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A quantitative analysis of the expression of α-smooth muscle actin in mesangioproliferative (GnMes) glomerulonephritis

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The aim of the study was to quantitatively assess the expression of α-SMA in the renal interstitium in GnMes cases. Two image analysis approaches (in grey scale and in spatial colour images) were used to determine correctly the presence of the reaction. In spatial evaluation, the mean area fraction of α-SMA in the renal interstitium was 3.63% $\pm$ 1.29%, while the results obtained from traditional quantitative method based on grey scale images were biased and too low.

key words: α-SMA, mesangioproliferative glomerulonephritis, immunohistochemistry, interstitial fibrosis

INTRODUCTION

In the course of mesangioproliferative glomerulonephritis (GnMes) overproduction of matrix and glomerulosclerosis occur. Additionally, tubular interstitial fibrosis may be found and there is proliferation of fibroblasts/miofibroblasts [2]. Macrophages of inflammatory infiltrates and tubular epithelial cells are the sources of miofibroblasts [6, 7]. In all these cells, smooth muscle actin is expressed. Because the extent of tubulointerstitial fibrosis is an important prognostic factor, we decided to evaluate quantitatively the α-SMA expression in the renal interstitium.

MATERIAL AND METHODS

This study used renal biopsy specimens obtained from patients with GnMes. The creatinine ranged from 68 to 114 μmol/l while the proteinuria varied from 3.2 to 6.8 g/day in the cases under investigation. Immunohistochemical staining for α-SMA was performed using monoclonal antibodies α-SMA Ab-1; clone 1A4 (Dako, Glostrup, Denmark). Part of the material was stained using Masson's trichrome to assess a degree of fibrosis in the renal interstitium.

Expression of α-SMA for each of the biopsy specimens was evaluated by a computer- assisted light microscope Olympus CX40 coupled with a Sony CCD--IRIS camera. Colour images of 768 $\times$ 576 pixels (24 bits per pixel) were acquired with the FAST.FORWARD program (Fast Forward Video Inc.). On average, 70 images per specimen were acquired. To extract and measure area fraction for α-SMA, colour images were first converted to grey scale and enhanced by using the mean filter of radius 1.5. The areas with α-SMA present were segmented by thresholding (Fig. 1) and measured using the "ImageJ" program (http://rsb.info.nih.gov/ij). The area fraction of α-SMA was determined as a percentage of the positive reaction in the renal interstitium. Using the "Analyzer 4D" program [5], planar colour images were extended to three-dimensional images, in which the intensity of colour of α-SMA was the third dimension (Fig. 2). In this way, we were able to extract the reac-

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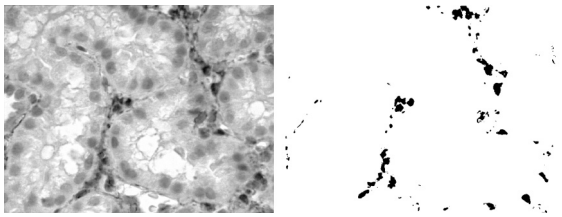

Figure 1. Expression of α-SMA in the renal interstitium (left) and its segmentation (right).

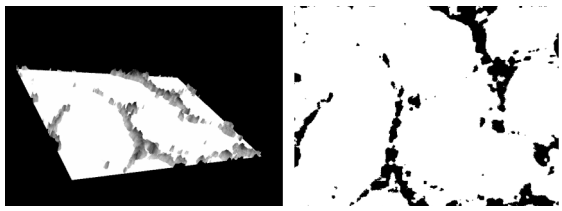

Figure 2. Spatial visualisation of α-SMA (left) and its binary planar image (right) as an analogue to the binary image in Figure 1.

tion with arbitrary intensity of its colour. For quantitative evaluation of α-SMA, spatial images in the HSB colour system were linearly converted to 256 colors, where pixels in red and yellow tones corresponded with α-SMA in raw images. These pixels were counted to assess the area fraction for α-SMA.

In Masson's trichrome stained material, colour images were first smoothed by the mean filter and then interstitial fibrosis was extracted by an interactive sampling of pixels in blue tones. Analogously, the normal part of the interstitium was extracted in red tones (Fig. 3). The ratio of selected pixels in blue tones and pixels in red tones expressed a degree of fibrosis.

RESULTS AND DISCUSSION

The staining intensities of α-SMA within the renal interstitium were relatively low and clustered.

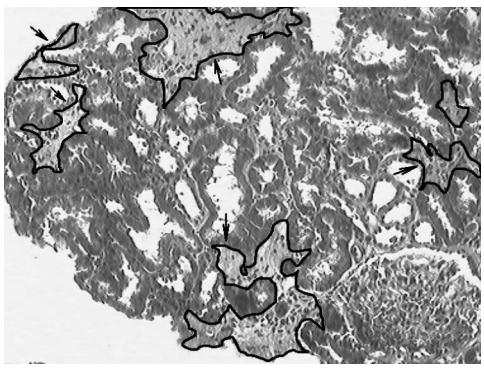

Figure 3. The renal interstitium in Masson's trichrome staining. The outlined fibrosis areas are indicated by arrows.

This caused difficulties in the segmentation of α-SMA expression in images converted to grey tones. The average area fraction of α-SMA detected in grey scale images was 1.11% ± 0.33% and ranged from 0.65% to 4.78%. These results seemed too low for GnMes cases. Therefore, we decided to assess the area fraction for α-SMA in the same raw images (without conversion to grey scale) by using the "Analyzer 4D" program. The results obtained were higher in colour images than in the same images converted to grey scale. The mean area fraction calculated from colour images was 3.63% ± 1.29% and ranged from 2.44% to 5.00%.

α-SMA can be present in very small amounts in the interstitium without fibrosis, while it can be detected, for instance, in the interstitium of fibrotic regions of allograft kidneys [1]. Hence, we expected an expression of α-SMA within the renal interstitium in GnMes cases. It should be mentioned that our results depended on the section thickness (3–5 μm in our study) and the quality of the immunostaining. Consequently, we also evaluated the renal interstitium in Masson's trichrome stained material. The mean degree of interstitial fibrosis was 30.96% ± ± 13.13% and ranged from 18.85 to 52.82%. In our studies, the area of normal architectural blue network surrounding the glomeruli and tubules increased the results in comparison with previous reports [3, 4], where such effects were manually removed before computation.

To conclude, the evaluation of α-SMA seems more stable and convenient for automatic image analysis than the evaluation of fibrosis in Masson's trichrome stained specimens.

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