Utility of ERG Immunohistochemistry for Evaluation of Lymphovascular Invasion in Testicular Germ Cell Tumors: A Retrospective Pilot Study

Aaron M. Udager, M.D., Ph.D.¹, Jonathan B. McHugh, M.D.¹, Todd M. Morgan, M.D.^{2,3}, Daniel E. Spratt, M.D.⁴, Arul M. Chinnaiyan, M.D., Ph.D.^{1,2,3,5,6}, Rohit Mehra, M.D.^{1,3,5}

¹Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109

²Department of Urology, University of Michigan Medical School, Ann Arbor, MI 48109

³Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI 48109

⁴Department of Radiation Oncology, University of Michigan Medical School, Ann Arbor, MI 48109

⁵Michigan Center for Translational Pathology, Ann Arbor, MI 48109

⁶Howard Hughes Medical Institute, Ann Arbor, MI 48109

Abstract

Lymphovascular invasion (LVI) of testicular germ cell tumors (GCT) is an important stage-determining variable in the evaluation of radical orchiectomy specimens. ERG endothelial cell expression, as detected by immunohistochemistry (IHC), robustly highlights lymphovascular spaces, and thus, we sought to assess the utility of ERG IHC for evaluation of GCT LVI. H&E slides from a retrospective cohort of twenty-five GCT radical orchiectomy specimens (emanating from a parent cohort of one-hundred fifty-nine radical orchiectomy GCT cases identified between 2003 and 2013) were reviewed, and sections with foci of positive or equivocal LVI were identified. ERG IHC was performed on sections off the surface of corresponding paraffin tissue blocks. All foci were then re-scored as positive, equivocal, or negative for LVI based on ERG endothelial cell expression. Twenty-three and thirteen foci were positive or equivocal for LVI by H&E staining, respectively. Of the H&E positive LVI foci, twenty (87%) were ERG IHC positive, while of the H&E equivocal LVI foci, five (38%) were ERG IHC positive, three (23%) were ERG IHC negative, and two (15%) were ERG IHC equivocal; all other foci were lost for evaluation. Overall, ERG IHC helped resolve the LVI status of 61% of foci deemed equivocal for LVI by H&E staining only. While ERG IHC is useful in confirming definitive LVI status in a subset of GCT cases, the overall clinical impact of ERG IHC is limited for H&E equivocal LVI foci in this specific retrospective patient cohort. Overall, in carefully selected clinical scenarios, these data suggest a supportive role for ERG IHC in evaluation of GCT LVI in radical orchiectomy specimens.

Keywords

ERG; lymphovascular invasion; germ cell tumor

Introduction

Testicular cancer accounts for approximately 1% of all malignant tumors in men but is the most common neoplasm in men aged 15–45 years^{1–3}. The vast majority (greater than 95%) of testicular cancers are germ cell tumors (GCT), a diverse histopathologic group of related neoplasms that include seminoma, embryonal carcinoma, yolk sac tumor, choriocarcinoma, and teratoma. These GCT subtypes can be found either as pure tumors or admixed in varying quantities, and clinically, testicular GCT are separated into two main groups: pure seminoma and non-seminomatous GCT (NSGCT)⁴.

Lymphovascular invasion (LVI) is a pathologic “T” stage-defining variable for testicular GCT². *Sandeman and Matthews* first demonstrated that the presence of LVI in otherwise organ-confined tumors helped define a subset of patients with worse overall prognosis⁵. Multiple follow-up studies in large cohorts of testicular GCT patients without clinical evidence of metastatic disease at presentation have confirmed that the presence of LVI in the radical orchiectomy specimen is an important prognostic factor for both occult metastasis and relapse after a negative retroperitoneal lymph node dissection (RPLND)^{6–11}.

In radical orchiectomy specimens for testicular GCT, the presence or absence of LVI can usually be determined by adequate sampling and routine hematoxylin and eosin (H&E) staining, however, some cases present challenging diagnostic dilemmas¹². How LVI status is determined in these equivocal cases is not currently standardized, and there is no gold standard for LVI determination. The recent growing armamentarium of ancillary techniques – most notably immunohistochemistry (IHC) – has spurred the development of novel methods for assessing LVI. Indeed, IHC for vascular and/or lymphatic endothelial cell markers, including CD31, CD34, and D2-40 (podoplanin), has been evaluated for the detection of LVI in melanoma, as well as breast, prostate, colon, gastric, and bladder cancer^{13–20}.

