify the YST and EC components.

While mixed GCTs are common in the testis, they remain rare in the ovary. In the ovary therefore, diagnostic issues will mainly be related to particular YST variants. We observed intense reactivity to HNF1β in the 2 ovarian tumors with major diagnostic issues, displaying respectively features of a pure endometrioid-like YST, and of a pure hepatoid YST. Clear cell carcinoma and endometrioid carcinoma of the ovary represent further diagnostic pitfalls, since HNF1β reactivity has been shown in 92% and 100% of the tumors respectively, albeit with weak intensity of staining in endometrioid carcinoma [3]. SALL4, a nuclear zinc finger transcription factor and a key player in self-renewal maintenance and embryonic stem cell pluripotency [19] will however display reactivity in YST [20] and allow correct diagnosis.

The "hepatoid pattern" of YST refers to focal or diffuse morphological features resembling malignant hepatocytes, and therefore raises the differential diagnosis of metastasis from a primary liver malignancy. SALL4 reactivity has been reported in the embryonal component of hepatoblastomas [21], a finding also observed in our hands (unpublished data). In the pure hepatoid YST we report in our series, age would however be uncommon for hepatoblastoma (21-year-old female) [22], and the patient had no liver mass, nor past relevant clinical history. In hepatocellular carcinoma (HCC), strong SALL4 nuclear staining has been shown, albeit
with a peculiar pattern of staining the authors describe as "punctuate/clumped" [23]. To further complicate the distinction between hepatoid YST and metastasis from a primary liver malignancy, expression of HNF1β has been described in HCC, with conflicting results. Whereas some series have described frequent HNF1β reactivity in HCC (62%, n=97), with however less than half the tumor cells displaying reactivity (in 92.8% of the cases) [24], others have reported an absence of HNF1β expression in HCC [25].

Immunohistochemical expression is an indicator of differentiation, and as such always requires integration with all other parameters, whether clinical, or morphological. Accordingly, we observed only rare HNF1β reactivity in less differentiated YST areas displaying a diffuse or diffuse/undifferentiated growth pattern. Tumors occurring in older age groups and discovered at unusual locations may prove diagnostically challenging. The rare adenocarcinomas with enteroblastic differentiation, recently recognized as a variant of α-fetoprotein (AFP)-producing carcinomas, also express SALL4 [26], and it is reasonable to hypothesize that these tumors would also express the early visceral endoderm differentiation marker HNF1β. We have seen one such tumor that ultimately proved to be a liver metastasis from a primary gastric adenocarcinoma with enteroblastic differentiation. The liver metastasis exclusively showed features of YST, and expressed both SALL4 and HNF1β (data not shown). Alternatively, tumors with a pure or predominantly YST pattern arising at unusual locations may represent mixed GCTs with overgrowth of the YST component [4]. These tumors mostly present at advanced clinical stages.

Chemotherapy regimens may alter the histological and immunohistochemical findings. Pre- and post-chemotherapy specimens were available in only one patient in our series: HNF1β reactivity was less diffuse in the post-chemotherapy primary tumor and liver metastasis. YST components may be difficult to identify in metastatic or recurrent tumors after chemotherapy. Although analysis of further cases is warranted, these results suggest that similar to other immunohistochemical markers, HNF1β may be an imperfect adjunct in such settings.

Finally, in normal male gonad and adnexae, we show that HNF1β stains spermatogonia, the rete testis cells, epididymis to some extent, and the epithelial cells of the vas deferens.

In summary, YST requires accurate identification but may easily be under-diagnosed on morphology alone. HNF1β represents a highly sensitive immunohistochemical marker of YST, with a high specificity in an adequate setting.
Acknowledgments

The authors thank Mrs Patrizia Bordignon for immunohistochemistry techniques.
Legends to Figures

Figure 1. Immunohistochemical staining of HNF1β in the YST component. Original magnification x200.
1A-C. Pure gonadal (testicular) prepubertal YST in a 14-month-old boy. A. Hematoxylin&Eosin (H&E) shows EE differentiation, with a Schiller-Duval body, and numerous hyaline globules. All tumor cells show strong nuclear expression of SALL4 (B) and HNF1β (C). 1D-F. Pure gonadal (ovarian) endometrioid-like YST in a 72-year-old female (x100). D. H&E shows pure SE differentiation, with a complex meshwork of anastomosing malignant glands. All tumor cells show strong nuclear expression of SALL4 (E) and HNF1β (F), whereas the two markers show no reactivity in the spindle cell stroma. 1G-I. Pure gonadal (ovarian) hepatoid YST in a 21-year-old female. G. H&E shows pure SE differentiation, reminiscent of liver cell plates. Tumor cells show mild by definite reactivity for SALL4 (H), and strong nuclear expression of HNF1β (I).

