MUC16 as a Sensitive and Specific Marker for Epithelial Downgrowth

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Objective: To compare immunohistochemical results of cytokeratin AE1/AE3, the traditional favored marker, with MUC16 and cytokeratin 19 for diagnostic sensitivity and specificity in epithelial downgrowth and control corneas.

Methods: Immunohistochemical analysis was performed in 5 cases of epithelial downgrowth and 5 control specimens for MUC16, cytokeratin AE1/AE3, and cytokeratin 19 using the immunoperoxidase method. The mean percentages of reactive cells on the epithelium and endothelium were compared for each antibody using the Wilcoxon rank sum test. The sensitivity and specificity for each marker were compared.

Results: All 3 antibodies showed high sensitivity (100%) in identifying epithelial downgrowth. However, the specificity was greatest for MUC16 (100%) compared with cytokeratin 19 (80%) and cytokeratin AE1/AE3 (0%). None of the endothelial cells in any case showed reactivity to anti-MUC16 compared with anti–cytokeratin AE1/AE3 (mean [SD], 0.0% [0.0%] vs 17.4% [10.4%]; P = .008). Cytokeratin 19 was positive in every case of epithelial downgrowth but showed focal staining of the endothelium (3.4% of cells) in 1 control.

Conclusions: Antibodies for MUC16, cytokeratin AE1/AE3, and cytokeratin 19 are equally sensitive for downgrowth. However, anti-MUC16 showed superior specificity compared with anti–cytokeratin 19 or anti–cytokeratin AE1/AE3 in this study.


Epithelial downgrowth, the invasion of epithelial cells into the eye, is a potentially devastating complication in the setting of intraocular surgery or trauma. Vision loss may result from corneal decompensation or glaucoma and may require enucleation for the treatment of intractable pain. The incidence of epithelial downgrowth is 0.076% to 0.27% after intraocular surgery.1,2 Early diagnosis and extirpation of epithelial downgrowth is crucial to preservation of vision. However, the diagnosis of epithelial downgrowth can be challenging and is often delayed because the clinical characteristics can mimic those of corneal edema, ocular inflammation, or glaucoma due to other causes. The diagnosis has been made by anterior chamber paracentesis with cytologic analysis, argon laser whitening of the epithelial membrane, confocal microscopy, or histopathologic examination of corneal transplant, evisceration, or enucleation specimens.3

The histopathologic findings of epithelial downgrowth typically consist of 1 to 3 layers of stratified nonkeratinized squamous epithelium extending over the posterior cornea and onto the iris.2 However, light microscopy alone may not be sufficient to establish a diagnosis of epithelial downgrowth due to attenuation of epithelial cells on the posterior corneal surface. In such cases, the epithelial cells may be difficult to distinguish from fibroblasts, retrocorneal membranes, or even endothelial cells.3 This problem is highlighted in epithelial downgrowth after Descemet stripping automated endothelial keratoplasty procedures. A thin layer of epithelium encountered on the failed graft mimics the endothelium (Figure 1). Conversely, the thin Descemet stripping automated endothelial keratoplasty specimens are prone to fold during processing, resulting in tangential sections in which a single endothelial layer may appear as multiple layers. Immunohistochemical analysis using anti–cytokeratin AE1/AE3 has been used in these cases for the diagnosis of epithelial downgrowth.4,5 Anti–cytokeratin AE1/AE3 detects multiple high- and low-molecular-weight cytokeratins and has broad reactivity for the detection of epithelial cells.5,6

In contrast, MUC16, also known as CA125, is expressed at the tips of the mi-
croplicae and only in the superficial epithelium at the corneal surface. MUC16 contributes to formation of the glyco-
calyx, providing a protective surface barrier against penetration by large molecules or pathogens. Previ-
ously, MUC16 was found to be expressed in superficial layers of corneal epithelium even when thinned after exfoliation. This observation suggested robust and conserved expres-
sion of MUC 16 in exposed superficial corneal epithelium. MUC16 could be a specific marker for the super-
ficial epithelium that constitutes epithelial downgrowth.

Herein, immunohistochemical staining for MUC16 and cyto-
keratin 19 is compared with cytokeratin AE1/AE3 for the detection of epithelial downgrowth. In addition, the hypothesis that epithelial downgrowth has a conjunc-
tival origin is interrogated by immunohistochemical reactivity to anti–cytokeratin 19.