Despite the central importance of LVI status in its pathologic staging, no extensive studies regarding the use of IHC for classifying LVI in primary testicular GCT have been reported in the published literature. ERG, a member of the ETS family of transcription factors, has recently been implicated in prostate cancer pathogenesis^{21, 22}. ERG is also expressed by normal vascular and lymphatic endothelium²³, where it plays an important role in vascular development²⁴. ERG IHC has been evaluated for the assessment of LVI in colon cancer²⁵. In that study, after a group consensus session, ERG IHC was found to increase LVI detection and decrease interobserver variability among pathologists. Interestingly, a recent abstract reported encouraging experience with ERG IHC for converting H&E LVI suspicious foci to positive LVI foci in NSGCT²⁶. In the current retrospective pilot study, we further sought to assess the potential utility of ERG IHC for evaluation of GCT LVI in radical orchiectomy specimens.

Materials and Methods

A comprehensive retrospective search of the Michigan Medicine pathology records database was performed to identify all radical orchiectomy specimens between January 2002 and

May 2013. H&E slides for all cases reporting positive or equivocal LVI were reviewed by three study pathologists (A.M.U., J.B.M. and R.M.), and sections with H&E positive or equivocal LVI foci were identified for further investigation. Based on current clinical practice standards, intravascular tumor cell aggregates with a smooth contour, attachment to endothelium, and admixed fibrin were designated as H&E positive for LVI¹²; for the purposes of this study, foci with some, but not all, of these features were designated as H&E equivocal for LVI.

For all H&E positive or equivocal foci, sections off the surface of corresponding paraffin tissue blocks were obtained (i.e., “off the top” sections), and ERG immunohistochemistry (EPR3864; pre-dilute, Ventana Medical Systems, Tucson, AZ) was performed using a BenchMark ULTRA automated stainer and the ultraView Universal DAB Detection Kit (Ventana Medical Systems). All foci were then re-scored as positive, equivocal, or negative for LVI based on ERG endothelial cell expression by three study pathologists (A.M.U., J.B.M. and R.M.). Foci which demonstrated all or some of the features for LVI (see above) and exhibited a complete peripheral layer of endothelial cells (as visualized by nuclear ERG protein expression) were designated ERG IHC positive for LVI, while foci with an incomplete peripheral endothelial cell layer or without endothelial cells entirely were designated ERG IHC equivocal and negative for LVI, respectively. All study investigators were blinded to specific clinical information (i.e., the presence or absence of clinical or pathologic metastasis, etc.) until after scoring ERG LVI foci, at which point additional clinicopathologic details for each case were obtained from the electronic medical record. Clinical staging for all patients at the time of diagnosis was based on the AJCC clinical staging system².

Results

Clinicopathologic features of a retrospective GCT cohort

Overall, one-hundred fifty-nine radical orchiectomy GCT cases were identified in the study period. Of these, forty (25%) reported positive or equivocal LVI, while the other one-hundred nineteen (75%) were negative for LVI. Upon retrospective evaluation of the H&E slides for cases reporting positive or equivocal LVI, twenty-five out of forty (63%) with H&E positive or equivocal LVI foci were identified and available for ERG IHC (Table 1). For the remaining fifteen cases, either the slides and/or blocks were not immediately available for inclusion in the study or the case was deemed negative for LVI (i.e., no positive or equivocal LVI foci were identified on retrospective review); the most common reason for discordance between reported and retrospectively reviewed LVI status was so-called “pseudolymphovascular invasion” – a well known diagnostic challenge in testicular GCT¹². Patient age at the time of radical orchiectomy ranged from 16 to 65 years (median = 25 years), and there were 10 right testicular and 15 left testicular GCT. The maximum tumor dimension ranged in size from 1.2 to 8.0 cm (median = 3.2 cm), and there were five pure seminomas and twenty NSGCT. Assignment of AJCC pathologic “T” stage classification yielded one “pT1” tumor, twenty-one “pT2” tumors, and three “pT3” tumors. Of the twenty-one “pT2” tumors, only two (10%) demonstrated invasion of the tunica vaginalis – the other “pT2” stage-defining pathologic feature besides LVI². Eighteen patients out of twenty-five

have had clinical and/or pathologic evidence of metastasis either at the time of diagnosis or during subsequent follow-up, while seven patients have had no evidence of metastatic spread (including two who underwent primary RPLND). One patient had persistently elevated serum tumor markers only (i.e., no definite metastatic site could be determined based on radiographic imaging). At last follow-up, all but one of the patients were alive and clinically free of disease, with a follow-up range of 1 to 114 months (median = 25 months).

