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TissueFAXS analysis as a novel method for semiquantitative assessment of intrarenal lymphocytes infiltration

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Objectives: Intrarenal infiltration of immune cells is part of a pathogenic mechanism in various inflammatory kidney diseases. Quantification of intrarenal lymphocytes using flow cytometry analysis of kidney mononuclear cells (KMNCs) has been a precise and useful method in a murine model of ischemic acute kidney injury. However, the amount of renal tissue required for flow cytometry analysis is often difficult to obtain by conventional kidney biopsy. We investigated the diagnostic potential of tissueFAXS as a tool for quantifying intrarenal lymphocytes infiltration.

Methods: A total of 20 cynomolgus monkeys with various renal function were included. Kidney samples for flow cytometry analysis of KMNCs and immunohistochemistry were collected simultaneously. Flow cytometry analysis of KMNCs was performed using FACSverse. Immunohistochemistry of CD3, CD20, and CD45 on formalin-fixed kidney tissues were performed and followed by tissueFAXS analysis. The proportion of lymphocytes positive for each CD marker was calculated using different denominators as follows: kidney mononuclear cells isolated with percoll in flow cytometry vs. all nucleated cells stained with hematoxylin in tissueFAXS. Linear regression analysis was performed to assess the association of CD3, CD20, and CD45 (+) lymphocytes evaluated with two different methods.

Results: Flow cytometry and tissueFAXS analyses showed positive correlation with the proportion of CD45 (+) lymphocytes ($R^2 = 0.5530$, $P < 0.01$). There was a positive correlation with the proportion of CD3 (+) T cells ($R^2 = 0.4584$, $P < 0.01$) and CD20 (+) B cells ($R^2 = 0.2374$, $P < 0.05$) between two methods.

Conclusions: Our study showed significant positive correlation between the proportion of intrarenal CD3, CD20, and CD45 (+) lymphocytes measured with flow cytometry and tissueFAXS in cynomolgus monkeys. These results suggest that immunohistochemistry followed by tissueFAXS analysis can be a useful tool for semiquantitative evaluation of intrarenal lymphocytes infiltration.