Figure 2. Immunohistochemical staining of HNF1β in YST intricately admixed with EC. Original magnification x100 (A-C), and x200 (D-F).
2A-C. Mixed testicular GCT, with intimately admixed YST and EC in a 39-year-old male. A. H&E shows mainly EC, whereas YST is more difficult to identify. B. The EC cells react to OCT4. C. HNF1β shows a perfect mirror image, not only confirming the presence of a YST component, but also highlighting the diffuse embryo (garland or necklace-like) pattern.
2D-F. Mixed testicular GCT, with intermingled YST and EC in a 38-year-old male. D. H&E shows YST and EC, realizing an embryoid body. E. OCT4 highlights the EC component. F. The YST component expresses HNF1β.

Figure 3. Immunohistochemical staining of HNF1β in the other mixed CGT components. Original magnification x100.
3A-B. Mixed testicular GCT, with intermingled teratoma and YST in a 19-year-old male. A. H&E shows teratoma, with squamous epithelium in continuity with glandular, enteric-type epithelium. B. The squamous epithelium is HNF1β-negative, whereas the glandular elements are highlighted by HNF1β.
3C-D. Choriocarcinoma component. C. H&E shows a malignant biphasic cytotrophoblastic and syncytiotrophoblastic proliferation, with hemorrhage. D. The tumor cell show only faint and focal cytoplasmic reactivity to HNF1β, as well as membranous staining.

Figure 4. Immunohistochemical staining of HNF1β in normal testis and adnexae. Original magnification x200 (A), and x100 (B). A. Spermatogonia show nuclear reactivity to HNF1β. B. Intense HNF1β nuclear reactivity and apical membranous staining in vas deferens.
Legends to tables

Table 1. Patient characteristics.
Abbreviations: N: numbers; mo: months; y: years; YST: yolk sac tumor; GCT: germ cell tumor; incl.: including

Table 2. Yolk sac tumor, immunohistochemical staining of HNF1β and classical YST markers. Results are expressed in percentages of positive cells, followed by staining intensity, and number of cases (in brackets)
Abbreviations: N: numbers; YST: yolk sac tumor; GCT: germ cell tumor; NA: non available

Table 3. HNF1b reactivity according to histological YST pattern (more than one pattern may be seen within each tumor). Results are expressed in percentages of positive cells, followed by staining intensity, and number of cases (in brackets)
#Female patient with metachronous ovarian pure YST and peritoneal metastasis; no intercurrent chemotherapy
Abbreviations: SE: somatic endoderm; EE: extraembryonal endoderm

Table 4. HNF1β reactivity according to the pattern of Yolk Sac Tumor vs. Embryonal Carcinoma (testis only)
Results are expressed in percentages of positive cells, followed by staining intensity, and number of cases (in brackets)
Abbreviations: N: numbers; YST: yolk sac tumor; EC: embryonal carcinoma

Table 5. HNF1β reactivity in other GCT components (testis only)
Results are expressed as number of positive cases on total number of available cases
References

TABLE 1. Patient characteristics.

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**Specimens available for IHC evaluation (N=49)**

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<th>Localisation, primary tumor</th>
<th>Pure YST</th>
<th>Mixed GCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>No prior chemotherapy</td>
<td>Testis (N=4, incl. 1 prepubertal)</td>
<td>Testis (N=32)</td>
</tr>
<tr>
<td>Mediastinum (N=2)</td>
<td>Mediastinum (N=1)</td>
<td>Lymph nodes (N=2)</td>
</tr>
<tr>
<td>Peritoneum (N=1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Liver (N=1)</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbrevations: N: numbers; mo: months; y: years; YST: yolk sac tumor; GCT: germ cell tumor; IHC : immunohistochemistry; incl.: including
TABLE 2. Yolk sac tumor, immunohistochemical staining of HNF1β and classical YST markers. Results are expressed in percentages of positive cells, followed by staining intensity, and number of cases (in brackets).

