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# H&E and OCT4/CD34 for the assessment of lympho-vascular invasion in seminoma and embryonal carcinoma

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ABSTRACT

*Background:* Lymphovascular invasion (LVI) is a relevant prognostic factor in germ cell tumors of the testis (GCTT), and it is included in the pT stage. However, its detection on hematoxylin and eosin (H&E) slides is very challenging, and previous studies reported fair to moderate inter-observer agreement among dedicated uropathologists. In the present study, we tested H&E and a recently developed in-house double staining for OCT4/CD34 to detect LVI in GCTT.

*Methods:* Nine authors [5 non-uropathologists and 4 uropathologists] independently evaluated 34 consecutive and retrospectively enrolled cases of GCTT. We assessed the inter-observer agreement (*Fleiss*'s Kappa) with both H&E and OCT4/CD34. Besides, we compared the consensus diagnosis on both H&E and OCT4/CD34-stained sections with the original diagnosis to evaluate the pT re-staging (*McNemar* test) and identify the sources of disagreement.

*Abbreviations:* LVI, lymphovascular invasion; GCTT, germ cell tumors of the testis; ISUP, International Society of Urological Pathology; UPs, uropathologists; non-UPs, non-uropathologists; GCNIS, germ cell neoplasia in situ; IrOA, inter-observer agreement; H&E, hematoxylin and eosin; IHC, immunohistochemistry; OCT4, octamer-binding transcription factor 4; CD34, cluster of differentiation 34; SALL4, sal-like protein 4; CD, consensus diagnosis/diagnoses; CD-H&E, consensus diagnosis on H&E slides; CD-OCT4/CD34, consensus diagnosis on OCT4/CD34 slides; S, seminoma/seminomas; EC, embryonal carcinoma/carcinomas; S-EC, mixed GCTT with S and EC components; AJCC, American Joint Committee on Cancer; RLND, retroperitoneal lymph node dissection; *ETS-related gene*, transcriptional regulator ERG encoded by ERG; D2–40, podoplanin; CD31, cluster of differentiation 31; LYVE-1, lymphatic vessel endothelial hyaluronan receptor 1; vWF, von Willebrand factor; vs, versus.

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*Results:* The inter-observer agreement among uropathologists plus non-uropathologists was fair with both H&E (KF=0.398; $p < 0.001$) and OCT4/CD34 (KF=0.312; $p < 0.001$). OCT4/CD34 (KF=0.290; $p < 0.001$) slightly reduces the inter-observer agreement compared to H&E (KF=0.321; $p < 0.001$) for non-uropathologists; in contrast, OCT4/CD34 (KF=0.293; $p < 0.001$) significantly reduces the inter-observer agreement compared to H&E (KF=0.529; $p < 0.001$) for uropathologists, changing it from moderate to fair. Consensus diagnosis with H&E modified the LVI status of the original diagnosis in 8/34 (23.5 %) cases (*p: 0.070*), with pT re-staging in 2/34 (5.9 %) cases (*p: 0.500*). Consensus diagnosis with OCT4/CD34 modified the LVI status of the original diagnosis in 8/34 (23.5 %) cases (*p: 0.289*), with pT re-staging in 3/34 (8.8 %) cases (*p: 0.250*). The consensus diagnosis with OCT4/CD34 modified the consensus diagnosis with H&E in 8/34 (23.5 %) cases (*p: 0.727*), and these findings resulted in pT-restaging in 3/34 (8.8 %) cases (*p: 0.500*). The sources of disagreement among uropathologists were: H&E [artefactual clefts misinterpreted as LVI in 4/6 (66.7 %) cases and true foci of LVI misinterpreted as clusters of histiocytes within the vessels in 2/6 (33.3 %) cases], OCT4/CD34 [artefactual clefts misinterpreted as LVI in 2/8 (25 %) cases, true LVI misinterpreted as artefactual clefts in 2/8 (25 %) cases or floaters in 4/8 (50 %) cases].
*Conclusions:* OCT4/CD34 does not improve the inter-observer agreement for the assessment of LVI in OCT4(+) GCTT. Consensus diagnosis with H&E modifies the LVI status in a significant number of cases, resulting in changes of the pT stage in a relatively small subgroup. Consensus diagnosis with OCT4/CD34 provides little additional benefit since it cannot exclude mimickers of LVI such as floaters and artefactual clefts. These results argue against the adoption of this diagnostic tool for the routine assessment of OCT4(+) GCTT.

## 1. Introduction

Lymphovascular invasion (LVI) is a crucial prognostic factor in seminoma (S) and embryonal carcinoma (EC), and it is incorporated in the pT stage of germ cell tumors of the testis (GCTT) [1,2]. More specifically, LVI is in and of itself sufficient to classify GCTT as pT2/IB stage and makes patients eligible for adjuvant treatments [1–9]. In contrast, orchiectomy followed by active surveillance is currently preferred for pT1/IA stage in patients without high-risk features, but there are histotype- and guideline-dependent differences [1–9]. For pure S, although LVI makes patients eligible for adjuvant treatments, active surveillance is still the "highly-preferred" strategy by the NCCN guidelines [9]. For nonseminomatous GCTT (and mixed GCTT with S component), LVI most likely results in adjuvant treatments, and active surveillance is a residual option for a small subgroup of patients [2–9]. The International Society of Urological Pathology (ISUP) recommended that LVI in GCTT should be assessed by dedicated uropathologists (UPs) on hematoxylin and eosin (H&E) slides using well-defined histologic criteria [10]. However, the assessment of LVI may be challenging due to several confounding factors such as the presence of histological mimickers [clusters of histiocytes within the vessels, germ cell neoplasia in situ (GCNIS), floaters, and artefactual clefts], and previous studies have reported a fair to moderate inter-observer agreement (IrOA) among UPs [10–16]. Only a few studies evaluated the utility of immunohistochemistry (IHC) for identifying LVI in GCTT, yielding somewhat conflicting results [16–19]. Therefore, ISUP has not issued formal recommendations regarding the use of IHC to aid in the detection of LVI [10]. In this study, we evaluated a double stain (DS) for OCT4/CD34 in a cohort of OCT4(+) GCTT [seminomas (S), embryonal carcinomas (EC), and mixed GCTT with S and EC components (S-EC)]. Prior data published by our group demonstrated that this combined stain (OCT4: tumor marker; CD34: vascular marker) is reliable and potentially useful to distinguish true LVI from mimickers, resulting in modifications of the pT stage [20,21]. In the present study, we evaluated the IrOA among UPs and non-uropathologists (non-UPs) for interpreting the LVI status of GCTT on H&E and OCT4/CD34 slides. Additionally, we compared the consensus diagnosis (CD) and the original diagnosis to analyze the effect of CD on tumor pT re-staging and discussed the potential sources of disagreement among UPs.