METHODS

CASE SELECTION

Five histopathologically confirmed cases of epithelial down-
growth were obtained from 2006 to 2008. The clinical fea-
tures are listed in Table 1. Five control corneal buttons were obtained from randomly selected penetrating keratoplasty speci-
mens from the epithelial and endothelial surfaces of the control corneal specimens. MUC16, cytokeratin AE1/AE3, and cytokeratin 19 were compared.

IMMUNOHISTOCHEMICAL ANALYSIS

Five control and 5 experimental corneal buttons were re-
embedded in the same paraffin block with a positive control specimen of eyelid skin obtained from an exenteration. Immu-
nohistochemical analysis was performed by the peroxidase-
antiperoxidase technique, as previously described. Five-
micrometer sections were deparaffinized in xylene, washed in graded alcohols, incubated for 13 minutes in TRIS-buffered sa-
lune (pH 6.3), blocked with 1% bovine serum albumin, rinsed briefly, and incubated with mouse monoclonal antibodies MUC16 (clone OC125; Cell Marque Corporation, Rocklin, Cali-
ifornia), cytokeratin AE1/AE3 (clone AE1 + AE3; Millipore Corporation, Billerica, Massachusetts), and cytokeratin 19 (clone RCK108; DAKO Inc, Carpinteria, California) diluted 1:50, 1/600, and 1:50, respectively (1 hour at room tempera-
ture). Secondary antibodies (goat antimouse) were obtained from DAKO. Omission of the primary antibody served as the negative controls.

The number of immunoreactive cells and nonimmunoreac-
tive cells were counted using a light microscope at original magnification ×250 and a laboratory counter (Fisher Scientific, Pitts-
burgh, Pennsylvania). All cells along the epithelial and endothelial surfaces were evaluated in control (range, 836-1890 for the epi-
thelium) and epithelial downgrowth (range, 29-167 for the endothelium) and epithelial down-
growth (range, 301-2243 for the epithelial surface and 63-1116 for the endothelial surface) specimens. Immunoreactive cells were defined as intensity of staining greater than the negative controls. The mean percentage of immunoreactive cells from all speci-
mens from the epithelial and endothelial surfaces of the control and epithelial downgrowth groups for MUC16, cytokeratin AE1/ AE3, and cytokeratin 19 were compared.

STATISTICAL ANALYSIS

Data were analyzed for differences in the percentage of immu-
noreactive cells for anti–MUC16 vs anti–cytokeratin AE1/AE3 vs anti–cytokeratin 19 for cells on the epithelial and endothelial surfaces of both control and epithelial downgrowth speci-
mens. The Wilcoxon rank sum test was used for analysis. P < .05 was considered statistically significant. Sensitivity (S) and speci-
ficity (SP) were calculated as: S = TP/TP + FN and SP = TN/ TP + TN, where TP = true positive, TN = true negative, FP = false positive, and FN = false negative cases of epithelial down-
growth.

RESULTS

All control corneal specimens showed immunoreactiv-
ity for MUC16, but limited to the superficial layers of ep-
thelium. Anti–cytokeratin AE1/AE3 reacted with all ep-
thelial layers in 3 of 5 control corneal specimens. The remaining 2 specimens showed reactivity of only the super-
ficial and basal epithelial layers. Quantitative analysis showed a lower percentage of positively stained epithelial cells in controls for MUC16 (mean [SD], 7.8% [10.5%]) compared with cytokeratin AE1/AE3 (mean [SD], 74.4% [61.2%]), which was significant (P = .008) (Figure 2). Anti-MUC16 showed no reactivity to endo-
thelial cells in controls (mean [SD], 0.0% [0.0%]) com-
pared with reactivity in all cases with anti–cytokeratin AE1/AE3 (mean [SD], 17.4% [10.4%]) (P = .008) (Figure 2).

Anti-MUC16 reacted to cells on both the epithelial (mean [SD], 20.0% [8.4%]) and endothelial (mean

Table 1. Clinical Characteristics of Patients With Epithelial Downgrowth

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age, y</th>
<th>Eye Operations/Diagnoses Prior to Diagnosis of ED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/67</td>
<td>ACIOL, PKP, Ahmed valve</td>
</tr>
<tr>
<td>2/M/41</td>
<td>PKP, repeated PKP with corneal melt</td>
</tr>
<tr>
<td>3/M/75</td>
<td>PPV with hemorrhage</td>
</tr>
<tr>
<td>4/F/69</td>
<td>Chronic corneal perforation with conjunctival flap</td>
</tr>
<tr>
<td>5/M/49</td>
<td>RG repair, PPV, choroidal drainage</td>
</tr>
</tbody>
</table>

