Standardized evaluation of tumor-infiltrating lymphocytes (TILs) in colorectal carcinoma for daily clinical and research practice

A tutorial based on the methodology of the International Immuno-Oncology Biomarker Working Group

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Aim of this tutorial

• To provide a guideline to pathologists for the standardized evaluation of tumor-infiltrating lymphocytes based on H&E slides of resected colorectal adenocarcinoma.

• Please consult the manuscript for more specific details.
Assessment of Tumor-infiltrating Lymphocytes Using International TILs Working Group (ITWG) System Is a Strong Predictor of Overall Survival in Colorectal Carcinoma

A Study of 1034 Patients

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Abstract: The presence of increased tumor-infiltrating lymphocytes (TILs) is established as a positive prognostic factor in many malignancies including colorectal carcinoma (CRC). However, multiple different approaches have been used to assess TILs. In 2014, the International TILs Working Group (ITWG) proposed a standardized methodology for evaluating TILs, initially in the context of breast cancer, but subsequently expanded to other malignancies. To date, the efficacy of the ITWG system has not been investigated in a large cohort of all-stage CRC. We, therefore, sought to validate this system in CRC. We used the ITWG system to assess the density of stromal TILs in an unselected cohort of 1034 CRC patients undergoing primary tumor resection at our institution. The percentage TILs' score was categorized into 3 groups: low (0% to 10%), intermediate (15% to 50%), and high (55% to 100%). The mean survival was 53, 67, and 75 months, respectively (P = 0.0001). This survival benefit remained statistically significant in multivariate analyses (P = 0.0001) and subgroup analyses of mismatch repair–proficient CRCs (P = 0.0001), mismatch repair–deficient CRCs (P = 0.031), BRAFV600E-mutant CRCs (P = 0.0001), and BRAF wild-type CRCs (P = 0.001). The predictive value of TILs assessed using the ITWG system was superior to the assessment of intraepithelial lymphocyte performed prospectively using a standard system requiring ≥5 lymphocytes per high-powered field in direct contact with tumor cells or between tumor clusters. We conclude that the ITWG system for assessing TILs is a powerful predictor of all-cause survival in CRC independent of many prognostic factors and superior to the assessment of intraepithelial lymphocytes using a traditional system.

Key Words: tumor-infiltrating lymphocytes (TILs), colorectal carcinoma (CRC), International TILs Working Group (ITWG)

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Assessment of the tumor microenvironment has grown in importance over the past decade, as strong evidence has emerged showing a significant correlation with patient survival in a number of different malignancies. In ad...
Step 1: Define area for TIL evaluation

- Only TILs within the borders of the invasive tumor are evaluated
- The invasive edge is included in the evaluation, but not reported separately
- Immune infiltrates outside of the tumor borders, e.g. tertiary lymphoid structures (TLS) in adjacent normal tissue are not included
Step 1: Define area for TIL evaluation

- Large areas of central necrosis or fibrosis are not included in the evaluation.
Step 2: Focus on stromal TILs

• In the diagnostic setting, only stromal TILs are relevant
**Step 2: Focus on stromal TILs**

- In the diagnostic setting, only stromal TIL are relevant

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*Include only TIL in this area = stromal TIL*

*Do not include TIL in this area*

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*Example 5*
Step 2: Scan tumor at low magnification – focus on the tumor stroma

- Stroma contains predominantly collagenous tissue, few round cells
- Stroma contains predominantly round cell infiltrate, collagenous tissue difficult to recognize

Example 6

Example 7
Step 2: Scan tumor at low magnification – focus on the tumor stroma

- Stroma contains predominantly collagenous tissue, few round cells
- Stroma contains predominantly round cell infiltrate, collagenous tissue difficult to recognize
Step 3: Determine type of inflammatory infiltrate

- Include only mononuclear infiltrate (lymphocytes & plasma cells)
- Do not include granulocytic infiltrate in areas of tumor necrosis

Example 12
Step 3: Determine type of inflammatory infiltrate

- Include only mononuclear infiltrate (lymphocytes & plasma cells)
- Do not include granulocytic infiltrate in areas of tumor necrosis

Example 13

Example 14
Step 4: As a first approach, include tumor in one of three groups based on low magnification and assess % stromal TILs (continue with Step 5 for percentage)

<table>
<thead>
<tr>
<th>Group A: tumor with no/minimal immune cells</th>
<th>Group B: tumor with intermediate / heterogeneous infiltrate</th>
<th>Group C: tumor with high immune infiltrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10% stromal TILs</td>
<td>10-40% stromal TILs</td>
<td>40-90% stromal TILs</td>
</tr>
</tbody>
</table>

For this intermediate group evaluate different areas at higher magnification.

Example 15

Example 16
Step 5: Report percentage of stromal lymphocytes

- Report the average of the stromal area, do not focus on hot spots.
- For intermediate group evaluate different areas at higher magnification.
- Please note that lymphocytes do not form solid aggregates, therefore even with 90-100% stromal TILs there will still be some space between the individual lymphocytes.
Please send any questions or comments to:

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