## 2. Materials and methods

### 2.1. Case series

We retrospectively collected 34 OCT4(+) GCTT [29 S, 3 EC, and 2 S-EC] diagnosed between January 1st 2019 and April 1st 2022 at our Institution (Pathology Unit, Maggiore Hospital-AUSL Bologna, Bologna). Fifteen additional cases were excluded based on predefined criteria: age < 18 years old (2 cases), patients with missing clinical-pathologic data (13 cases), and cases with no archival tissue available (5 cases). Clinical parameters (age and tumor size) were retrieved from the digital records of the Urology Department, Maggiore Hospital-AUSL Bologna. All cases had been diagnosed and staged according to the 5th edition of the WHO classification of urinary and male genital tumors and the 8th edition of the AJCC Cancer Staging Manual [2,21,22]. Some cases in this cohort have been previously published by our group [19,20,23,24].

### 2.2. Datasets (H&E and OCT4/CD34), LVI assessment, and consensus diagnoses (CD)

All cases were reviewed to confirm the original diagnosis and select a representative block, as previously defined [19,20]. Two consecutive 3-μm sections were cut from each paraffin-embedded tissue block and stained with H&E and OCT4/CD34 (BenchMark ULTRA automated immunostainer; Ventana Medical Systems-Roche Diagnostics, Switzerland), respectively. Immunohistochemical protocols, antibody clones, and other technical data are summarized in Supplementary Material 1-Table S1. All slides were de-identified to blind the participating pathologists to the clinicopathologic data, and the H&E and OCT4/CD34-stained sections were separated into two independent datasets. All pathologists [5 non-UPs (Path1, 2, 3, 4, and 5) and 4 UPs (Path6, 7, 8, and 9)] reviewed the datasets independently (over the course of several weeks) and scored the LVI as positive (LVI +) or negative (LVI -), with no distinction regarding the type of the involved vessel (lymphatic or blood). Pathologists were asked to adopt diagnostic criteria that had been previously agreed upon within our group (Supplementary Material 2-Table S2) [19,20]. Subsequently (two months after the first assessment), two UPs (Path8 and Path9) reviewed the datasets on a multi-head microscope and a consensus diagnosis (CD) was reached for H&E (CD-H&E) and OCT4/CD34-stained sections (CD-OCT4/CD34). Finally, cases assessed by Path8 and Path9 with discordant initial and CD results were further analyzed by these pathologists on a multi-head microscope to identify the sources of disagreement.

### 2.3. Statistical analyses

This is an observational retrospective cohort study. A sample size calculation was not performed and all eligible patients were included

(*Materials and methods-Case series*). The IrOA was evaluated with *Fleiss's* Kappa (FK) for all pathologists (UPs plus non-UPs), as well as for the individual subgroups (UPs and non-UPs) [25]. CD-H&E, CD-OCT4/CD34, and the original diagnoses were compared to evaluate the changes in pT stage (*McNemar* test). Statistical analyses were performed using the IBM SPSS software, with a *p-value* $< 0.05$ (two-sided) indicating statistical significance.

### 2.4. Ethics committee

All clinical-pathological investigations were conducted according to the principles of the Declaration of Helsinki and all information regarding the human material used in this study has been managed using anonymous numerical codes. The study has been approved by the local ethics committee/CE-AVEC Bologna-Emilia Romagna (463-2022-AUSLBO-22092-ANAPAT TESTIS 03).

## 3. Results

### 3.1. Case series

Thirty-four OCT4(+) GCTT [29/34 (85.3 %) S, 3/34 (8.8 %) EC, 2/34 (5.9 %) S-EC] were included in the study. The mean age at diagnosis was 40.8 years (range: 23–64 years) and the mean tumor size was 4.2 cm (range: 0.9–8 cm). The pathologic stage (AJCC Cancer Staging Manual, 8th edition) was pT1 in 19/34 (55.9 %) tumors and pT2 in 15/34 (44.1 %) tumors; in the subgroup of pT1 S, 8/16 (50 %) were pT1a and 8/16 (50 %) pT1b. In the original diagnostic report, LVI was present in 12/34 (35.3 %) cases. Clinical-pathological data of the case series are listed in Supplementary Material 3-Table S3.

### 3.2. LVI assessment: IrOA

The IrOA among all pathologists (UPs plus non-UPs) was fair both on H&E (KF=0.398; $p < 0.001$) and OCT4/CD34-stained sections (KF=0.312; $p < 0.001$), with the former being closer to the cut-off for moderate agreement (0.4). OCT4/CD34 (KF=0.290; $p < 0.001$) slightly reduced the IrOA among non-UPs compared to H&E (KF=0.321; $p < 0.001$), but the agreement remained fair with both stains. In contrast, OCT4/CD34 (KF=0.293; $p < 0.001$) significantly reduced the IrOA among UPs compared to H&E (KF=0.529; $p < 0.001$), with the agreement changing from moderate (H&E) to fair (OCT4/CD34). These results are summarized in Supplementary Material 4-Table S4. The IrOA results are shown in Table 1.

### 3.3. LVI assessment: CD and pT re-staging

CD-H&E modified the LVI status of the original diagnosis in 8/34 (23.5 %) cases (*p: 0.070*), including 7/34 (20.6 %) reclassified as LVI - and 1/34 (2.9 %) reclassified as LVI + . These findings resulted in changes of pT stage in 2/34 (5.9 %) cases (*p: 0.500*), both down-staged from pT2 to pT1b. CD-OCT4/CD34 modified the LVI status of the original diagnosis in 8/34 (23.5 %) cases (*p: 0.289*), including 6/34 (17.6 %) reclassified as LVI - and 2/34 (5.9 %) reclassified as LVI + . These findings resulted in changes of pT stage in 3/34 (8.8 %) cases (*p: 0.250*), all of which down-staged from pT2 to pT1b or pT1. The comparisons between CD and original diagnosis with the resulting changes of pT stage are summarized in Tables 2 and 3. The CD-OCT4/CD34 modified the CD-H&E LVI status in 8/34 (23.5 %) cases (*p: 0.727*), including 5/34 (14.7 %) reclassified as LVI + and 3/34 (8.8 %) reclassified as LVI -. These findings resulted in changes of pT stage in 3/34 (8.8 %) cases (*p: 0.500*): 1/35 (2.9 %) up-staged from pT1b to pT2, and 2/34 (5.9 %) down-staged from pT2 to pT1b or pT1. The comparison between CD-H&E and CD-OCT4/CD34, with the resulting changes of pT stage are summarized in Table 4.