Abbreviations: ACIOL, anterior chamber intraocular lens; ED, epithelial downgrowth; PKP, penetrating keratoplasty; PPV, pars plana vitrectomy; RG, ruptured globe.
[SD], 19.5% [13.1%]) surfaces of all epithelial downgrowth specimens but again was confined to the superficially exposed cell layers. Anti–cytokeratin AE1/AE3 reacted with cells on both epithelial (mean [SD], 41.2% [37.7%]) and endothelial (mean [SD], 48.6% [42.0%]) surfaces of all epithelial downgrowth specimens. No significant differences were detected in the percentage of anti-MUC16–reactive cells vs cells stained with anti–cytokeratin AE1/AE3 compared on either the epithelial or endothelial surfaces of epithelial downgrowth specimens (Figure 2). Comparison of the sensitivity and specificity of the immunomarkers is shown in Table 2. All markers were sensitive for downgrowth, but MUC16 was more specific than cytokeratin AE1/AE3 and cytokeratin 19 for epithelial downgrowth specimens.

In 2 control specimens, anti–cytokeratin 19 reacted with cells in the superficial and basal epithelium. In 3 specimens, reactivity was confined to occasional cells in the superficial epithelium. The endothelium was positive in 1 specimen (3.4% of cells) for anti–cytokeratin 19. Quantitative analysis showed mean [SD] 3.4% [4.4%] of positively stained cells on the epithelial side and 0.7% [1.5%] of positively stained cells on the endothelial side of the control specimens. In epithelial downgrowth specimens, anti–cytokeratin 19 reacted with mean [SD] 20.0% [15.4%] of cells on the superficial epithelium and 44.1% [43.7%] of cells on the endothelial surface. Anti–cytokeratin 19 reacted to a significantly greater percentage of cells on the endothelial surface of epithelial downgrowth specimens compared with control specimens (P = .02).

In controls, anti–cytokeratin 19 reacted with a lower percentage of epithelial cells (mean [SD], 3.4% [4.4%]) than anti–cytokeratin AE1/AE3 (mean [SD], 74.4% [61.2%]) (P = .008). Also, fewer endothelial cells were stained with anti–cytokeratin 19 in controls (mean [SD], 0.7% [1.5%]) compared with anti–cytokeratin AE1/AE3 (mean [SD], 17.4% [10.4%]) (P = .02) (Figure 2). However, there was no significant difference in the percentage of positively stained cells on either the epithelial or endothelial side between cytokeratin 19 and cytokeratin AE1/AE3 in epithelial downgrowth specimens (Figure 3).

Representative sections of staining on the endothelial side of control corneal and epithelial downgrowth specimens with MUC16, cytokeratin AE1/AE3, and cytokeratin 19 are shown in Figure 4.

**Table 2. Sensitivity and Specificity of MUC16, Cytokeratin AE1/AE3, and Cytokeratin 19 for Epithelial Downgrowth**

<table>
<thead>
<tr>
<th>No. of positive cases/total No. of cells</th>
<th>MUC16</th>
<th>Cytokeratin AE1/AE3</th>
<th>Cytokeratin 19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0/84.8</td>
<td>18.2/99.8</td>
<td>0.2/76.2</td>
</tr>
<tr>
<td>Epithelial downgrowth</td>
<td>66.4/438</td>
<td>147.4/497.6</td>
<td>103.4/336</td>
</tr>
<tr>
<td>Controls</td>
<td>0/5</td>
<td>5/5</td>
<td>1/5</td>
</tr>
<tr>
<td>Epithelial downgrowth</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>100</td>
<td>0</td>
<td>80</td>
</tr>
</tbody>
</table>

aThe mean numbers of cells or cases.

bThe endothelial surface.

