**ABSTRACT**

Introduction: Studies have detected telomerase activity in up to 90% of urothelial carcinomas (UC), and telomerase activity can be detected in urinary tract cytology (UTC) specimens, and indicating an increased risk of UC. hTERT is the catalytic subunit component of the telomerase ribonucleoprotein complex. The majority of UCs have mutations in the TERT promoter. We evaluated the performance of a commercially available antibody on 500 consecutive UTC specimens.

Methods: Unstained Cytospin™ preparations were created from residual urine specimens and were stained using the anti-hTERT Antibody (SCG-A7). Two algorithms were developed for concatenating the hTERT result and cytologic diagnosis: a "no indeterminates algorithm" in which a negative cytology and positive hTERT result is considered positive, and a "high specificity algorithm", in which a negative cytology and positive hTERT result is considered indeterminate (and thus negative for comparison to the gold standard).

Results: 410 specimens were considered to have interpretable hTERT stains; 297 had sufficient follow up data to determine a gold standard diagnosis. The "no indeterminates algorithm" and "high specificity algorithm" yielded a sensitivity of 56.9% and 50.8%, a specificity of 68.5% and 89.2%, a positive predictive value of 56.9% and 33.6%, and a negative predictive value of 86.6% and 85.0%, respectively.

Conclusion: A positive hTERT result may identify a subset of patients with an increased risk of HGUC who may otherwise not be closely followed, while a negative hTERT IHC result is associated with a slight reduction in risk for HGUC.

**MATERIALS AND METHODS**

- Unstained Cytospin preparations created from leftover urine specimens
- Slides stained with anti-hTERT mouse monoclonal antibody (SCG-A7)
- Antibody interpretation by one board-certified cytopathologist (see figure for examples)
- Concurrent cytospin findings, concurrent and subsequent tissue biopsies, and subsequent UTC results considered gold standard for comparison
- Data analyzed with two algorithms, one "high specificity" and one "no indeterminates"

**RESULTS**

- **Urine Cytology**
  - Positive
  - Negative

<table>
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<th>Urine Cytology Result</th>
<th>hTERT ICC Result</th>
<th>Overall Urine Result</th>
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<td>Negative</td>
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<tr>
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<td>Negative (NGAM)</td>
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</tr>
<tr>
<td>Negative</td>
<td>&quot;Atypical&quot; (Negative)</td>
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Table 1. Final test interpretation for comparison to gold standard based on concatenation of urinary cytology specimen clinical diagnosis and hTERT result.

**CONCLUSIONS**

- hTERT antibody staining may help to identify a sub-population of patients with HGUC who could be missed by cytocentric screening alone.
- Negative hTERT staining is associated with a decreased risk of HGUC compared to cytology alone.

Abbreviations: HGUC, high grade urothelial carcinoma; LGUC, low grade urothelial carcinoma; UTC, urinary tract specimen; N/A: negative for urothelial atypia or malignancy; AUC-US, atypical urothelial cells of undetermined significance; AUC-H, atypical urothelial cells, cannot exclude HGUC.

**Figure 1. Examples of hTERT staining.** A. A cluster of three cells (top center) stain positive, note the negative cell at the right side of the field. The corresponding diagnostic specimen was called “Negative for Urothelial Atypia or Malignancy”; the patient was subsequently found to have a low grade urothelial neoplasm. B. A cluster of atypical urothelial cells, the majority of which stain positive. The corresponding diagnostic specimen was called “Atypical Urothelial Cells of Uncertain Significance”; the patient was subsequently found to have high grade urothelial carcinoma. C-H. Additional specimens in which hTERT was interpreted to be positive. I. An example of a specimen containing high grade urothelial carcinoma cells in which hTERT was negative.

**Figure 2. Examples of hTERT interpretation.** A. Numerous malignant cells are seen, more than half demonstrating positive nuclear staining. The cells have recognizable and intact cytoplasm on the cytocentrin and are morphologically compatible with urothelial cells; thus it is appropriate to interpret them as hTERT positive. The presence of cytoplasmic staining in some of these cells does not invalidate the result. B. Two cells (lower left) have nuclear staining and recognizable, intact cytoplasm. The cells are morphologically compatible with urothelial cells; a mature squamous cell (upper right) acts as an internal negative control. C. A cluster of bacteria stain positively by hTERT and approximate the size of a urothelial cell nucleus. The stain was not interpreted as positive if a cell’s cytoplasm was not clearly seen. D-F. Numerous positive-staining bacteria are present in the background and stuck to mature squamous cells. In cases where positive-staining inflammatory cells and/or bacteria obscured the preparation, the test was considered non-interpretable. F. Despite positive-staining bacteria at the bottom of the field, a positive urothelial cell is clearly seen in the center of the field. In such instances, cases could be interpreted as positive.

**Figure 3. Table 1.**

**Figure 4.** Evaluation of Sienna Cancer Diagnostics hTERT Antibody on 500 Consecutive Urinary Tract Specimens

**Figure 5.** Overall performance of cytology plus hTERT immunocytochemistry (ICC) compared to the study’s gold standard. The "high specificity" algorithm considers a benign cytology specimen with a positive hTERT ICC result as indeterminate and thus as an overall negative result when compared to the gold standard.

**Figure 6.** Overall performance of cytology plus hTERT immunocytochemistry (ICC) compared to the study’s gold standard.