**Table 1**
The IrOA of LVI assessment.

| | UPS | non-UPS | UPs plus non-UPs |
|---|---|---|---|
| H&E | 0.529 ($p < 0.001$) | 0.321 ($p < 0.001$) | 0.398 ($p < 0.001$) |
| OCT4/CD34 | 0.293 ($p < 0.001$) | 0.290 ($p < 0.001$) | 0.312 ($p < 0.001$) |

inter-observer agreement (IrOA); lymphovascular invasion (LVI); hematoxylin and eosin (H&E); octamer-binding transcription factor 4 (OCT4); cluster of differentiation 34 (CD34); uropathologists (UPs); non-uropathologists (non-UPs).

### 3.4. LVI assessment: histological sources of disagreement among UPs

Path8 and Path9 compared the CD with their initial assessment and reassessed the slides to identify the sources of disagreement. On H&E-stained sections, the sources of disagreement included artefactual clefts misinterpreted as LVI in 4/6 (66.7 %) cases and foci of true LVI misinterpreted as clusters of histiocytes within the vessels in 2/6 (33.3 %) cases. On OCT4/CD34-stained sections, the sources of disagreement included artefactual clefts misinterpreted as LVI in 2/8 (25 %) cases, and foci of true LVI misinterpreted as artefactual clefts in 2/8 (25 %) cases or floaters in 4/8 (50 %) cases. The sources of disagreement between UPs are shown in Table 5. Illustrative examples of cases and potential diagnostic pitfalls are illustrated in Figs. 1 and 2.

**Table 2**
The comparison between CD-H&E and original diagnosis. CD-H&E have been rendered by two UPs (Path8 and Path9) on a multi-head microscope adopting H&E-stained sections [*Materials and methods-Datasets (H&E and OCT4/CD34), LVI assessment, and consensus diagnosis (CD)*]. *Italic*: cases for which CD-H&E modified the original diagnosis; Underlined: cases for which CD-H&E modified the original diagnosis and resulted in pT re-staging;.

| Case number | LVI-original diagnosis | pT (LVI-original diagnosis) | CD-H&E | pT (CD-H&E) |
|---|---|---|---|---|
| 1 | + | *pT2* | - | *pT2* |
| 2 | + | *pT2* | - | *pT2* |
| 3 | - | pT1a | - | pT1a |
| 4 | - | pT1b | - | pT1b |
| 5 | - | pT1 | - | pT1 |
| 6 | + | pT2 | + | pT2 |
| 7 | - | pT1a | - | pT1a |
| 8 | + | *pT2* | - | *pT2* |
| 9 | - | pT2 | - | pT2 |
| 10 | - | pT1b | - | pT1b |
| 11 | - | pT1 | - | pT1 |
| 12 | - | pT1b | - | pT1b |
| 13 | - | pT1 | - | pT1 |
| 14 | - | pT1b | - | pT1b |
| 15 | - | pT1a | - | pT1a |
| 16 | - | pT2 | - | pT2 |
| 17 | + | pT2 | + | pT2 |
| 18 | + | pT2 | + | pT2 |
| 19 | - | pT1b | - | pT1b |
| 20 | - | pT1b | - | pT1b |
| 21 | + | *pT2* | - | *pT2* |
| 22 | - | pT1a | - | pT1a |
| 23 | - | *pT2* | + | *pT2* |
| 24 | + | *pT2* | - | *pT2* |
| 25 | + | pT2 | + | pT2 |
| 26 | + | pT2 | + | pT2 |
| 27 | - | pT1b | - | pT1b |
| 28 | - | pT1b | - | pT1b |
| 29 | - | pT1a | - | pT1a |
| 30 | - | pT1a | - | pT1a |
| 31 | + | *pT2* | - | *pT1b* |
| 32 | + | *pT2* | - | *pT1b* |
| 33 | - | pT1a | - | pT1a |
| 34 | - | pT1a | - | pT1a |

lymphovascular invasion (LVI); negative (-); positive (+); hematoxylin and eosin (H&E); consensus diagnosis on H&E slides (CD-H&E).

**Table 3**
The comparison between CD-OCT4/CD34 and original diagnosis. CD-OCT4/CD34 have been rendered by two UPs (Path8 and Path9) on a multi-head microscope adopting OCT4/CD34-stained sections [*Materials and methods-Datasets (H&E and OCT4/CD34), LVI assessment, and consensus diagnosis (CD)*]. *Italic*: cases for which CD-OCT4/CD34 modified the original diagnosis; Underlined: cases for which CD-OCT4/CD34 modified the original diagnosis and resulted in pT re-staging.

| Case number | LVI-original diagnosis | pT (LVI-original diagnosis) | CD-OCT4/CD34 | pT (CD-OCT4/CD34) |
|---|---|---|---|---|
| 1 | + | *pT2* | - | *pT2* |
| 2 | + | pT2 | + | pT2 |
| 3 | - | pT1a | - | pT1a |
| 4 | - | pT1b | - | pT1b |
| 5 | - | pT1 | - | pT1 |
| 6 | + | *pT2* | - | *pT1* |
| 7 | - | pT1a | - | pT1a |
| 8 | + | pT2 | + | pT2 |
| 9 | - | *pT2* | + | *pT2* |
| 10 | - | pT1b | - | pT1b |
| 11 | - | pT1 | - | pT1 |
| 12 | - | pT1b | - | pT1b |
| 13 | - | pT1 | - | pT1 |
| 14 | - | pT1b | - | pT1b |
| 15 | - | pT1a | - | pT1a |
| 16 | - | pT2 | - | pT2 |
| 17 | + | pT2 | + | pT2 |
| 18 | + | *pT2* | - | *pT2* |
| 19 | - | pT1b | - | pT1b |
| 20 | - | pT1b | - | pT1b |
| 21 | + | *pT2* | - | *pT2* |
| 22 | - | pT1a | - | pT1a |
| 23 | - | *pT2* | + | *pT2* |
| 24 | + | pT2 | + | pT2 |
| 25 | + | pT2 | + | pT2 |
| 26 | + | *pT2* | - | *pT1b* |
| 27 | - | pT1b | - | pT1b |
| 28 | - | pT1b | - | pT1b |
| 29 | - | pT1a | - | pT1a |
| 30 | - | pT1a | - | pT1a |
| 31 | + | *pT2* | - | *pT1b* |
| 32 | + | pT2 | + | pT2 |
| 33 | - | pT1a | - | pT1a |
| 34 | - | pT1a | - | pT1a |