Impact of ERG IHC on LVI assessment of individual LVI foci

First, we decided to determine the impact of ERG IHC on LVI assessment of individual LVI foci in this GCT cohort. From the twenty-five cases with H&E positive or equivocal LVI foci, retrospective evaluation of the H&E slides revealed thirty-six H&E positive or equivocal LVI foci: twenty-three H&E positive LVI foci and thirteen H&E equivocal LVI foci. Of the twenty-three H&E positive LVI foci, twenty (87%) were designated as positive for LVI based upon ERG IHC, three were lost for evaluation, and none were ERG IHC equivocal or negative (Figure 1; Table 2). The GCT component identified in these H&E positive LVI foci were comprised of embryonal carcinoma (17 foci), seminoma (2 foci), yolk sac tumor (2 foci), and choriocarcinoma (2 foci), which corresponded to the dominant GCT component (in terms of percentage of volume of the entire tumor) in all cases except the two foci of yolk sac tumor; in these two cases, the dominant GCT component was embryonal carcinoma. Of the thirteen H&E equivocal LVI foci, five (39%) were designated as positive based on ERG IHC, three (23%) were ERG IHC negative, two (15%) were ERG IHC equivocal, and three (23%) were lost upon sectioning for evaluation (Figures 2 and 3; Table 2; see Materials and Methods for details). The GCT component present in these equivocal H&E LVI foci included embryonal carcinoma (6), seminoma (5), and choriocarcinoma (2), which again corresponded to the dominant GCT subtype in all cases except one focus of embryonal carcinoma; in that case, the dominant GCT component was teratoma.

Impact of ERG IHC on LVI assessment of individual cases

After determining the utility of ERG IHC for LVI assessment of individual LVI foci, we next sought to establish whether ERG IHC had an impact on the overall LVI status of individual cases, which, in turn, could impact pathologic and clinical classification. All told, nineteen cases were positive for LVI based on H&E and/or ERG IHC evaluation, while the remaining six were either negative or equivocal for LVI. Of the nineteen LVI positive cases, all were originally classified as either “pT2” or “pT3”, and therefore, ERG IHC would not have affected the pathologic staging of these cases. In contrast, of the six LVI negative or equivocal cases, one was originally classified as “pT1” and the other five as “pT2”; none of these tumors demonstrated tunica vaginalis invasion, and therefore, the presence (or absence) of LVI in cases was pathologic “T” stage-defining. Based on our retrospective analysis, ERG IHC would have confirmed the absence of LVI in two cases, only one of which was originally classified as “pT2”. Therefore, ERG IHC would have had a positive albeit limited impact on the overall pathologic staging on this cohort.

Overall, sixteen out of twenty-five cases had at least one H&E positive LVI focus – fifteen (94%) of which also had at least one ERG IHC positive LVI focus. Fifteen (88%) of these

H&E positive foci were also ERG LVI positive, while the other two were lost for evaluation upon sectioning. Both H&E equivocal foci were positive for LVI by ERG IHC. The four clinical stage I tumors corresponded to six H&E positive or equivocal LVI foci (all involving embryonal carcinoma): five H&E positive LVI foci and one H&E equivocal LVI focus. Four of these foci (from three total cases) were ERG IHC positive, while the other two (including the H&E equivocal focus) were lost for evaluation upon sectioning. Neither of the clinical stage IB tumors had evidence of tunica vaginalis or spermatic cord invasion (i.e., LVI was pathologic “T” stage-defining). Three of these clinical stage I patients ultimately underwent RPLND, which confirmed GCT metastasis in retroperitoneal lymph nodes; the fourth patient – who had persistently elevated serum tumor markers only – was treated with chemotherapy, after which his serum tumor markers normalized.

Of the nine remaining cases (out of twenty-five) without at least one H&E positive LVI focus, five (56%) could be classified as either positive or negative for LVI based on ERG IHC. One clinical stage IIIA tumor had only a single H&E equivocal focus (involving seminoma), which was positive for LVI based on ERG IHC. Four clinical stage I tumors corresponded to five H&E equivocal LVI foci, four involving seminoma cells and one involving choriocarcinoma cells. Two of these foci were ERG IHC positive (one each involving seminoma and choriocarcinoma), while the other three were ERG IHC negative (all involving seminoma). Three of the clinical stage I tumors had no evidence of tunica vaginalis or spermatic cord invasion (i.e., LVI was stage-defining), however, the other tumor invaded the spermatic cord (and, thus, LVI was not stage-defining). At last follow-up (range 4–73 months), none of these clinical stage I patients had developed clinical or pathologic evidence of GCT metastasis, including a patient with an ERG IHC positive LVI focus of choriocarcinoma cells who subsequently underwent RPLND.