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Location</th>
<th>HNF1β</th>
<th>CDX2</th>
<th>CK19</th>
<th>GATA3</th>
<th>GPC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure YST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis (N=4)</td>
<td>0 0 0</td>
<td>&lt;90%-100%</td>
<td>40%-100%</td>
<td>30%-90%</td>
<td>15%-80%</td>
<td></td>
</tr>
<tr>
<td>Mediatinum* (N=2)</td>
<td>0 0 0</td>
<td>&lt;80%-100%</td>
<td>70%-100%</td>
<td>20%-80%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovary (N=6)</td>
<td>0 0 0</td>
<td>&lt;80%-100%</td>
<td>70%</td>
<td>20%-80%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peritonueum (N=1)</td>
<td>0 0 0</td>
<td>&lt;100%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>YST as component of a mixed GCT</td>
<td>Testis (N=32)</td>
<td>0 0 0</td>
<td>&lt;80%-100%</td>
<td>10%-30%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-chemotherapy specimens</td>
<td>Lymph nodes (2x)</td>
<td>0 0 0</td>
<td>40% (2nd procedure)</td>
<td>30% (1st procedure)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Metastasis Mediatinum</td>
<td>0 0 0</td>
<td>30%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinum*</td>
<td>100% 3+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Abbreviations: N: numbers; Neg:Negative; YST: yolk sac tumor;
GCT: germ cell tumor; NA: non available; ND: not done
*Male patient with pure mediastinal YST tumor (primary tumor assessed before and after chemotherapy; liver metastasis resected after chemotherapy)
#Female patient with metachronous ovarian pure YST and peritoneal metastasis; no intercurrent chemotherapy
Table 3. HN1β reactivity according to histological YST pattern (more than one pattern may be seen within each tumor)
Results are expressed in percentages of positive cells, followed by staining intensity, and number of cases (in brackets)

<table>
<thead>
<tr>
<th>Primary resection</th>
<th>YST Pattern</th>
<th>HN1β</th>
<th>HNF1β</th>
<th>HNF1β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>80-100% 3+</td>
<td>10%-70% 3+</td>
<td>80-100% 2+</td>
</tr>
<tr>
<td></td>
<td>Reticular/microcystic (EE)</td>
<td>(35/41)#</td>
<td>(3/41)</td>
<td>(3/41)</td>
</tr>
<tr>
<td></td>
<td>Endodermal (EE)</td>
<td>80%-100% 3+</td>
<td>10% 3+ (1/5)</td>
<td>100% 2+ (1/5)</td>
</tr>
<tr>
<td></td>
<td>Macrocystic (EE)</td>
<td>100% 3+ (4/4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myxomatous (EE)</td>
<td>Neg (7/7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solid (EE)</td>
<td>100% 3+ (1/4)</td>
<td>0% (3/4)#</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glandular/Alveolar (SE)</td>
<td>100% 3+ (4/5)#</td>
<td>70% 3+ (1/5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hepatoid (SE)</td>
<td>80% 3+ (2/2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endometrioid-like (SE)</td>
<td>100% 3+ (1/1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solid/undifferentiated (SE)</td>
<td>Neg (1/1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post-chemotherapy specimens</th>
<th>YST Pattern</th>
<th>HN1β</th>
<th>HNF1β</th>
<th>HNF1β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node (2x)</td>
<td>Reticular/microcystic (EE)</td>
<td>30% 3+ &amp; 40% 2+</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solid/undifferentiated (SE)</td>
<td>Neg &amp; 40% 1+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinum#</td>
<td>Reticular/microcystic (EE)</td>
<td>40% 3+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solid (EE)</td>
<td>Neg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glandular/Alveolar (SE)</td>
<td>40% 3+</td>
<td>Mediastinum#</td>
<td></td>
</tr>
<tr>
<td>Liver metastasis#</td>
<td>Reticular/microcystic (EE)</td>
<td>30% 3+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glandular/Alveolar (SE)</td>
<td>30% 3+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#Female patient with metachronous ovarian pure YST and peritoneal metastasis; no intercurrent chemotherapy
Abbreviations: SE:somatic endoderm ; EE:Extraembryonal endoderm
Table 4. HN1β reactivity according to the pattern of Yolk Sac Tumor vs. Embryonal Carcinoma (testis only)
Results are expressed in percentages of positive cells according to staining intensity, and number of cases (in brackets)

<table>
<thead>
<tr>
<th>YST/EC pattern</th>
<th>HNF1β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Diffuse embryoma (N=17)</td>
<td>0</td>
</tr>
<tr>
<td>Polyembryoma (N=9)</td>
<td>0</td>
</tr>
<tr>
<td>Diffuse embryoma and polyembryoma (N=2)</td>
<td>0</td>
</tr>
<tr>
<td>Indistinct (N=4)</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: N: numbers; YST: yolk sac tumor; EC: embryonal carcinoma
Table 5. HNF1β reactivity in other GCT components (testis only)
Results are expressed as number of positive cases on total number of available cases

<table>
<thead>
<tr>
<th>Component</th>
<th>HNF1β</th>
<th>Choriocarcinoma</th>
<th>Seminoma</th>
<th>Teratoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonal carcinoma</td>
<td>0 (0/28)</td>
<td>Cytoplasmic only, faint and focal (6/7)</td>
<td>0 (0/6)</td>
<td>Enteric-type glands only (11/16)</td>
</tr>
</tbody>
</table>
Figure 2