lymphovascular invasion (LVI); negative (-); positive (+); hematoxylin and eosin (H&E); octamer-binding transcription factor 4 (OCT4); cluster of differentiation 34 (CD34); consensus diagnosis on OCT4/CD34 slides (CD-OCT4/CD34).

**Table 4**
The comparison between CD (CD-H&E and CD-OCT4/CD34). CD-H&E and CD-OCT4/CD34 have been rendered by two UPs (Path8 and Path9) on a multi-head microscope adopting H&E and OCT4/CD34-stained sections, respectively [*Materials and methods-Datasets (H&E and OCT4/CD34), LVI assessment, and consensus diagnosis (CD)*]. *Italic*: cases for which CD-OCT4/CD34 modified the CD-H&E; Underlined: cases for which CD-OCT4/CD34 modified the CD-H&E and resulted in pT re-staging;.

| Case number | CD-H&E | pT (CD-H&E) | CD-OCT4/CD34 | pT (CD-OCT4/CD34) |
|---|---|---|---|---|
| 1 | - | pT2 | - | pT2 |
| 2 | - | *pT2* | + | *pT2* |
| 3 | - | pT1a | - | pT1a |
| 4 | - | pT1b | - | pT1b |
| 5 | - | pT1 | - | pT1 |
| 6 | + | *pT2* | - | *pT1* |
| 7 | - | pT1a | - | pT1a |
| 8 | - | *pT2* | + | *pT2* |
| 9 | - | *pT2* | + | *pT2* |
| 10 | - | pT1b | - | pT1b |
| 11 | - | pT1 | - | pT1 |
| 12 | - | pT1b | - | pT1b |
| 13 | - | pT1 | - | pT1 |
| 14 | - | pT1b | - | pT1b |
| 15 | - | pT1a | - | pT1a |
| 16 | - | pT2 | - | pT2 |
| 17 | + | pT2 | + | pT2 |
| 18 | + | *pT2* | - | *pT2* |
| 19 | - | pT1b | - | pT1b |
| 20 | - | pT1b | - | pT1b |
| 21 | - | pT2 | - | pT2 |
| 22 | - | pT1a | - | pT1a |
| 24 | + | pT2 | + | pT2 |
| 25 | - | *pT2* | + | *pT2* |
| 26 | + | pT2 | + | pT2 |
| 27 | + | *pT2* | - | *pT1b* |
| 28 | - | pT1b | - | pT1b |
| 29 | - | pT1b | - | pT1b |
| 30 | - | pT1a | - | pT1a |
| 31 | - | pT1a | - | pT1a |
| 32 | - | pT1b | - | pT1b |
| 33 | - | *pT1b* | + | *pT2* |
| 34 | - | pT1a | - | pT1a |
| 35 | - | pT1a | - | pT1a |

negative (-); positive (+); hematoxylin and eosin (H&E); octamer-binding transcription factor 4 (OCT4); cluster of differentiation 34 (CD34); consensus diagnosis/diagnoses (CD); consensus diagnosis on H&E slides (CD-H&E); consensus diagnosis on OCT4/CD34 slides (CD-OCT4/CD34).

## 4. Discussion

LVI represents one of the most relevant parameters used to predict risk of disease relapse in stage I GCTT, mainly in stage I non-seminomatous GCTT [1–8]. As result, LVI has been incorporated in the pT stage of GCTT and used to identify patients at high risk of disease recurrence that may benefit from adjuvant treatments [2]. In contrast, active surveillance is preferred for patients with pT1/IA stage GCTT and absence of other significant risk factors (70–85 % across different studies) [3–8]. Detection of LVI is often challenging and represents the most important source of discrepancy between the diagnoses rendered at peripheral communities and specialized centers [9–13]. *Purshouse K* et al. found that the review of GCTT in specialized centers modified the original diagnosis of GCTT in up to 28 % of the cases, with LVI being the most common source of disagreement [13]. These changes resulted in changes of the pT stage and clinical management in 9 % and 6.5 % of patients, respectively [13]. Similarly, *Nicolai N* et al. found that the agreement for LVI between original and consult diagnoses was poor, and only the latter were significantly associated with lymph node status at retroperitoneal lymph node dissection (RLND) [10]. Although the IrOA in GCTT diagnosis was moderate to substantial among UPs overall, the IrOA for some specific parameters (including LVI) was significantly lower even among experts [9–14]. Our results mirror those published in prior studies, with IrOA for LVI being moderate among UPs (KF=0.529), but only fair among all pathologists (non-UPs plus UPs, KF=0.398) and non-UPs (KF=0.321) on H&E slides. Reevaluation of discordant cases demonstrated that the most common sources of disagreement among UPs (Path8 and 9) were artefactual clefts containing tumor cells and clusters of histiocytes within the vessels rather than floaters (i.e., so-called “pseudovascular invasion”) [14,26]. This could be partially explained by our initial request to interpret the findings on H&E critically (Supplementary Material 2- Table S2). Notwithstanding the relevant prognostic and therapeutic implications of LVI in GCTT, as well as the problems associated with its interpretation, only a few prior studies have explored the potential utility of IHC in this context [15–18]. The results of these studies are difficult to compare due to differences in the antibodies (ERG, D2–40, CD31, LYVE-1, vWF), the tumor types [S and nonseminomatous GCTT, only nonseminomatous GCTT, OCT4(+) GCTT], type of cases (routine cases vs metastatic patients, patients treated with active surveillance vs RLND), disease recurrence criteria (biochemical recurrence, metastasis), and skills of the pathologists involved (not-UPs, UPs, GCTT-dedicated pathologists) [15–18]. Moreover, no study has investigated whether IHC improves the IrOA for the assessment of LVI in GCTT; consequently, this topic has not been discussed in prior Testicular Cancer Consultation Recommendations and ISUP meetings [9]. The adoption of DS is increasing in both academic

