Short Communication

An image analysis-based approach for the evaluation of glomerulonephritis in a lupus model

Didier Merciris

Merciris D. An image analysis-based approach for the evaluation of glomerulonephritis in a lupus model. J Histol. Histopathol 2021;5(1):1-4.

Systemic lupus erythematosus (SLE) is a typical systemic autoimmune disease which can affect multiple organ systems, especially kidney. Patients can display severe and progressive kidney damage, namely glomerulonephritis (GN), leading to morbidity and mortality. The search of new medicines against SLE requires pertinent animal model reproducing the pathology and robust readouts for GN evaluation. Until now, in animal lupus models pathologists used to evaluate GN by different scoring systems on a limited number of glomeruli per kidney section. Moreover, the scoring process is time consuming, providing data liable to reproducibility and subjectivity possible issues. The aim of the current study was to propose a digital pathology approach for glomerulonephritis quantification that was able to discard human interpretation while improving the workload.

Key Words:

Glomerulonephritis GN, Whole slide imaging, Immunofluorescence, machine learning algorithm

INTRODUCTION

SLE, a disease with unknown etiology [1], is an autoimmune disease that can affect any organs of the body [2]. SLE is mainly due to deposits of immune complexes in various organs, which trigger mediators of inflammation [3]. Symptoms and severity of the disease varies from patient to patient but in one third of cases [4], severe kidney inflammation leading progressively to severe glomerulonephritis is observed [5]. GN is characterized by enlarged and hypercellular glomeruli with extended mesangial matrix and peritubular leucocyte infiltration [6]. Evaluation of GN severity in animal models is usually done by scoring systems that are time consuming, tricky to perform while offering poorly discriminative data [7]. To circumvent this, we have chosen a digital approach that was able to generate accurate morphometric data in a faster and more reliable manner [8].

METHODS

In 8-9 week old Balb/c female mice (Janvier Labs, France) were housed in a dedicated in house animal facility under specific pathogen-free conditions according to the Federation for Laboratory Animal Science Associations guidelines. The study was performed according to ethical guidelines approved by the Animal Institutional Care and Use Committee of Galapagos controlled by the French Authorities. Animals were housed in filter top cages, provided ad libitum with filtered tap water and standard chow and were maintained humidity on a 12h light/dark cycle [9].

The skin of right ears was treated topically, 3 times weekly with 1.25mg Imiquimod IMQ or with Vaseline officinale cream. For systemic dosing, IMQ-treated mice received prophylatic treatment of Mycophenolate mofetil (USP, USA) at 100mg/kg diluted in 0.5% methylcellulose (VWR, USA) by oral gavage for 11 weeks.

HISTOLOGY PROCESS

At sacrifices, kidneys were collected, fixed with neutral buffered formalin 4% and embedded in paraffin according the sagittal plane. For histopathological evaluation, 5µm thick kidney sections were stained with periodic acid-Schiff (PAS) and scored in a blinded. Glomerular lesions were scored semi-quantitatively on a scale from 0 to 2 for mesangial proliferation, endocapillary proliferation, mesangial matrix expansion, and segmental sclerosis. Then, for each sample, all scores were summed up giving one total score (score 0 to 8). For each mouse, the glomerular lesion was scored with 50 glomeruli per kidney.

IMMUNOFLUORESCENCE STAININGS

Kidney paraffin sections were double immunostained either with anti-podocin (Sigma-Aldrich, USA) and anti-collagen type (Southern Biotech, USA) or with anti-podocin and Ki67 (ebioscience, USA), followed by incubation with alexa fluor 488 or 594 conjugated secondary antibody. Autofluorescence was reduced by incubating sections in 1% Sudan black in 70% ethanol. Slides were mounted with Vectashield with DAPI (Vector, USA). For podocin/collagen 4 staining, podocin was in green and collagen 4 was in red. For podocin/Ki67 staining, podocin was in red and Ki67 was in green.

RESULTS

GN EVALUATION POST PAS STAINING

In spite of these good correlations, glomeruli detection in PAS stained slides was not considered optimal because sustained manual correction was required after the first segmentation provided by Tissue recognition algorithm [10]. As these data were encouraging and in order to speed up the imaging process, we decided to improve the process with immunofluorescent staining allowing a more accurate and discriminant approach.

GN EVALUATION POST DOUBLE IMMUNOSTAINING

The immunofluorescence approach was done through 2 double immunostainings: podocin+collagen 4 and podocin+Ki67. Podocin is a protein expressed by podocytes which are the major actors of the glomerular blood filtration barrier [11]. Podocytes, as revealed with podocin immunostaining, were arranged all around the glomeruli by marrying their global shape. Podocin immunostaining detected with Tissue recognition

Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad, India

***Correspondence to:** *Didier Merciris, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad, India, E-mail: didier.merciris@glpg.com*

Received: February 04, 2021; **Accepted:** February 18, 2021; **Published:** February 25, 2021

algorithm provided an accurate glomerular recognition that required almost no manual correction [12]. The quantification of collagen type 4 extent and Ki67+ cells was done with the same algorithm.

CENTRALISE

During the setting of lupus, a glomerular inflammation (i.e. glomerulonephritis GN) is induced and constitutes the main mandatory parameter for the evaluation of the disease. In the current study, a mouse model of lupus was assayed displaying a clear and pertinent GN induction. In the first attention, GN was evaluated by a scoring method using kidney slides post PAS or HE staining. This manual method was unfortunately very time consuming whilst being tricky to perform as gathering multiple parameters. Moreover, the scoring approach was offering poorly discriminant data for the evaluation of experimental groups.

CONCLUSION

The current study is proposing a fast and easy process for an automated quantification of GN based on the combination of immunofluorescence staining and a machine learning algorithm. This method could be useful for the selection of future therapeutics for SLE and also towards kidney diseases in a general way.

LIST OF ABBREVIATIONS

GN: Glomerulonephritis; IMQ: Imiquimod; MMF: mycophenolate mofetil; MOA: mode of action; MRL: Murphy Roths large; PAS: periodic acid-Schiff; ROA: region of interest

ACKNOWLEDGEMENTS

We thank the in vivo pharmacology and Histology department facility of Galapagos (Romainville, France).

AVAILABILITY OF DATA AND MATERIALS

Data generated or analyzed in this study are included in this article and associated supplementary information files and raw data can be obtained from the corresponding author.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

All authors are Galapagos employees.

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