Use of Immunohistochemical Markers to Confirm the Presence of Vas Deferens in Vasectomy Specimens

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Abstract

CD10 has recently been described as a marker that can distinguish wolffian duct derivatives from müllerian remnants but has yet to be tested in vasectomy specimens. We sought to determine if CD10 and pankeratin could corroborate the presence of vas deferens (VD). For the study, 103 consecutive vasectomy specimens were immunohistochemically analyzed for CD10, pankeratin, and CD31 expression in luminal and basal layer cells. In all cases with optimal epithelial histologic features (92/103), CD10 demonstrated intense apical membranous expression in all VD and weak basal cytoplasmic staining in about 98% of cases. Pankeratin demonstrated cytoplasmic and membranous expression in apical and basal layers in 99% of VD. In cases with suboptimal epithelial histologic features (11/103), the detection of epithelia was 100% for CD10 and pankeratin. Our data show that CD10 and pankeratin can be used to confirm the presence of VD in vasectomy specimens in which the epithelial histologic features are suboptimal.

Vasectomy is a common male surgical sterilization procedure in which a segment of vas deferens (VD) is removed to prevent sperm from passing through the reproductive tract. More than 40 million couples worldwide use vasectomy as a contraceptive method.1 Many urology practitioners routinely send the resected portions of the VD for histologic confirmation.2-4 Although the success of vasectomy is eventually confirmed by azoospermia in a semen specimen, histologic examination is beneficial for detecting certain surgical mishaps, such as mistaking another structure for the VD.4

For pathologists, it is important to ensure that the VD has been transected by identifying a complete cross-section of the VD, which is composed of pseudostratified apical columnar epithelium with stereocilia and cuboidal basal cells overlying loose connective tissue stroma and a well-developed, thick, 3-layered muscular coat. However, in rare cases, the presence of VD is difficult to confirm by routine H&E-stained sections owing to suboptimal histologic features such as denuded or attenuated epithelium or specimens that have been embedded incorrectly. No studies have been reported to address how these cases should be handled.

Studies have demonstrated that CD10, which is commonly used in the categorization of hematopoietic malignancies, also serves as a useful marker with high sensitivity and some specificity for wolffian-derived structures, such as glandular epithelium of the prostate, seminal vesicles, VD, and epididymis.5,6 Our goal was to assess issues in VD histologic examination and identify an immunohistochemical marker that could corroborate the presence of VD in specimens that are not ideal. In the present study, we examined whether CD10 in conjunction with a nonspecific epithelial marker, pankeratin, could help confirm the presence...
of surgically transected VD. The endothelial marker CD31 was also tested.

Materials and Methods

For the study, 103 consecutive vasectomy specimens processed in 2008 at the University of Pittsburgh Department of Pathology (Pittsburgh, PA) were retrieved. All tissues were fixed in 10% formalin. The VD segments are submitted unsectioned in a cassette to the histology laboratory and are sectioned by a histotechnologist at the time of paraffin embedding. Four-micrometer sections were stained with H&E. All slides were reviewed by pathologists involved in this study (S.I.B., D.L.Z., and A.V.P.). In cases in which histologic confirmation was difficult on the original section owing to incorrect embedding, poor epithelial histologic features, or incomplete lumen, the step-section recuts obtained at the time the case was managed were also evaluated. Sections from a radical prostatectomy and orchectomy were also obtained and included distal VD, seminal vesicle, ejaculatory duct, prostate, and spermatic cord.

Immunohistochemical stains were performed on deparaffinized, formalin-fixed sections. Primary antibodies, including manufacturer, clone, and dilution, were as follows: CD10 (56C6, 1:50, Novocastra/Vector, Burlingame, CA), pankeratin (1:100, panceroatkeratin cocktail, Chemicon, Temecula, CA), and CD31 (M0823, JC/70A, 1:25, DAKO, Carpinteria, CA). A standard, automated, streptavidin-biotin-peroxidase technique was performed using the Ventana Immunostainer (Ventana, Tucson, AZ) and a biotin-Vector Elite ABC kit with the Cadenza Immunostainer (Shandon, Pittsburgh, PA).

Positive staining in each structural compartment of VD (surface columnar and basal epithelial cell layers and inner, middle, and outer muscle layers) was semiquantitatively classified as 0 (<5% of cells stained), 1+ (5%-10% of cells stained), 2+ (11%-50% of cells stained), and 3+ (>50% of cells stained). In cases in which the discrimination of basal cells vs surface columnar cells was not possible owing to crushed or attenuated/partially denuded epithelium (such that only a single layer of cells is identified), a single epithelial score was given. Immunohistochemical scoring of the muscle was not done in cases in which discrimination of muscle layers was not possible.

