the diagnostic pathology journal DIAGNOSTIC PATHOLOGY

13th European Congress on Digital Pathology Proceedings, diagnostic pathology 2016, 8:144
ISSN 2364-4893
DOI: http://dx.doi.org/10.17629/www.diagnosticpathology.eu-2016-8:144

Proceedings

SY14.02 | **Image Analysis III**

A GRAPH-BASED DIGITAL PATHOLOGY APPROACH TO DESCRIBE LYMPHOCYTE CLUSTERING PATTERNS AFTER RENAL TRANSPLANTATION

N.S. Schaadt*[1], A. Uvarovskii[2], M. Meyer-Hermann[2, 3], R. Schönmeyer[4], N. Brieu[4], J.H. Bräsen[1], W. Gwinner[5], F. Feuerhake[1]

[1]Institute for Pathology, Hannover Medical School, Hannover, Germany, [2]Helmholtz Centre for Infection Research, Systems Immunology and Integrated Centre of Systems Biology, Braunschweig, Germany, [3]Institute for Biochemistry, Biotechnology and Bioinformatics, TU Braunschweig, Braunschweig, Germany, [4]Definiens AG, Munich, Germany, [5]Clinic for Nephrology, Hannover Medical School, Hannover, Germany

Introduction/ Background

Renal transplantation (rTx) induces an adaptive immune response against foreign donor antigens mediated by lymphocytes of the recipient. Local accumulation of B- and T-cells is an important component of this response enabling and controlling immune cell interactions [1]. Combining digital microscopic images with network analysis [2,3] opens new perspectives to study the spatial dimension of lymphocyte clustering and to model their potential interactions.

Aims

The aim of this study is to characterize the range of B- and T-lymphocytic infiltrates below the threshold of rejection defined by the Banff classification [4,5] and to propose a mathematical description of immune cell clustering for use in systems medicine approaches.

Methods

We established a workflow to comprehensively characterize lymphocyte clusters and compare their morphological features with organized structures such as secondary or tertiary lymphoid organs (TLO/SLO) [6]. 51 renal protocol and indication biopsies from 13 patients without evidence for severe rejection over 10 years were stained by CD3/CD20 duplex immunohistochemistry. Whole slide images (WSIs) were acquired to automatically detect biologically relevant regions of interest (ROIs) by means of density maps for lymphocytes (image analysis workflow illustrated in <Figure 1a>). They are generated from single nuclei identification using an au- to-adaptive random forest pixelwise classifier ("nucleus container" module [7], Definiens, Germany). We implemented a graph-based tool in Java using individual cell coordinates to identify cell compartments <Figure 1b> and applied it to each selected ROI. For this, a neighborhood graph is built by Delaunay triangulation and Euclidean distances. This analysis allows describing their specific clustering behavior based on features as described in [8]. The convex hull of the neighborhood graph allows a visualization of B- and T-cell compartments.

Results

We identified B-cell rich compartments in about 55% of 150 ROIs in kidney tissue after successful transplantation (examples in <Figure 2>). The B-cell compartments in rTx tended towards smaller overall size with on average about 90 cells in a B-cell cluster compared to more than 600 B-cells observed in mature TLOs and SLOs and they showed less prominent spatial organization (average degree on average 3.92 instead of 4.97; degree shows generally Poisson distribution as illustrated in <Figure 3A>). Further, the graph analysis confirmed lower B-cell density (Fig. 3B displays the exponential character of the spatial B-cell distribution in a selected ROI), a different ratio between T- and B-cell compartments, and more frequent overlap between both regions than in mature lymphoid structures.

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We conclude that the graph-based approach is feasible to distinguish relevant immune cell patterns in rTx and provides a useful mathematical description of neighborhood relationships between immune cells and their spatial organization. The workflow has the potential to improve throughput and robustness of immune cell evaluation for use in translational science.