Four cases (out of nine without at least one H&E positive LVI focus) were unable to be definitively designated as either positive or negative for LVI based on H&E and ERG IHC evaluation. One clinical stage IIA tumor had only a single H&E equivocal focus (involving choriocarcinoma), which was equivocal for LVI based on ERG IHC. Three clinical stage I tumors corresponded to three H&E equivocal LVI foci (all involving embryonal carcinoma); one focus was ERG IHC equivocal, and the other two foci were lost for evaluation. None of the clinical stage I tumors had evidence of tunica vaginalis or spermatic cord invasion (i.e., LVI was stage-defining), and at last follow-up (range 9–89 months), none of these clinical stage I patients had developed clinical or pathologic evidence of GCT metastasis.

Discussion

For surgical pathologists, our data suggest at least three possible scenarios for which ERG IHC may be useful: confirmation of H&E positive LVI foci (group 1); converting H&E equivocal LVI foci to positive for LVI (group 2); and, converting H&E equivocal LVI foci to negative for LVI (group 3). For group 1, our data indicate a high rate of concordance between H&E and ERG IHC, as nearly 90% of H&E positive LVI foci were also ERG IHC positive (Figure 1 and Table 2). Only three H&E positive LVI foci were not confirmed by ERG IHC, and in all of these instances, the positive focus was lost for evaluation upon sectioning for IHC. (Importantly, no H&E positive LVI foci were ERG IHC equivocal or

negative.) For groups 2 and 3, our data suggest that more than half of H&E equivocal LVI foci may be resolved by ERG IHC (Figures 2 and 3 and Table 2). Indeed, of the H&E equivocal LVI foci available for ERG IHC evaluation (i.e., not lost for evaluation upon sectioning), eight (80%) were ultimately able to be designated as either positive or negative for LVI by ERG IHC. Therefore, we propose a possible algorithmic approach to utilizing ERG IHC for evaluation of LVI in testicular GCT, whereby ERG IHC is employed predominantly for the classification of H&E equivocal LVI foci and only sparingly for confirmation in H&E positive LVI cases, as deemed clinically appropriate or necessary (Figure 4).

While our data suggest possible utility of ERG IHC for confirming or clarifying LVI status of individual LVI foci in testicular GCT at the time of radical orchiectomy, the potential patient-level impact is not as clear and needs to be resolved by additional studies. For treating clinicians, the presence or absence of LVI in testicular GCT at the time of radical orchiectomy is most important in the context of organ-confined tumors without clinical evidence of metastasis by staging radiographs or serum tumor markers^{2, 5–11}. For these patients, LVI – in addition to other clinicopathologic variables, such as the overall percentage of embryonal carcinoma, absence of teratoma, rete testis invasion, etc. – helps determine the overall risk of occult metastasis and/or recurrence, which may impact the treatment options offered (i.e., primary RPLND or chemotherapy). Indeed, presence or absence of LVI has been demonstrated to be a very powerful predictor of relapse for clinical stage I NSGCT patients^{11, 27}. While our study wasn't specifically designed to evaluate the prognostic value of ERG IHC for LVI evaluation in clinical stage IA or IB testicular GCT tumors, there were nine such cases in our cohort: two with at least one H&E positive LVI focus; and, seven with only H&E equivocal foci. For both of the tumors with at least one H&E positive LVI focus, ERG IHC confirmed the presence of LVI, and these patients subsequently developed pathologic evidence of GCT metastasis. Of the seven tumors with only H&E equivocal foci, two (29%) were LVI positive by ERG IHC, two (29%) were LVI negative by ERG IHC, one (13%) was LVI equivocal by ERG IHC, and two (29%) were lost for evaluation, and at last follow-up, none of these patients have developed clinical or pathologic evidence of GCT metastasis. This is a particularly important finding given the relatively low frequency, in our cohort, of H&E positive LVI foci in patients without clinical evidence of metastasis at diagnosis compared to patients with evidence of metastasis (22% vs. 88%, respectively). Therefore, surgical pathologists should tread carefully in cases where only H&E equivocal foci are present, as these tumors in our current retrospective cohort of GCT did not show subsequent clinical or pathologic evidence of GCT metastasis in the limited follow up time available for these patients. These data suggest that the presence of LVI in such cases should only be reported when unequivocally confirmed by ERG IHC. Further studies are warranted to investigate such specific clinical scenarios.