**Table 5**
The sources of disagreement among UPs. Cases assessed by Path8 and Path9 with discordant initial and CD results were analyzed on a multi-head microscope to identify the sources of disagreement [*Materials and methods-Datasets (H&E and OCT4/CD34), LVI assessment, and consensus diagnosis (CD)*]. *Italic*: cases for which CD and initial assessment by UPs (Path8 or Path9) were discordant.

| Case number | Path8: LVI-H&E (initial assessment) | Path9: LVI-H&E (initial assessment) | CD-H&E | Notes | Path8: LVI-OCT4/CD34 (initial assessment) | Path9: LVI-OCT4/CD34 (initial assessment) | CD-OCT4/CD34 | Notes |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 2 | *1* | *0* | *0* | *Artefactual clefts misinterpreted as LVI by Path8* | *0* | *1* | *1* | *LVI misinterpreted as floaters by Path8* |
| 3 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 4 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 5 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 6 | 1 | 1 | 1 | | *0* | *1* | *0* | *Artefactual clefts misinterpreted as LVI by Path9* |
| 7 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 8 | 0 | 0 | 0 | | *0* | *1* | *1* | *LVI misinterpreted as floaters by Path8* |
| 9 | *0* | *1* | *0* | *Artefactual clefts misinterpreted as LVI by Path9* | *0* | *1* | *1* | *LVI misinterpreted as floaters by Path8* |
| 10 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 11 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 12 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 13 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 14 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 15 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 16 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 17 | *1* | *0* | *1* | *LVI misinterpreted as cluster of histiocytes within the vessels by Path9* | *0* | *1* | *1* | *LVI misinterpreted as artefactual clefts by Path8* |
| 18 | 1 | 1 | 1 | | 0 | 0 | 0 | |
| 19 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 20 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 21 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 22 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 23 | 0 | 1 | 1 | *LVI misinterpreted as clusters of histiocytes within the vessels by Path8* | *0* | *1* | *1* | *LVI misinterpreted as artefactual clefts by Path8* |
| 24 | *0* | *1* | *0* | *Artefactual clefts misinterpreted as LVI by Path9* | *0* | *1* | *1* | *LVI misinterpreted as floaters by Path8* |
| 25 | 1 | 1 | 1 | | 1 | 1 | 1 | |
| 26 | 1 | 1 | 1 | | *0* | *1* | *0* | *Artefactual clefts misinterpreted as LVI by Path9* |
| 27 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 28 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 29 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 30 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 31 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 32 | *0* | *1* | *0* | *Artefactual clefts misinterpreted as LVI by Path9* | 1 | 1 | 1 | |
| 33 | 0 | 0 | 0 | | 0 | 0 | 0 | |
| 34 | 0 | 0 | 0 | | 0 | 0 | 0 | |

lymphovascular invasion (LVI); positive (+); hematoxylin and eosin (H&E); octamer-binding transcription factor 4 (OCT4); cluster of differentiation 34 (CD34); consensus diagnosis/diagnoses (CD); consensus diagnosis on H&E slides (CD-H&E); consensus diagnosis on OCT4/CD34 slides (CD-OCT4/CD34); uropathologists (UPs); inter-observer agreement (IrOA); lymphovascular invasion (LVI); hematoxylin and eosin (H&E); octamer-binding transcription factor 4 (OCT4); cluster of differentiation 34 (CD34); uropathologists (UPs); non-uropathologists (non-UPs).

and private practice settings [19,20,27–32]. We recently developed an in-house DS for OCT4/CD34 and tested it on a small cohort of GCTT [19,20]. We found that OCT4/CD34 was reliable and led to changes of the LVI status and pT stage when compared to the original diagnosis and consensus evaluation of H&E slides [19,20]. We adopted OCT4 instead of sal-like protein 4 (SALL4) for both the following reasons: a) we had performed some preliminary tests with SALL4 (before our previous publication) but it was weak/barely perceptible in many tested cases, probably due to the antigenic degeneration after multiple steps required for DS (increased number of cycles at high temperature); b) OCT4 allows to stain S, EC, and S-EC components (spanning more than 75 % of GCTT) and, according to the literature, also in mixed GCTT with other components (teratoma, yolk sac tumor of postpubertal-type, and choriocarcinoma) LVI foci are almost all related to EC components (fitting with the model according to which metastatic cells are typically neoplastic embryonic stem cells) [16,20,22]. In the present study, we assessed the utility of OCT4/CD34 to distinguish LVI from its histologic mimickers and improve the IrOA among UPs and non-UPs. Unexpectedly, we found that not only did OCT4/CD34 not improve IrOA, but it reduced it in all the groups analyzed herein: UPs (KF=0.293), non-UPs (KF=0.290), UPs plus non-UPs (KF=0.312). A possible explanation for this result is that all the participating pathologists (and UPs in particular) were familiar

