ACHIEVING REPRODUCIBLE MATURATION STAGING OF TERTIARY LYMPHOID STRUCTURES: FROM IMAGING MASS CYTOMETRY DATA TO PATHOLOGY APPLICATIONS

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Résumé

Background: In cases of persistent inflammation, the migration and positioning of immune cells follow the organogenesis of secondary lymphoid organs (SLO) within the organs themselves, leading to the formation of tertiary lymphoid structures (TLS). These structures have become increasingly important as diagnostic and prognostic markers in chronic diseases and cancers. However, there is ongoing debate regarding how to assess the maturation of TLS in tissue samples, both in terms of determining the stages themselves and achieving agreement among pathologists.
Objective: Based on the composition and organization of TLS revealed through imaging mass cytometry (IMC), we aimed to propose a reproducible classification system consisting of three maturation stages for TLS, which can be applied in pathology practice.

Methods: We utilized formalin-fixed and paraffin-embedded gastric samples (45 tissues with 134 TLS) and colonic samples (50 tissues with 159 TLS), representing inflammatory, cancerous, and control conditions. These samples were subjected to IMC analysis using a panel of 39 markers. The IMC images were segmented into individual cells and analyzed using QuPath software. These data gained insights into the expression and localization of multiple immune markers at a single-cell resolution within TLS. Additionally, we sought correlations between markers that reflect the maturation of TLS in terms of cell composition and architecture. The two markers from the IMC panel that exhibited the strongest correlation with TLS maturation were selected to transfer the TLS maturation staging from IMC to dual-staining immunohistochemistry (IHC). This transfer was done to investigate the level of agreement among pathologists when classifying TLS using a set of 60 tissue IHC images.

Results: Through the use of IMC, we were able to comprehensively characterize the maturation of TLS and propose three distinct stages: 1) Early TLS, 2) Primary Follicle-Like TLS, and 3) Secondary Follicle-Like TLS. These stages were identified as IHC CD21-CD23-, IHC

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CD21+CD23-, and IHC CD21+CD23+, respectively. The analysis of the 60 dual CD21-CD23 IHC images by three pathologists showed a high level of inter-pathologist agreement in classifying TLS into the three maturation stages (Cohen's Kappa values: > 0.8).
Conclusions: With a strong correlation to cellular and architectural changes during TLS maturation, the dual CD21-CD23 IHC marker allows for the staging of TLS in tissue samples with a high level of inter-pathologist agreement. This dual-marker TLS staging holds potential for prognostic and predictive purposes.

Mots-Clés: Tertiary lymphoid structure, Imaging mass cytometry, Digestive tract, Immunohistochemistry, Oncology, Immunology