In this study, we investigated the utility of ERG immunohistochemistry for evaluation of GCT LVI in radical orchiectomy specimens, however, there are a number of other options available for surgical pathologists to address this issue, including submitting additional tissue for histopathologic review and obtaining “off the top” H&E stained sections. Obviously, in a retrospective study, submitting additional tissue for histopathologic review is not feasible, but even in the prospective setting, there is a practical limit to this approach

(i.e., eventually all possible useful tissue will be expended). It seems reasonable that decisions regarding additionally submitted tissue be handled on a case-by-case basis, at the signing pathologist's discretion. Our study was not specifically designed to compare "off the top" ERG IHC and H&E stained sections for GCT LVI evaluation, but at least in some instances, our data seem to indicate that an additional "off the top" H&E stained section alone may help resolve LVI status of H&E equivocal LVI foci (see Figure 2C-F). Of course, even in these cases, ERG IHC may provide an extra layer of confirmation of LVI status. Finally, other IHC markers, including CD31, CD34, and D2-40, have been evaluated for LVI assessment in other tumors^{13–20}. In contrast to ERG, all of these markers have non-nuclear staining patterns, which may be less helpful for LVI evaluation. To date, only one published report with colon cancer cases has compared these markers (CD31, D2-40, and ERG) for LVI assessment²⁵. In that study, endothelial cell discrimination by nuclear ERG IHC was preferred to non-nuclear CD31 and D2-40 IHC, and ERG IHC increased LVI detection and decreased interobserver variability among pathologists, after a group consensus session. Finally, given the known strong, robust expression of D2-40 by seminomas^{28–30}, any utility of D2-40 IHC for GCT LVI would likely be limited to foci with non-seminomatous components. In any event, the superiority of ERG IHC for evaluation of GCT LVI, as compared to other available LVI IHC markers, was not addressed here and remains an area of possible future exploration.

Acknowledgments

The authors would like to thank Tina Fields and the staff of the Department of Pathology Immunohistochemistry Laboratory at Michigan Medicine for technical assistance.

Funding: None.

References

1. Eble, JN. Pathology and genetics of tumours of the urinary system and male genital organs. Lyon: IARC; 2004. International Agency for Research on Cancer.
2. Edge, SB. AJCC cancer staging manual. New York: Springer; 2010. American Joint Committee on Cancer.
3. Ulbright, TM, Young, RH. Tumors of the testis and adjacent structures. Silver Spring, MD: American Registry of Pathology; 2013. Armed Forces Institute of Pathology (U.S.).
4. International Germ Cell Cancer Collaborative Group. International Germ Cell Consensus Classification: a prognostic factor-based staging system for metastatic germ cell cancers. *J Clin Oncol*. 1997; 15:594–603. [PubMed: 9053482]
5. Sandeman TF, Matthews JP. The staging of testicular tumors. *Cancer*. 1979; 43:2514–2524. [PubMed: 455237]
6. Moriyama N, Daly JJ, Keating MA, et al. Vascular invasion as a prognosticator of metastatic disease in nonseminomatous germ cell tumors of the testis. Importance in "surveillance only" protocols. *Cancer*. 1985; 56:2492–2498. [PubMed: 2412688]
7. Freedman LS, Parkinson MC, Jones WG, et al. Histopathology in the prediction of relapse of patients with stage I testicular teratoma treated by orchidectomy alone. *Lancet*. 1987; 2:294–298. [PubMed: 2886764]
8. Klepp O, Olsson AM, Henrikson H, et al. Prognostic factors in clinical stage I nonseminomatous germ cell tumors of the testis: multivariate analysis of a prospective multicenter study. Swedish-Norwegian Testicular Cancer Group. *J Clin Oncol*. 1990; 8:509–518. [PubMed: 1689773]