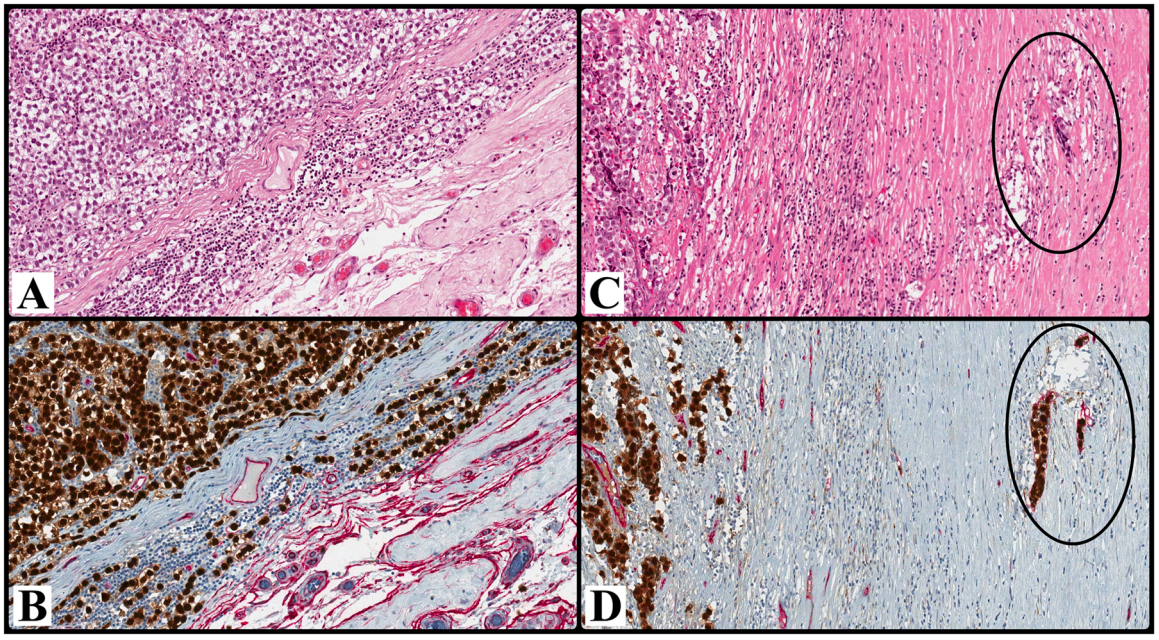


**Fig. 1.** Germ cell tumor of the testis (GCTT); seminoma (S); lymphovascular invasion (LVI); positive (+); negative (-); hematoxylin and eosin (H&E); cluster of differentiation 34 (CD34); octamer-binding transcription factor 4 (OCT4); consensus diagnosis on H&E slides (CD-H&E); consensus diagnosis on OCT4/CD34 slides (CD-OCT4/CD34); Two cases of GCTT with assessment of LVI (H&E and OCT4/CD34). *Case#3* (Supplementary Material 4-Table S4 and Table 5): S (A: H&E, original magnification x200; B: OCT4/CD34, original magnification x200). Path8-first assessment (H&E): LVI -; Path9-first assessment (H&E): LVI -; Path8-first assessment (OCT4/CD34): LVI -; Path9-first assessment (OCT4/CD34): LVI -; CD-H&E: LVI -; CD-OCT4/CD34: LVI -; *Case#8* (Supplementary Material 4-Table S4 and Table 5): S (C: H&E, original magnification x200; D: OCT4/CD34, original magnification x200). Path8-first assessment (H&E): LVI -; Path9-first assessment (H&E): LVI -; Path8-first assessment (OCT4/CD34): LVI -; Path9-first assessment (OCT4/CD34): LVI + ; CD-H&E: LVI -; CD-OCT4/CD34: LVI + ; OCT4/CD34 highlights multiple foci of LVI not easily identifiable with H&E (black ovals) and misinterpreted as floaters.

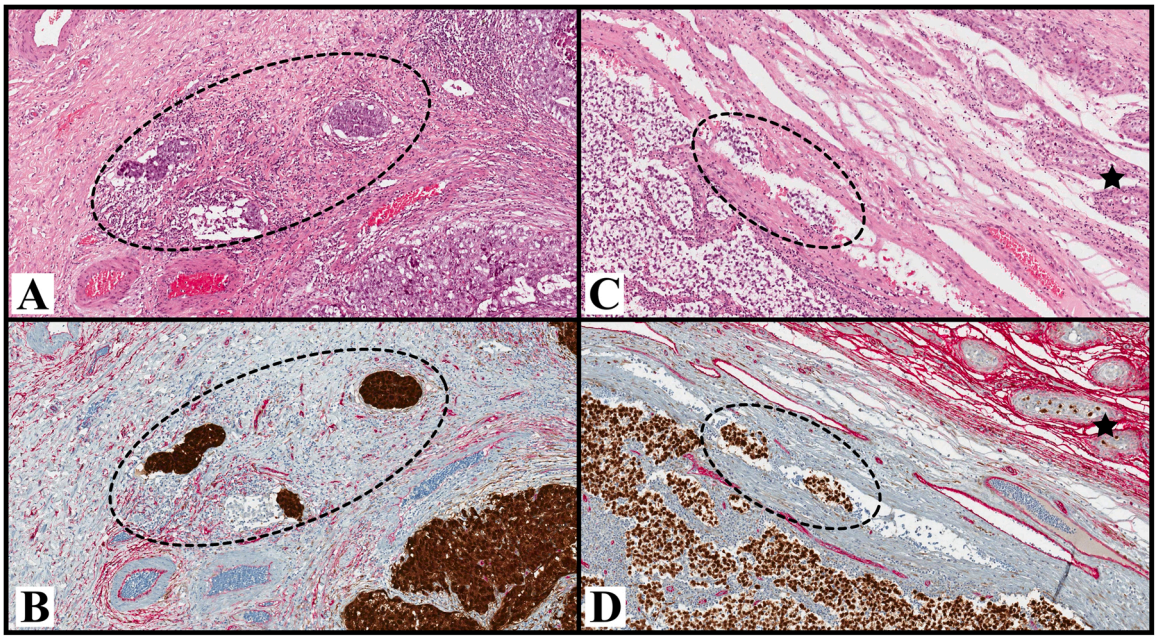


**Fig. 2.** Seminoma (S); embryonal carcinoma (EC); lymphovascular invasion (LVI); positive (+); negative (-); hematoxylin and eosin (H&E); cluster of differentiation 34 (CD34); octamer-binding transcription factor 4 (OCT4); consensus diagnosis on H&E slides (CD-H&E); consensus diagnosis on OCT4/CD34 slides (CD-OCT4/CD34); Mimickers of LVI with H&E and OCT4/CD34. *Case#6* (Supplementary Material 4-Table S4 and Table 5): EC (A: H&E, original magnification x100; D: OCT4/CD34, original magnification x100). Path8-first assessment (H&E): LVI + ; Path9-first assessment (H&E): LVI + ; Path8-first assessment (OCT4/CD34): LVI -; Path9-first assessment (OCT4/CD34): LVI + ; CD-H&E: LVI + ; CD-OCT4/CD34: LVI -; *Case#4* (Supplementary Material 4-Table S4 and Table 5): S (C: H&E, original magnification x100; D: OCT4/CD34, original magnification x100). Path8-first assessment (H&E): LVI -; Path9-first assessment (H&E): LVI -; Path8-first assessment (OCT4/CD34): LVI -; Path9-first assessment (OCT4/CD34): LVI -; CD-H&E: LVI -; CD-OCT4/CD34: LVI -; In both cases, OCT4/CD34 reveals as the artefactual clefts (dotted black ovals) are not bordered by a peripheral layer of lymphatic-endothelial cells CD34(+). GCNIS (black stars) could represent a potential mimicker of LVI at both H&E and OCT4/CD34 assessment, but it does not fulfill all the diagnostic criteria to diagnose LVI (Supplementary Material 2-Table S2).

