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OCT4 staining increases the detection of lymphatic/vascular invasion in pure seminoma of the testis obscured by prominent lymphohistiocytic inflammation

Andrew A. Renshaw and Edwin W. Gould

Department of Pathology, Baptist Hospital, Miami, FL, United States

Summary

Lymphatic/vascular invasion (LVI) is an important prognostic feature in pure seminoma of the testis, a tumour that may be associated with lymphohistiocytic inflammation (LHI). Traditionally, LVI is identified on routine haematoxylin and eosin (H&E) staining. We sought to determine if staining of LHI near vessels in cases of pure seminoma with OCT4 could improve detection of LVI. All available cases of pure seminoma of the testis at our institutions were reviewed and correlated with clinicopathological features. A total of 67 cases were reviewed. Traditional LVI was identified in five cases by routine H&E evaluation. LHI was associated with identifiable vessels in 13 cases. In six of these cases, neoplastic cells were identified in the lumen of vessels by H&E staining. In five additional cases, OCT4 identified neoplastic cells in the lumen of vessels. Two foci did not have neoplastic cells identified. Patients with LVI had larger tumours, were less often limited to the testicular parenchyma (other than the LVI), and more often had metastatic disease than patients without LVI. Traditional LVI was found most often in the testis, while LVI with LHI was found most often in the spermatic cord. LVI in the spermatic cord of patients with pure seminoma may be obscured by prominent LHI, and staining of such foci with OCT4 may increase the detection of LVI by 45%.

Key words: Testis; seminoma; lymphatic vascular invasion; metastasis; OCT4.

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INTRODUCTION

Lymphatic/vascular invasion (LVI) is an adverse prognostic feature in pure seminoma of the testis.[1–4] It results in classifying an otherwise organ confined tumour as stage T2 rather than T1, and patients with this finding are less often selected for surveillance only therapy.[5] While contamination of lymphatic/vascular spaces with germ cell tumours in general, including seminoma, has been well described,[6] little else has been written until recently about the morphological features of LVI in these tumours, and to our knowledge, identification of LVI is generally performed using routine haematoxylin and eosin (H&E) stains and based on identifying neoplastic cells within the lumen of vessels that do not represent contamination. Recently we had a case where the LVI by pure seminoma was obscured by abundant lymphohistiocytic inflammation, and neoplastic cells were very difficult to identify without immunohistochemical stains. In addition, a recent report suggests that other pathologists have also encountered this scenario.[7] To further investigate whether the routine use of OCT4 staining could improve the detection of LVI in pure seminoma, specifically when applied to tumours with prominent lymphohistiocytic inflammation associated with identifiable vessels, we reviewed a series of pure seminomas of the testis and characterised the histological features of LVI using OCT4 staining.

METHODS

The study was approved by the institutional review board.

All cases of pure seminoma received at Baptist Hospital, Homestead Hospital, and West Kendall Hospital, Florida, from 2001 through October 2014 for which slides were available, were identified. Slides could not be retrieved in four cases and these were excluded from further analysis. All slides were reviewed by one author (AAR) to confirm the diagnosis and stage and to identify traditional LVI which was unassociated with lymphohistiocytic inflammation (Fig. 1). In addition, the presence of prominent lymphohistiocytic inflammation consisting of islands and nests of lymphocytes and histiocytes (LVI with LHI) within the lumen of a vascular or lymphatic space were identified (Fig. 2 and 3). In most cases, when the inflammation involved a large vessel, a nest or island of inflammatory cells was surrounded by what appeared to be an endothelial layer, and often the nest appeared to be protruding into the lumen from the edge of the vessel. When present in smaller vessels, there was no lining of the nest, and the cells appeared to be set in a proteinaceous background. In cases in which LVI was identified, a thorough search of the slide for evidence of contamination was performed. In no case with LVI with LHI was there any evidence of contamination on the slide other than the focus identified within the vascular lumen. In cases in which contamination was identified, the cells were typically discohesive and present as only tumour cells or tumours cells admixed with single lymphocytes and histiocytes. Discrete aggregates of lymphocytes and histiocytes forming islands were not identified.

Clinical information concerning the presence of metastatic disease (based on imaging studies) was obtained form the medical record.

Immunohistochemistry for OCT4 (clone MRQ-10; Cell Marque, USA) was performed without dilution and with high retrieval, and for CD31 (clone JC70A; Dako, Denmark) was performed without dilution and with high pH.

Statistical analysis was performed using a two-tailed Fisher's exact test for categorical data and the two-tailed Mann–Whitney U test for continuous data. A p value of <0.05 was considered significant.

RESULTS

A total of 67 cases were reviewed (Table 1). Traditional LVI was identified in five cases, all in the testis itself (Fig. 1). Lymphohistiocytic inflammation was present near