9. Albers P, Siener R, Kliesch S, 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.* 2003; 21:1505–1512. [PubMed: 12697874]
10. Vergouwe Y, Steyerberg EW, Eijkemans MJ, et al. Predictors of occult metastasis in clinical stage I nonseminoma: a systematic review. *J Clin Oncol.* 2003; 21:4092–4099. [PubMed: 14559885]
11. Divrik RT, Akdogan B, Ozen H, et al. Outcomes of surveillance protocol of clinical stage I nonseminomatous germ cell tumors-is shift to risk adapted policy justified? *J Urol.* 2006; 176:1424–1430. [PubMed: 16952649]
12. Ye H, Ulbright TM. Difficult differential diagnoses in testicular pathology. *Arch Pathol Lab Med.* 2012; 136:435–446. [PubMed: 22458906]
13. Niakosari F, Kahn HJ, Marks A, et al. Detection of lymphatic invasion in primary melanoma with monoclonal antibody D2-40: a new selective immunohistochemical marker of lymphatic endothelium. *Arch Dermatol.* 2005; 141:440–444. [PubMed: 15837861]
14. Roma AA, Magi-Galluzzi C, Kral MA, et al. Peritumoral lymphatic invasion is associated with regional lymph node metastases in prostate adenocarcinoma. *Mod Pathol.* 2006; 19:392–398. [PubMed: 16400321]
15. Mohammed RA, Martin SG, Gill MS, et al. Improved methods of detection of lymphovascular invasion demonstrate that it is the predominant method of vascular invasion in breast cancer and has important clinical consequences. *Am J Surg Pathol.* 2007; 31:1825–1833. [PubMed: 18043036]
16. Harris EI, Lewin DN, Wang HL, et al. Lymphovascular invasion in colorectal cancer: an interobserver variability study. *Am J Surg Pathol.* 2008; 32:1816–1821. [PubMed: 18779725]
17. Rose AE, Christos PJ, Lackaye D, et al. Clinical relevance of detection of lymphovascular invasion in primary melanoma using endothelial markers D2-40 and CD34. *Am J Surg Pathol.* 2011; 35:1441–1449. [PubMed: 21881483]
18. Kryvenko ON, Epstein JI. Histologic criteria and pitfalls in the diagnosis of lymphovascular invasion in radical prostatectomy specimens. *Am J Surg Pathol.* 2012; 36:1865–1873. [PubMed: 23154772]
19. McQuitty E, Ro JY, Truong LD, et al. Lymphovascular invasion in micropapillary urothelial carcinoma: a study of 22 cases. *Arch Pathol Lab Med.* 2012; 136:635–639. [PubMed: 22646270]
20. Gresta LT, Rodrigues-Junior IA, de Castro LP, et al. Assessment of vascular invasion in gastric cancer: a comparative study. *World J Gastroenterol.* 2013; 19:3761–3769. [PubMed: 23840114]
21. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science.* 2005; 310:644–648. [PubMed: 16254181]
22. Mehra R, Tomlins SA, Shen R, et al. Comprehensive assessment of TMPRSS2 and ETS family gene aberrations in clinically localized prostate cancer. *Mod Pathol.* 2007; 20:538–544. [PubMed: 17334343]
23. Miettinen M, Wang ZF, Paetau A, et al. ERG transcription factor as an immunohistochemical marker for vascular endothelial tumors and prostatic carcinoma. *Am J Surg Pathol.* 2011; 35:432–441. [PubMed: 21317715]
24. Sato Y. Role of ETS family transcription factors in vascular development and angiogenesis. *Cell Struct Funct.* 2001; 26:19–24. [PubMed: 11345500]
25. Kim S, Park HK, Jung HY, et al. ERG Immunohistochemistry as an Endothelial Marker for Assessing Lymphovascular Invasion. *Korean J Pathol.* 2013; 47:355–364. [PubMed: 24009631]
26. Huang H, Jadallah S, Chen Y, 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.* 2013; 93:219A.
27. Ondrus D, Ondrusova M, Hornak M, et al. Nonseminomatous germ cell testicular tumors clinical stage I: differentiated therapeutic approach in comparison with therapeutic approach using surveillance strategy only. *Neoplasma.* 2007; 54:437–442. [PubMed: 17688374]
28. Lau SK, Weiss LM, Chu PG. D2-40 immunohistochemistry in the differential diagnosis of seminoma and embryonal carcinoma: a comparative immunohistochemical study with KIT (CD117) and CD30. *Mod Pathol.* 2007; 20:320–325. [PubMed: 17277761]

29. Yu H, Pinkus GS, Hornick JL. Diffuse membranous immunoreactivity for podoplanin (D2-40) distinguishes primary and metastatic seminomas from other germ cell tumors and metastatic neoplasms. *Am J Clin Pathol.* 2007; 128:767–775. [PubMed: 17951198]
30. Iczkowski KA, Butler SL, Shanks JH, et al. Trials of new germ cell immunohistochemical stains in 93 extragonadal and metastatic germ cell tumors. *Hum Pathol.* 2008; 39:275–281. [PubMed: 18045648]

Figure 1. Confirmation of testicular GCT LVI by ERG IHC in foci positive by H&E (A, C, E) H&E and (B, D, F) ERG IHC. The H&E image in (A) shows a focus of embryonal carcinoma in a small vessel with associated blood and fibrin; the corresponding ERG IHC image (B) highlights the endothelial cells lining the vessel (arrowhead). The H&E images in (C) and (E) show foci of yolk sac and choriocarcinoma, respectively, in medium vessels with associated blood and fibrin; the corresponding ERG IHC images (D, F) shows the vessel endothelial cells (arrowheads). Magnification: (A, B) 200X and (C-F) 100X.