with the criteria for diagnosing LVI on H&E slides, but not with OCT4/CD34. This discrepancy may account for the greater diagnostic variability and lower IrOA obtained with OCT4/CD34. We found similar results in a recent study that compared H&E and CD34/SOX10 for the assessment of LVI in cutaneous melanoma [28]. On a retrospective review of the discordant cases, Path8 and 9 found that the major sources of discordance with OCT4/CD34 were foci of true LVI misinterpreted as artefactual clefts and/or floaters, and artefactual clefts misinterpreted as LVI. As expected, clusters of histiocytes within vessels did not represent a source of disagreement on OCT4/CD34-stained sections. Another relevant finding of our study pertains to the results of the comparison between the original diagnosis and CD (CD-H&E and CD-OCT4/CD34). We found that the CD on both H&E and OCT4/CD34-stained sections changed the LVI status of the original diagnosis in 8/34 (23.5 %) cases, with most of them reclassified as LVI - [CD-H&E: 7/34 (20.6 %); CD-OCT4/CD34: 6/34 (17.6 %)]. Although these changes did not reach statistical significance (likely due to the limited sample size), CD-H&E (*p: 0.070*) approached statistical significance. Reclassification of LVI status had a relatively small effect on the pT re-staging [2/34 (5.9 %) for CD-H&E; 3/34 (8.8 %) for CD-OCT4/CD34] because most cases reclassified as LVI – had additional histologic findings that justified a pT2/IB stage (i.e., invasion of hilar soft tissue, epididymis, and visceral mesothelial layer) [2]. Udager et al. reported similar results, which they attributed to selection bias (the break-down of their series was pT1: 4 %, pT2: 84 %, pT3: 12 %; 72 % of the patients had clinically and/or pathologically confirmed metastasis) [17]. Our study confirmed that the low frequency of pT re-staging obtained with CD (both CD-H&E and CD-OCT4/CD34) is also observed with unselected cases (routine scenario). Finally, CD-OCT4/CD34 was discordant with CD-H&E in 8/34 (23.5 %) cases, but the overall effect on pT re-staging was also relatively small (3/34 cases, 8.8 %) and not statistically significant (*p: 0.500*). These data suggest that consensus review of foci suspicious for LVI on H&E slides may be clinically impactful on a small subset of patients (less than 10 %), without additional benefits derived from the evaluation of OCT4/CD34-stained sections (either independently or at consensus). Taking into account all this results, we concluded that for the evaluation of such relevant prognostic data as LVI, CD-H&E among UPs (so providing the best value of IrOA and assuring that pathologists "speak the same language" when defining LVI) is still the most effective method. The limitations of our study include (a) a small sample size, (b) the absence of prognostic data (follow-up was too short to evaluate outcomes), (c) the characteristics of the case series (retrospective from a single institution), and (d) the adoption of a GCTT-marker that highlights only S, EC, and S-EC.

In conclusion, the results presented herein show that OCT4/CD34 does not improve the IrOA for the assessment of LVI assessment in OCT4 (+) GCTT. CD-H&E rendered by two UPs on a multi-head microscope modifies the LVI status in a significant number of cases, resulting in changes of the pT stage in a relatively small subset. In this scenario, consensus evaluation of OCT4/CD34-stained slides provides little additional benefit since it cannot exclude mimickers of LVI such as floaters and artefactual clefts. These results argue against the implementation with OCT4/CD34 for the routine assessment of LVI in OCT4 (+) GCTT. Further studies are needed to evaluate the utility of OCT4/CD34 in selected diagnostic scenarios.

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## CRediT authorship contribution statement

**Costantino Ricci:** Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Francesca Ambrosi, Tania Franceschini, Francesca Giunchi, Maria Eugenia Maracci, Maria Sirolli, Agnese Orsatti, Federico Chiarucci:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology. **Eugenia Franchini, Matteo Borsato:** Data curation, Formal analysis, Investigation, Methodology. **Francesco Massari, Veronica Mollica, Federico Mineo Bianchi:** Conceptualization, Data curation, Methodology. **Maurizio Colecchia, Andres Martin Acosta, Michelangelo Fiorentino:** Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review & editing.

## Author's statement

All Authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is an author's original work and that it has been presented (smaller case series with a low number of cases, partial results, and different discussion) as Oral Presentation-Uropathology session at the ECP (European Congress of Pathology) Meeting 2021, and as Poster-Uropathology session as Siapec (Società Italiana di Anatomia Patologica e Citodiagnostica) Meeting 2022.

## Note

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## Declaration of interests

All the authors present in this article declare no conflict of interests.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prp.2023.154337.

## References

[1] F. Honecker, J. Aparicio, D. Berney, et al., ESMO Consensus Conference on testicular germ cell cancer: diagnosis, treatment and follow-up, Ann. Oncol. 29 (2018) 1658–1686.
[2] M.B. Amin, S.B. Edge, F.L. Greene, et al.. AJCC Cancer Staging Manual, eightth ed., Springer, Chicago, 2017.
[3] P. Albers, R. Siener, S. Kliesch, et al., Risk factors for relapse in clinical stage I nonseminomatous testicular germ cell tumors: results of the German Testicular Cancer Study Group Trial, J. Clin. Oncol. 21 (2003) 1505–1512.
[4] C. Kollmannsberger, C. Moore, K.N. Chi, et al., Non-risk-adapted surveillance for patients with stage I nonseminomatous testicular germ-cell tumors: diminishing treatment-related morbidity while maintaining efficacy, Ann. Oncol. 21 (2010) 1296–1301.
[5] P. Dong, Z.W. Liu, X.D. Li, et al., Risk factors for relapse in patients with clinical stage I testicular nonseminomatous germ cell tumors, Med. Oncol. 30 (2013) 494.
[6] J.L. Boormans, J. Mayor de Castro, L. Marconi, et al., Testicular tumour size and rete testis invasion as prognostic factors for the risk of relapse of clinical stage I seminoma testis patients under surveillance: a systematic review by the testicular cancer guidelines panel, Eur. Urol. 73 (2018) 394–405.
[7] P. Warde, M.K. Gospodarowicz, D. Banerjee, et al., Prognostic factors for relapse in stage I testicular seminoma treated with surveillance, J. Urol. 157 (1997) 1705–1709.
[8] J. Aparicio, Prognostic factors for relapse in stage I testicular seminoma: tumor size and rete testis invasion revisited, Clin. Transl. Oncol. 20 (2018) 1358–1359.