**COMMENT**

Anti–cytokeratin AE1/AE3 identifies most acidic and basic cytokeratins of multiple molecular weights and has broad sensitivity for epithelial cells. The antibody is the most commonly used immunohistochemical stain for the identification of epithelial downgrowth. Previous studies promulgated that corneal endothelium might be morphologically similar to the keratin-containing cells in normal cornea. However, later studies have demonstrated that the corneal endothelium does not express keratins. Weinreb and Ryder demonstrated general anticytokeratin staining of normal corneal endothelium using indirect immunofluorescence. These studies postulated that corneal endothelium might be morphologically similar to simple epithelial cells found in other tissues. Shamsuddin et al found that normal corneal endothelium lacked some of the ultrastructural characteristics of vascular endothelium using electron microscopy. They also
demonstrated immunohistochemical reactivity of the endothelium with a number of epithelial markers including general anticytokeratin and proposed that corneal endothelium may not be vascular endothelium. Further studies have found that specific cytokeratins, such as cytokeratin 7, 8, 18, and 19, are expressed in human corneal endothelial cells and that there are differences in the degree of expression of these cytokeratins even in normal corneal specimens.9,18,19 Cytokeratins, such as cytokeratin AE1, AE3, and 7, are also expressed in the endothelium of various ocular diseases, such as posterior polymorphous dystrophy, congenital hereditary endothelial dystrophy, iridocorneal endothelial syndrome, and Fuchs endothelial dystrophy.20-22 Our data indicate that keratoconus can be added to the list.

In this study, we found that while MUC16 and cytokeratin AE1/AE3 were both highly sensitive for detecting epithelial downgrowth, MUC16 was much more specific. Surprisingly, cytokeratin AE1/AE3 appeared to lack specificity; some degree of positive endothelial staining was observed in all control cases. Cytokeratin AE1/AE3 does in fact overlap with cytokeratin classes that are expressed in normal corneal endothelium. Cytokeratin 19 is a 40-kDa cytokeratin that reacts with anti–cytokeratin AE1 and cytokeratin 8 is a 52-kDa cytokeratin that reacts with anti–cytokeratin AE3. Therefore, cytokeratin AE1/AE3 may be a sensitive and robust immunohistochemical stain for detecting epithelial downgrowth; however, it may also lead to false-positive results. Our data did suggest that although the endothelium in the controls showed positive staining for cytokeratin AE1/AE3, there was a higher percentage of staining in the endothelium of epithelial downgrowth specimens. Therefore, a quantitative rather than a qualitative approach to interpretation of anti–cytokeratin AE1/AE3 in the assessment of epithelial downgrowth may mitigate false-positive interpretation.

Cytokeratin 19 has been touted as being specific for epithelial cells of conjunctival origin and has been used to study cicatricial conjunctivitis and limbal stem cell deficiency but, to our knowledge, had not yet been used to study epithelial downgrowth. The source of epithelial cells in epithelial downgrowth, conjunctival vs corneal epithelium, has been debated and both have been suggested as possible sources. In this study, cytokeratin 19 was applied in an attempt to differentiate whether the causative cells responsible for epithelial downgrowth arose from corneal or conjunctival epithelial cells. Cytokeratin 19 was previously reported to stain only conjunctival and not corneal epithelial cells. However, our data show occasional expression in superficial and/or basal epithelial cells in control corneal specimens. Previous studies support our finding that cytokeratin 19 expression may not be limited to conjunctival epithelium. Our limited number of epithelial downgrowth cases all involved either complicated injuries or multiple operations, which may account for the variability in staining and does not clarify the source(s) of the epithelial cells. Cytokeratin 19 was also highly sensitive for epithelial downgrowth, but was less specific than MUC16.

The percentage of immunoreactive cells for MUC16, cytokeratin AE1/AE3, and cytokeratin 19 in control and epithelial downgrowth specimens did vary considerably, which was evident in the large standard deviations observed. An effort was made to minimize variability in staining by embedding all control and epithelial downgrowth specimens in 1 block. There may have been different levels of expression of MUC16, cytokeratin AE1/AE3, and cytokeratin 19 depending on the mechanism of disease or the number or types of previous surgeries. Another limitation of our study is the relatively small sample sizes. The results, however, were sufficient to identify clear qualitative and statistically significant differences between the immunohistochemical stains.

Epithelial downgrowth is a rare, yet serious complication of ocular surgery or trauma. MUC16 seems to be a sensitive and much more specific immunohistochemical stain compared with cytokeratin AE1/AE3 and may assist in the timely and definite diagnosis of epithelial downgrowth.

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REFERENCES


Ophthalmic Images

Unusual Findings After Vitrectomy
Thekla G. Papadaki, MD, PhD
Marco Mura, MD
Mirjam E. J. van Velthoven, MD, PhD
H. Stevie Tan, MD, PhD

Slitlamp biomicroscopy findings 1 day after 25-gauge pars plana vitrectomy for epiretinal membrane in a pseudophakic eye. Triamcinolone acetonide was given intravitreally at the end of the case. Note the triamcinolone crystals staining a vitreous tag attaching to the phacoemulsification incision.