Figure 2. Positive testicular GCT LVI by ERG IHC in foci equivocal by H&E

(A, C, E) H&E and (B, D, F) ERG IHC. The H&E image in (A) shows a focus of seminoma in a possible small vessel but without associated blood and fibrin; the corresponding ERG IHC image (B) highlights the endothelial cells lining the vessel (arrowhead). The H&E image in (C) shows a focus of seminoma in a possible small vessel surrounded by lymphocytes but without blood or fibrin; the corresponding ERG IHC image (D) demonstrates the vessel endothelial cells (arrowhead in lower portion of image). For this focus, an additional H&E section alone may have helped resolved the LVI status, however, ERG IHC added an additional layer of confirmation. The H&E image in (E) shows a focus

of embryonal carcinoma in a possible medium vessel with associated fibrin; the corresponding ERG IHC image (F) highlights endothelium surrounding tumor cells (arrowheads in right central portion of image). Similarly, for this focus, an additional H&E section alone may have helped resolved the LVI status, however, ERG IHC added an additional layer of confirmation. Magnification: (A, B) 200X and (C-F) 100X.

Figure 3. Negative or equivocal GCT LVI by ERG IHC in cases equivocal by H&E

(A, C, E) H&E and (B, D, F) ERG IHC. The H&E image in (A) shows a focus of seminoma; the corresponding ERG IHC image (B) demonstrates scattered small vessels (arrowheads) but no vascular invasion. The H&E image in (C) shows a small focus of seminoma associated with and possibly invading small vessels (arrowheads); the corresponding ERG IHC image (D) reveals scattered small vessels (arrowhead) but no vascular invasion. The H&E image in (E) shows a focus of embryonal carcinoma in a possible medium vessel; the corresponding ERG IHC image (F) shows an incomplete peripheral endothelial cell layer

(arrowheads). This focus was considered equivocal for LVI by ERG IHC. Magnification: (A-D) 200X and (E, F) 40X.

Figure 4. Proposed algorithm for ERG IHC in evaluation of testicular GCT LVI

ERG IHC may be useful when definitive confirmation of an H&E positive LVI focus is clinically appropriate or necessary. In addition, in select clinical scenarios, ERG IHC may be helpful in resolving the LVI status of H&E equivocal LVI foci.

Table 1

Clinicopathologic characteristics of study testicular GCT cases.

Patient #	Age (years)	Side	Size (cm)	Type	Histology	AJCC Stage	Tunica vaginalis invasion?	Clinical stage (at diagnosis)	Metastasis?	Metastasis Location	Follow-up (months)	Status
1	16	Right	6.0	NSGCT	EC (50%), YS (40%), chorio (10%)	pT2	No	IIA	Yes (pathologic)	Retropertitoneal LN	22	Alive
2	17	Left	2.0	NSGCT	EC (75%), YS (15%), teratoma (10%)	pT2	No	IIIA	Yes (clinical)	Pulmonary, retropertitoneal LN	59	Alive
3	19	Right	6.0	NSGCT	Chorio (60%), teratoma (15%), sem (15%), EC (10%)	pT2	Yes	IIIC	Yes (clinical)	Pulmonary	<1	DOD
4	19	Left	2.8	NSGCT	Teratoma (50%), EC (40%), YS (10%), Chorio (<1%)	pT2	No	IB	No (clinical)		89	Alive
5	20	Right	1.5	NSGCT	Chorio (70%), EC (25%), YS (5%)	pT2	No	IB	No (pathologic)		4	Alive
6	20	Right	1.2	NSGCT	EC (100%)	pT2	No	IIA	Yes (clinical)	Retropertitoneal LN	40	Alive
7	20	Left	3.0	NSGCT	EC (90%), YS (10%)	pT2	No	IB	Yes (pathologic)	Retropertitoneal LN	114	Alive
8	21	Left	2.4	NSGCT	EC (90%), teratoma (5–10%), sem (<5%)	pT2	No	IS	Yes (clinical)	Elevated serum tumor markers only	53	Alive
9	21	Right	3.9	Sem	Sem (100%)	pT2	No	IIB	Yes (clinical)	Retropertitoneal LN	26	Alive
10	22	Right	2.2	NSGCT	EC (60%), YS (40%)	pT2	No	IB	No (pathologic)		22	Alive
11	23	Left	8.0	NSGCT	EC (60%), sem (25%), YS (10%), chorio (5%)	pT3	No	IIB	Yes (pathologic)	Retropertitoneal LN	9	Alive
12	24	Left	3.6	NSGCT	EC (80%), teratoma (20%)	pT2	Yes	IIA	Yes (pathologic)	Retropertitoneal LN	25	Alive
13	25	Left	3.0	NSGCT	EC (100%)	pT2	No	IIA	Yes (pathologic)	Retropertitoneal LN	30	Alive
14	25	Left	3.2	NSGCT	EC (100%)	pT2	No	IIB	Yes (pathologic)	Retropertitoneal LN	14	Alive
15	25	Right	2.0	Sem	Sem (100%)	pT2	No	IIB	Yes (clinical)	Retropertitoneal LN	11	Alive
16	27	Left	3.5	NSGCT	EC (85%), sem (10%), YS (5%), teratoma (<1%)	pT2	No	IB	No (clinical)		9	Alive
17	28	Right	2.8	NSGCT	EC (90%), teratoma (10%)	pT2	No	IS	Yes (pathologic)	Retropertitoneal LN	5	Alive
18	31	Left	6.3	NSGCT	EC (95%), YS (5%)	pT2	No	IIB	Yes (clinical)	Retropertitoneal LN	17	Alive
19	35	Left	7.8	NSGCT	Chorio (70%), teratoma (30%)	pT2	No	IIA	Yes (clinical)	Retropertitoneal LN	32	Alive
20	35	Left	7.8	NSGCT	EC (80%), YS (10%), teratoma (10%)	pT2	No	IIIA	Yes (clinical)	Pulmonary, hilar LN	72	Alive
21	35	Left	1.5	NSGCT	EC (95%), YS (5%)	pT2	No	IB	Yes (pathologic)	Retropertitoneal LN	46	Alive
22	40	Right	6.0	Sem	Sem (100%)	pT3	No	IB	No (clinical)		19	Alive