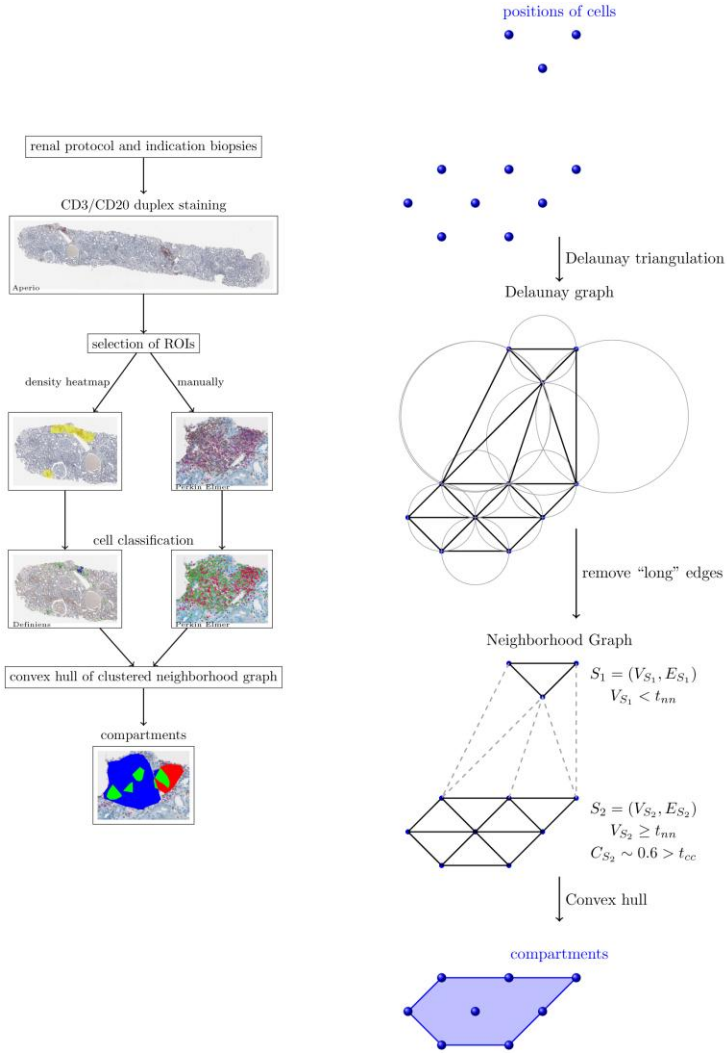

Figure 1.