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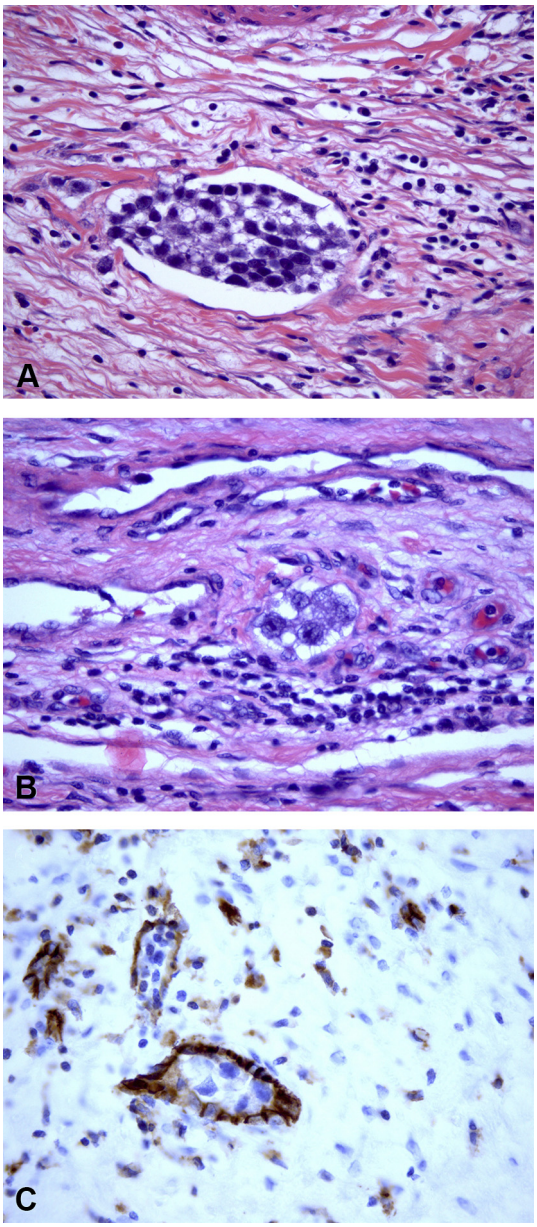

Fig. 1 Examples of traditional lymphatic invasion. The foci consist of cohesive clusters of neoplastic cells the outline of which mirrors that of the surrounding blood vessel. The diameter of the blood vessels were all <1 mm. (A,B) H&E. (C) CD31 stain highlights the blood vessel wall (same case as B).

identifiable vessels in 13 cases. In six cases neoplastic cells could be identified within the lumen of vessels by H&E staining. Neoplastic cells were identified within the lumen of vessels with OCT4 staining in all six cases in which neoplastic cells could be seen on H&E staining, as well as an additional five cases in which they could not (increasing detection rate for LVI from 11 to 16 cases or 45%). Two cases with lymphohistiocytic inflammation did not have neoplastic cells identified by either routine H&E or OCT4 staining.

LVI with prominent lymphohistiocytic inflammation (LVI with LHI) involved large vessels in six cases (Fig. 2) and lymphatic spaces next to large vessels in five cases (Fig. 3).

The ages of the patients with traditional LVI, LVI with LHI, and no LVI were not statistically different (all $p > 0.24$).

The diameter of the tumours with traditional LVI and LVI with LHI were larger than those without either (means 6.3 cm, 5.2 cm, versus 3.5 cm, respectively; $p = 0.06$ for traditional LVI versus neither and $p = 0.05$ for LVI with LHI versus neither).

Tumours with no LVI were more likely to be limited to the testicular parenchyma (not involving the base of cord) (98%) than those with traditional LVI (40%, $p = 0.001$) or with LVI with LHI (82%, $p = 0.08$).

All of the foci of traditional LVI were located in the testis itself either immediately adjacent to the tumour itself or in the tunica albuginea. In contrast, 10 of 11 of the foci of LVI with LHI were located in the spermatic cord ($p < 0.001$).

The percentage of patients with metastatic disease was higher for patients with traditional LVI than those without any LVI (40% versus 4.3%, $p = 0.04$). The percentage of patients with metastatic disease was significantly higher for patients with LVI with LHI than those without any LVI (55% versus 4.3%, $p < 0.001$).

DISCUSSION

In this report we demonstrate that OCT4 staining of lymphohistiocytic foci associated with identifiable vessels can increase the detection of LVI in pure seminoma by 45% compared with detection by routine H&E staining. Most previous reports describing the histological features of LVI have emphasised distinguishing true invasion from artefactual contamination.[6] The previous report by Nazeer *et al.*, which examined all germ cell tumours and not just seminoma, emphasised that true vascular invasion consists of tumour plugs that are attached to the vessel wall and/or conform to the shape of the vascular lumen.[6] The illustrations in that report document very large cohesive clusters of seminoma cells present in very large vessels. No clinicopathological correlation was performed in that prior study. In contrast, in the current study we noted that while lymphatic invasion consisting of nests of neoplastic cells (traditional LVI) was the most common histological finding when LVI was identified in the testis itself, most often LVI in the spermatic cord was associated with lymphohistiocytic inflammation, and in nearly half of these cases the neoplastic cells could only be identified with the use of OCT4 staining. Importantly, we were unable to identify any evidence of contamination on the slides in which LVI with LHI was identified. In addition, in cases in which contamination was identified, the contaminant consisted of individual tumour cells, lymphocytes and histiocytes, and did not consist of cohesive clusters of such cells. These results are very similar to those of another recent report on the same subject.[7]

The finding of prominent lymphohistiocytic inflammation obscuring seminoma cells is not unique to this study. Previous studies have shown that the same issue can arise when trying to diagnose germinomas in the central nervous system.[8] In the testis, lymphocytes are quite common and giant cells are not uncommon in seminomas, but rarely does this inflammation obscure the underlying neoplasm. In contrast, within the vascular spaces of the spermatic cord, this inflammation appears to be more common, and the identification of the neoplastic cells more difficult.

While this study focussed on the use of OCT4 to improve the sensitivity of detection of LVI in seminoma, it is not the only immunohistochemical stain that may be of value in this process. When the vascular structure that is involved by LVI is small, or when it may be difficult to decide if a lumen is