Patient #	Age (years)	Side	Size (cm)	Type	Histology	AJCC Stage	Tunica vaginalis invasion?	Clinical stage (at diagnosis)	Metastasis?	Metastasis Location	Follow-up (months)	Status
23	48	Left	5.5	Sem	Sem (100%)	pT2	No	IB	No (clinical)		31	Alive
24	49	Left	2.7	NSGCT	Sem (80%), EC (20%)	pT1	No	IA	No (clinical)		12	Alive
25	69	Right	5.6	Sem	Sem (100%)	pT3	No	IIIA	Yes (clinical)	Retroperitoneal and mediastinal LN	73	Alive

Sem = seminoma; EC = embryonal carcinoma; YS = yolk sac tumor; chorio = choriocarcinoma; NSGCT = non-seminomatous germ cell tumor; LN = lymph node; AJCC = American Joint Committee on Cancer; DOD = died of disease

Table 2

Results of ERG IHC in positive or equivocal LVI foci in 25 testicular GCT cases.

Focus #	Patient #	H&E LVI	ERG LVI	Dominant Component	LVI Component
1	1	Pos	Pos	EC	YS
2	2	Pos	Pos	EC	YS
3	3	Pos	Pos	Chorio	Chorio
4	3	Pos	Pos	Chorio	Chorio
5	4	Equiv	Lost	Teratoma	EC
6	5	Equiv	Pos	Chorio	Chorio
7	6	Pos	Pos	EC	EC
8	6	Pos	Pos	EC	EC
9	6	Pos	Pos	EC	EC
10	6	Pos	Lost	EC	EC
11	7	Pos	Pos	EC	EC
12	8	Pos	Pos	EC	EC
13	8	Pos	Pos	EC	EC
14	9	Pos	Pos	Sem	Sem
15	10	Equiv	Lost	EC	EC
16	11	Pos	Pos	EC	EC
17	12	Pos	Pos	EC	EC
18	13	Pos	Pos	EC	EC
19	14	Pos	Lost	EC	EC
20	14	Equiv	Pos	EC	EC
21	15	Pos	Pos	Sem	Sem
22	16	Equiv	Equiv	EC	EC
23	17	Pos	Lost	EC	EC
24	18	Pos	Pos	EC	EC
25	18	Pos	Pos	EC	EC
26	18	Pos	Pos	EC	EC
27	19	Equiv	Equiv	Chorio	Chorio

Focus #	Patient #	H&E LVI	ERG LVI	Dominant Component	LVI Component
28	20	Pos	Pos	EC	EC
29	20	Equiv	Pos	EC	EC
30	21	Pos	Pos	EC	EC
31	21	Equiv	Lost	EC	EC
32	22	Equiv	Pos	Sem	Sem
33	23	Equiv	Neg	Sem	Sem
34	24	Equiv	Neg	Sem	Sem
35	24	Equiv	Neg	Sem	Sem
36	25	Equiv	Pos	Sem	Sem

EC = embryonal carcinoma; sem = seminoma; chorio = choriocarcinoma; YS = yolk sac tumor; pos = positive; neg = negative; equiv = equivocal