Results

Immunohistochemical Reactivity in Vasectomy Specimens

CD10, pankeratin, and CD31 were tested on vasectomy specimens with results summarized in Table 1. Of the 103 vasectomy specimens examined, surface columnar epithelial cells and basal cells were identified in 92 cases (89.3%) Image 1A. CD10 immunohistochemical analysis demonstrated a well-defined, apical, membranous staining pattern in surface columnar epithelial cells outlining the lumen in all cases (2+, 1/92 [1%]; 3+, 91/92 [99%]) and cytoplasmic staining in basal epithelial cells in the vast majority of cases (0, 2/92 [2%]; 1+, 1/92 [1%]; 2+, 2/92 [2%]; and 3+, 87/92 [95%]) Image 1B. Similarly, pankeratin immunostaining revealed strong membranous and cytoplasmic expression in apical (0, 1/92 [1%]; 1+, 0/92 [0%]; 2+, 6/92 [7%]; and 3+, 85/92 [92%]) and basal epithelial layers (0, 1/92 [1%]; 1+, 0/92 [0%]; 2+, 3/92 [3%]; and 3+, 88/92 [96%]) Image 1C. CD31 did not show apical or basal epithelial reactivity in any specimens Image 1D.

CD10 did not stain any muscular structures of VD. There was weak patchy background reactivity in the middle oblique/circular or outer longitudinal coats in 39 of 101 specimens (0, 62/101 [61%]; 1+, 39/101 [39%]) using pankeratin. Muscle layers were negative for CD31 in all cases. In 4 of 103 cases, scoring of all 3 muscle layers was not possible because the muscle layers appeared sclerotic and were not distinguishable. In 1 of these 4 cases, the epithelium was also attenuated, further obscuring the histologic features.

VD With Suboptimal Epithelial Histologic Features

Even after recutting or re-embedding, 11 (10.7%) of 103 cases had poor epithelial histologic features, preventing identification of apical vs basal cell layers. The 11 cases exhibited one or more of the following issues: dilated lumen lined by a single layer of attenuated cells (n = 2) Image 2A, partially denuded epithelia (n = 6), crush Image 2B, cautery (n = 3) Image 2C, and/or a compressed tight lumen obscuring epithelial histologic features (n = 3). Within this subset, CD10 (11/11 [100%]) and pankeratin (11/11 [100%]) highlighted the thinned, single-layer of epithelium Image 2D and Image 2E or distorted epithelium Image 2F, Image 2G, Image 2H, and Image 2I.
Immunohistochemical Expression in Additional Genitourinary Specimens

All wolffian duct derivatives, including distal VD, seminal vesicles, prostate glands, and ejaculatory ducts, showed strong positive staining for CD10 (well-defined apical membranous and basal cytoplasmic staining pattern) and pankeratin (strong apical and basal membranous and cytoplasmic staining pattern), similar to the staining seen in the vasectomy specimens. CD31 was restricted to blood vessels and did not show epithelial staining. The spermatic cord soft tissue was negative for pankeratin, had CD31 reactivity in endothelial cells, and showed CD10 reactivity restricted to lymphocytes and neutrophils.

Discussion

Vasectomy-related cases are the subject of a substantial amount of litigation owing to the emotional and economic impact of the failure to induce sterility. Vasectomy failure is attributed to 2 mechanisms: failure to divide vas or vasal recanalization, caused by the formation of microchannels.
between the divided ends. Histologic examination could detect the former and provides solid evidence of vas transection, which gives some protection to a surgeon in the event of a medicolegal complaint, although the pathology cannot establish laterality of the transected VD. Moreover, histologic examination may also be useful in cases of repeated vasectomies to identify cause(s) of vasectomy failure or recanalization, such as sperm granuloma. In 1 study, histologic confirmation of the resected VD could not be done in 4 of 92 patients, and repeated vasectomies were performed. One of the patients in this study without histologic verification of surgical transection impregnated his wife and was found to have normospermia without evidence of recanalization on repeated vasectomy. For these reasons, although it is not required to submit the excised length of VD for histologic confirmation of its structure following vasectomy, it is common practice for some urologists. Pathologists confirm the presence of successfully transected VD by light microscopic findings, via the presence of a ductal structure lined by folded epithelium composed of apical columnar and cuboidal basal cells, surrounded by 3 muscular coats. However, these structures are sometimes destroyed or incomplete due to the surgical procedure or histologic processing of the specimen. No studies delineate

**Image 2** Immunohistochemical staining of vas deferens (VD) with suboptimal epithelial histologic features. A, Epithelium composed of 1 or 2 attenuated epithelial cell layers lining a dilated lumen without folding (H&E, ×40). B, Strong staining (CD10, ×40). C, Pankeratin stain positive in the remaining epithelial cells (×40). D, Partially crushed epithelium composed of a few squashed epithelial layers (H&E, ×20). E, Distinctive apical intense membranous reactivity (CD10, ×20). F, Pankeratin is positive throughout the epithelial layers (×20). G, Crushed/cauterized epithelium lining in a distorted lumen (H&E, ×40). H, CD10 shows surface membranous positivity and identifies some basal cells that have cytoplasmic reactivity (×40). I, Pankeratin is positive throughout the distorted epithelial layers (×40).
the appropriate workup of such cases, and the usefulness of immunohistochemical analysis has not been described.