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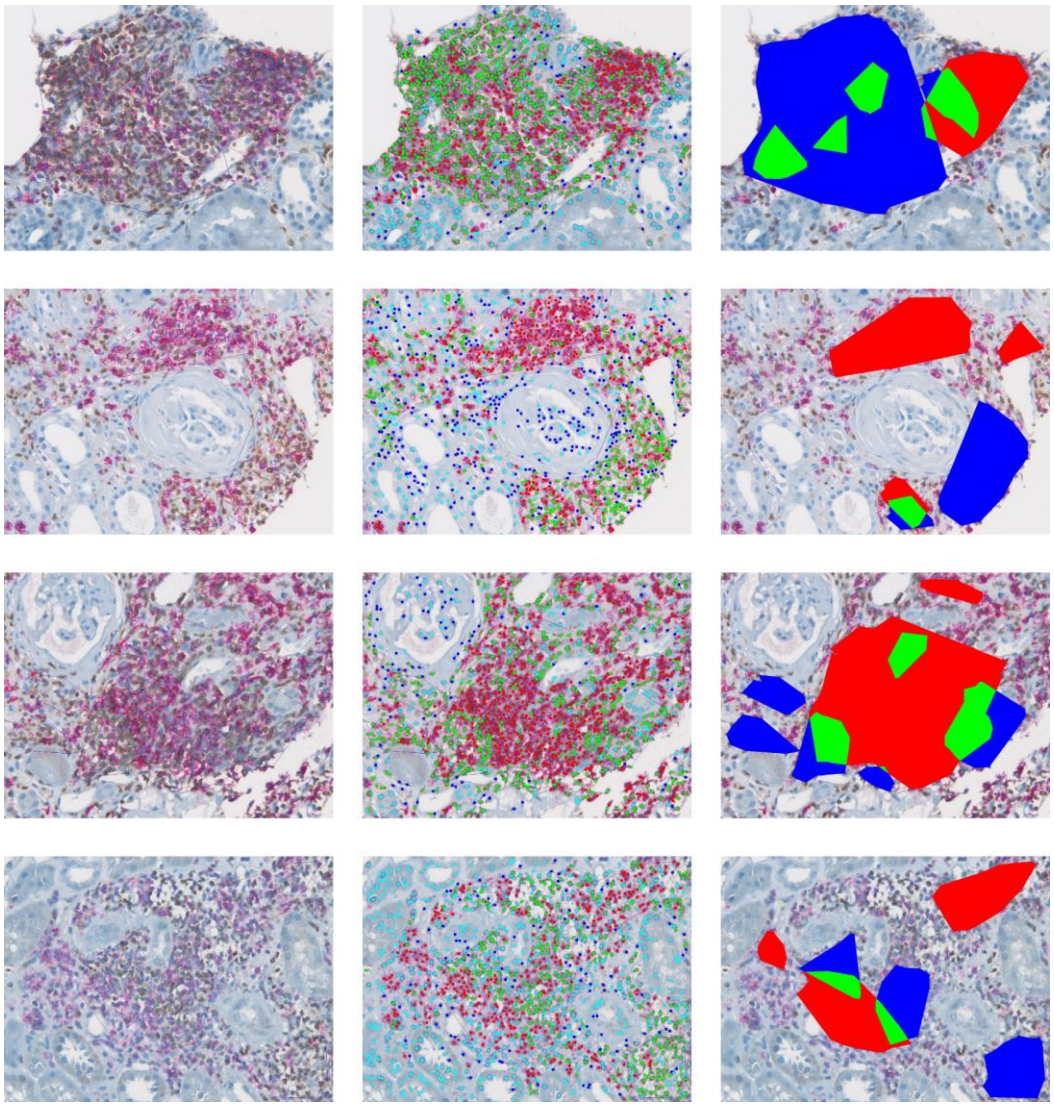

Figure 2.

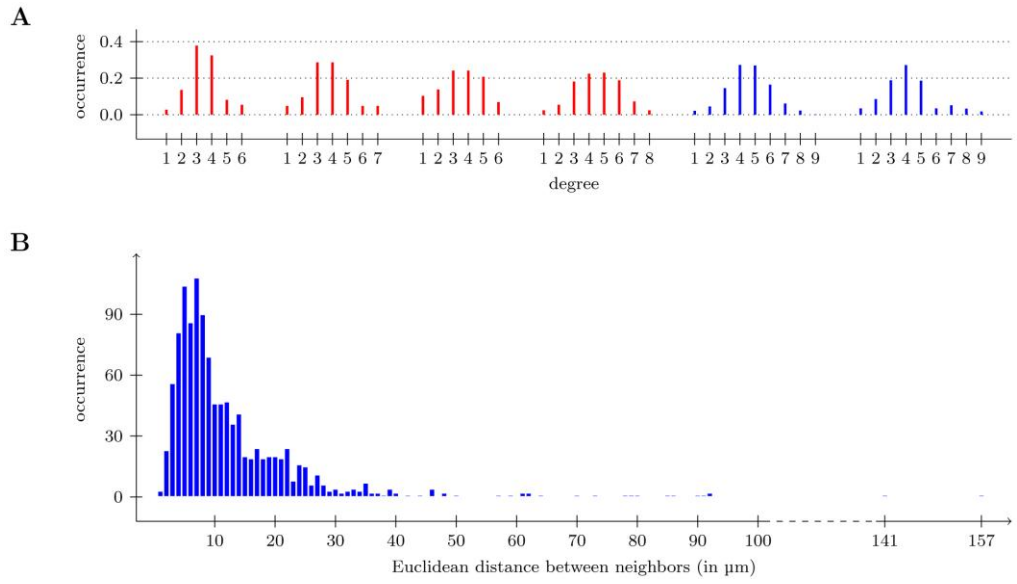

Figure 3.

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