[9] T. Gilligan, D.W. Lin, R. Aggarwal, et al., Testicular cancer, version 2.2020, NCCN clinical practice guidelines in oncology, J. Natl. Compr. Cancer Netw. 17 (2019) 1529–1554.
[10] C. Verrill, A. Yilmaz, J.R. Srigley, et al., Reporting and staging of testicular germ cell tumors: the International Society of Urological Pathology (ISUP) Testicular Cancer Consultation Conference Recommendations, Am. J. Surg. Pathol. 41 (2017) e22–e32.
[11] N. Nicolai, M. Colecchia, D. Biasoni, et al., Concordance and prediction ability of original and reviewed vascular invasion and other prognostic parameters of clinical stage I nonseminomatous germ cell testicular tumors after retroperitoneal lymph node dissection, J. Urol. 186 (2011) 1298–1302.
[12] D.M. Berney, F. Algaba, M. Amin, et al., Handling and reporting of orchidectomy specimens with testicular cancer: areas of consensus and variation among 25 experts and 225 European pathologists, Histopathology 67 (2015) 313–324.
[13] P. Sharma, J. Dhillon, G. Agarwal, et al., Disparities in interpretation of primary testicular germ cell tumor pathology, Am. J. Clin. Pathol. 144 (2015) 289–294.
[14] K. Purshouse, R.A. Watson, D.N. Church, et al., Value of supraregional multidisciplinary review for the contemporary management of testicular tumors, Clin. Genitourin. Cancer 15 (2017) 152–156.
[15] B.L. French, D.L. Zynger, Do histopathologic variables affect the reporting of lymphovascular invasion in testicular germ cell tumors? Am. J. Clin. Pathol. 145 (2016) 341–349.
[16] J. Lobo, H. Stoop, A.J.M. Gillis, et al., Interobserver agreement in vascular invasion scoring and the added value of immunohistochemistry for vascular markers to predict disease relapse in stage I testicular nonseminomas, Am. J. Surg. Pathol. 43 (2019) 1711–1719.
[17] J. Heinzelbecker, M. Gross-Weege, C. Weiss, et al., Microvascular invasion of testicular nonseminomatous germ cell tumors: implications of separate evaluation of lymphatic and blood vessels, J. Urol. 192 (2014) 593–599.
[18] A.M. Udager, J.B. McHugh, T.M. Morgan, et al., Utility of ERG immunohistochemistry for evaluation of lymphovascular invasion in testicular germ cell tumors: a retrospective pilot study, Appl. Immunohistochem. Mol. Morphol. 27 (2017) 392–401.
[19] H. Huang, S. Jadallah, Y. Chen, et al., The utility of ERG immunohistochemistry in converting "Suspicious for" to "Definite" lymphovascular invasion in non-seminomatous germ cell tumor of testis, Mod. Pathol. 93 (2013) 219A.
[20] C. Ricci, T. Franceschini, F. Giunchi, et al., A preliminary study investigating the detection of lymphovascular invasion in germ cell tumors of the testis with double staining for OCT4/CD34, Pathol. Res Pract. 227 (2021), 153637.
[21] C. Ricci, M. Borsato, T. Franceschini, et al., Novel double staining (OCT4/CD34) for the detection of lymphovascular invasion in OCT4(+) Germ Cell Tumors: technical aspects, results and future prospective, Virchow Arch. 479 (2021) S1–S320.
[22] WHO Classifications of Tumors Editorial Board. Urinary and Male Genital Tumours, fifth ed., IARC Press, Lyon, 2022.
[23] H. Moch, M.B. Amin, D.M. Berney, et al., The 2022 World Health Organization classification of tumours of the urinary system and male genital organs-part A: renal, penile, and testicular tumours, Eur. Urol. 82 (2022) 458–468.
[24] A. Orsatti, M. Sirolli, F. Ambrosi, et al., SOX2 and PRAME in the "reprogramming" of seminoma cells, Pathol. Res Pract. 237 (2022), 154044.
[25] C. Ricci, T. Franceschini, F. Giunchi, et al., Immunohistochemical expression of preferentially expressed antigen in melanoma (PRAME) in the uninvolved background testis, germ cell neoplasia in situ, and germ cell tumors of the testis, Am. J. Clin. Pathol. 157 (2022) 644–648.
[26] J.M. Bland, D.G. Altman, Statistical methods for assessing agreement between two methods of clinical measurement, Lancet 1 (1986) 307–310.
[27] H. Ye, T.M. Ulbright, Difficult differential diagnoses in testicular pathology, Arch. Pathol. Lab Med. 136 (2012) 435–446.
[28] M. Tetzlaff, C.A. Torres-Cabala, P. Pattanaprichakul, et al., Emerging clinical applications of selected biomarkers in melanoma, Clin. Cosmet. Investig. Dermatol. 8 (2015) 35–46.
[29] C. Ricci, E. Dika, M. Lambertini, et al., Hematoxylin and eosin or double stain for CD34/SOX10: which is better for the detection of lymphovascular invasion in cutaneous melanoma? Pathol. Res Pr. 233 (2022), 153876.
[30] C. Ricci, E. Dika, F. Ambrosi, et al., Cutaneous melanomas: a single center experience on the usage of immunohistochemistry applied for the diagnosis, Int. J. Mol. Sci. 23 (2022) 5911.
[31] C. Ricci, E. Capizzi, F. Giunchi, et al., Reliability of programmed death ligand 1 (PD-L1) tumor proportion score (TPS) on cytological smears in advanced non-small cell lung cancer: a prospective validation study, Ther. Adv. Med. Oncol. 12 (2020), 1758835920954802.
[32] M. Grillini, C. Ricci, V. Pino, et al., HMB45/PRAME, a novel double staining for the diagnosis of melanocytic neoplasms: technical aspects, results, and comparison with other commercially available staining (PRAME and Melan A/PRAME), Appl. Immunohistochem. Mol. Morphol. 30 (2022) 14–18.