In recent years, various studies have reported mesonephric structures and mesonephric-derived tumors to have consistent positivity for epithelial markers such as low-molecular-weight cytokeratins, including CK7, and epithelial membrane antigen.10-12 These authors stress that the epithelial immunophenotype for these markers is not substantially different between müllerian and mesonephric structures and that there is not yet a marker specific for mesonephric structures.

CD10, or CALLA, is a cell surface neutral endopeptidase that inactivates several biologically active neuropeptides.13 This antigen has been known largely because it is expressed by lymphoid precursor cells, germinal center B lymphocytes, and some myelocytes and is used as a cell surface marker for the classification of acute leukemia and lymphoma.14,15 A CD10 monoclonal antibody (clone 56C6) is commercially available for paraffin-embedded tissue that provides satisfactory results after heat-induced epitope retrieval.16 Using this antibody, CD10 has been shown to be a useful marker with strong and reliable staining for wolffian-derived structures.5,6 Notably, these studies demonstrated strong staining of CD10 for glandular epithelium of the prostate, seminal vesicles, VD, and epididymis, with 100% sensitivity, and the lack of CD10 expression in normal müllerian-derived epithelium, stimulating our interest in investigating this marker for the identification of VD in routine vasectomy specimens.

In this study, we demonstrated that CD10 and pankeratin are reliable markers with very high sensitivity to confirm the presence of VD in optimal vasectomy specimens. Moreover, these immunohistochemical markers consistently highlight the epithelial cells of VD, even in cases with suboptimal morphologic features. The usefulness of cytokeratin immunohistochemical stains to evaluate distorted epithelia has been previously well documented.17-19 In the workup of necrotic carcinoma, we frequently use pankeratin in our daily practice. However, to our knowledge, this is the first report demonstrating the usefulness of CD10 immunostaining in the analysis of distorted epithelia.

The majority of suboptimal epithelial histologic features appeared to be related to the vasectomy procedure, eg, cautery, crush, and denuded epithelium are likely surgical artifacts and should not interfere with identifying a cross-section of VD. However, a few rare specimens were seen with a single attenuated layer of epithelia surrounding a dilated lumen, lacking the characteristic histologic features of VD. When the epithelial histologic features are poor in a routine vasectomy specimen, a 3-layered muscular coat might be an adjunctive hallmark to confirm the presence of VD.

However, 3 specimens also had a uniformly dense sclerotic muscular coat together with a single attenuated layer of epithelium, which hampers discrimination from other structures with a lumen, such as blood vessels, with a similar tubular structure surrounded by muscle. None of the 3 patients had a history of previous surgical manipulation in the scrotal or inguinal area, which might cause the sclerotic changes, and no fibroblastic or inflammatory response was seen. We are uncertain of the cause of this luminal dilatation, epithelial attenuation, and sclerotic change of the muscular coat, but, regardless of the cause, in these cases, the remaining single layer of epithelium was positive for CD10 and pankeratin and negative for CD31, consistent with the properties of normal VD. Although it was difficult to classify remaining single-layered epithelial cells in partially denuded or attenuated specimens by H&E, the intracytoplasmic staining pattern of CD10 in certain samples suggests that these are likely basal cells. Similar to thinned or partially denuded VD, embedding errors may result in only a remaining minimal amount of epithelium. In these cases, CD10 and pankeratin may be useful to corroborate that the sample is VD.

Other structures potentially misinterpreted as VD might be glandular inclusions. These glandular inclusions are rarely found in inguinal hernia sacs and bear a striking morphologic resemblance to the epididymis (epididymis-like inclusions) or VD (VD-like inclusions).5 Cerilli et al demonstrated that none of the VD-like inclusions showed CD10 positivity and 50% of epididymis-like inclusions had epithelial positivity of CD10. However, epididymis-like inclusions are composed of closely arranged clusters of numerous tubules and are morphologically relatively easily differentiated from VD.

Our data show that CD10 and pankeratin are reliable markers to highlight the epithelium of VD. We demonstrated that these markers can be used to confirm the presence of VD in vasectomy specimens in which the epithelial histologic features are suboptimal. CD10 may provide additional reassurance that the specimen is a wolffian-derived epithelial-lined structure. These markers may prove to be useful in the workup in rare cases in which recutting or re-embedding does not yield adequate histologic features to identify the specimen as VD.

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