α -SM actin expression as prognostic indicator in IgA nephropathy (Berger's Disease)¹

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Abstract. Recent studies have demonstrated that α -Smooth Muscle actin expression in glomerular and tubulointerstitial compartments of renal tissue could represent a prognostic marker in several renal diseases. Our objective was to identify the prognostic value of α -SM actin expression on the evolution of renal damage in Primary IgA nephropathy (Berger's Disease). 43 patients followed up from 1988 to 1999 at the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil, was studied. Clinical-laboratory data were obtained from the medical records of the patients using a protocol containing name, race, gender, origin, profession, age at clinical presentation of the disease and personal and family history. The parameters assessed in the approach to IgA nephropathy were serum creatinine, creatinine clearance, serum albumin, total serum protein, 24 hours proteinuria, glycaemia, serum sodium, potassium, calcium and phosphorus ions, analysis of urinary sediment, serum complement profile, blood count, and renal biopsy. Morphological evaluation was performed by renal biopsy using common light and immunofluorescence microscopy. Immunohistochemical studies were performed using a murine monoclonal antibody to α -SM actin. Our data showed that α -SM actin expression in the glomerular and tubulointerstitial compartments are not correlated with unfavorable clinical course of primary IgA nephropathy.

Keywords: Berger's disease, primary IgA nephropathy, glomerulonephritis, hematuria, α -SM actin, prognostic factors, chronic renal progression

1. Introduction

Since its original description in 1968 by Jean Berger and Nicole Hinglais [4], nephropathy due to intercapillary IgA deposits, also known as Berger's Disease (BD), has been extensively studied in terms of clinical, pathological and immunological aspects and today is recognized as the most common primary glomerulopathy worldwide [12]. In general, 3.7% of BD patients develop long-lasting spontaneous clinical remis-

sion [10] indicating that, as is the case for other "primary" glomerulonephritis, the initial disorder of BD can revert spontaneously even after a long disease course. On this basis, the possible factors involved in its prognosis have been extensively studied. With respect to the clinical parameters, frequently recurring macroscopic hematuria, the absence of persistent proteinuria, the absence of hypertension, and age of less than 16 years at the onset of the disease favor a better prognosis for the patient [13,14,24]. With respect to the microscopic parameters, the lower the extent of the lesion in the glomerular [8], tubulointerstitial [5] and vascular [27] compartments and the presence of IgA restricted to the mesangium without reaching the capillary loops [16] the better the prognosis.

The accumulation of extracellular matrix (ECM) leading to glomerulosclerosis and interstitial fibrosis is

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a frequent finding in the course of many clinical and experimental renal diseases [15,22]. Renal fibrosis (along with glomerulosclerosis) is the final common pathway of a relatively uniform response of the kidney to sustained inflammation independent of its origin [29]. The ultimate anatomical feature of glomerular sclerosis is the accumulation of extracellular matrix in the glomerulus and collapse of capillary lumina [28,29]. Tubulointerstitial scarring is characterized by tubular atrophy and an accumulation of interstitial matrix, which may emanate from fibroblasts or tubular cells themselves and occurs with any number of different primary diseases, whether immune, metabolic or degenerative [7, 28,29]. Recent studies have demonstrated that progressive glomerulonephritis is associated with phenotypic changes in glomerular and interstitial cells. α -Smooth Muscle actin (α -SM actin) is expressed normally by vascular smooth muscle cells. In pathological conditions leading to fibrosis including renal scarring α -SM actin is also expressed by stromal fibroblastic and mesangial cells [1,31].

In various types of glomerular injury, the mesangial cells acquire the characteristics of myofibroblasts, which may in fact be injurious to the glomerulus. Like myofibroblasts in other tissues, the mesangial cell acquires smooth muscle cell-like properties, characterized by *de novo* expression of α -SM actin, and the development of fibroblast-like properties characterized by the production of interstitial collagens in addition to normal mesangial matrix constituents [6,19].

The objective of the present study was to identify if the over expression of α -SM actin in the glomerular and tubulointerstitial areas can predict unfavorable renal clinical course in patients with primary IgA nephropathy (Berger's Disease).

2. Materials and methods

2.1. Sample

Forty-three patients with a diagnosis of BD confirmed by renal biopsy seen at the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil, between January 1988 and December 1999 were selected, and represent the population of the present study. Important to say that in our service no renal biopsy is indicated for patients with microscopic hematuria without proteinuria or with asymptomatic proteinuria. On this basis, no real "benign" cases of BD will be present in our series.

2.2. Clinical-laboratory evaluation

Clinical-laboratory data were obtained from the medical records of the patients using a protocol containing name, race, gender, origin, profession, age at clinical presentation of the disease and personal and family history.

The parameters assessed in the approach to BD were serum creatinine, creatinine clearance, serum albumin, total serum protein, 24 hours proteinuria, glycaemia, measurement of serum sodium, potassium, calcium and phosphorus ions, analysis of urinary sediment, serum complement profile, blood count, and renal biopsy.

The clinical presentation of BD [9] was considered to be the first manifestation of the disease as: (I) nephrotic syndrome when proteinuria was more than 3 g/day, total serum protein less than 6 g/dL and serum albumin less than 3 g/dL, or in the presence of a well characterized history of anasarca; (II) non-nephrotic proteinuria when proteinuria was higher than 200 mg/dL and lower than 3 g/dL discovered during clinical evaluation not related to nephrology; (III) macroscopic haematuria was defined as the report of a well characterized history of urine darkness; microscopic haematuria was defined as the presence of three or more red blood cells per large magnification microscopic field in at least two examinations of urinary sediment at any time during the course of the disease; (IV) nephritic syndrome was defined as arterial hypertension, plus microscopic haematuria, plus azotemia (serum creatinine more than 1.5 mg/dL); and (V) arterial hypertension was defined as blood pressure more than 140/90 mmHg in three measurements made at different times.

The following evolutionary forms of BD were considered during the clinical course of the disease [9]: (I) clinical remission (CR): proteinuria below 200 mg/day with normal renal function; (II) clinical improvement (CI): regression of nephrotic or nephritic syndrome or reduced proteinuria with normal renal function; (III) unchanged (UN): clinical and/or laboratory picture without significant modification during the course of the disease; (IV) clinical worsening (CW): aggravation of nephrotic or nephritic syndrome or increased proteinuria with normal renal function; and (V) chronic renal insufficiency (CRI): serum creatinine of 1.5 mg/dL or more and/or creatinine clearance of less than 80 mL/min per 1.73 m², or the need for dialysis or renal transplant (end stage renal disease – ESRD). We considered CR and CI to indicate a favorable clinical course and CW plus CRI (including ESRD) to indicate an unfavorable clinical course. All

patients with BD were treated receiving corticosteroids (methyl-prednisolone or prednisone) combined or not with an immunosuppressive drug (cyclophosphamide), according to different protocols.

2.3. Morphological evaluation

Morphological evaluation was performed by renal biopsy using common light microscopy and immunofluorescence microscopy [9]. Tissue obtained by renal biopsy from 43 patients with BD and control sections from preserved renal areas of kidney from nephrectomized patients were fixed in Bouin's solution, embedded in paraffin, cut into 4- μ m-thick sections, and stained with hematoxylin and eosin (HE), Masson trichrome and methenamine silver (PAMS). For immunofluorescence microscopy frozen sections were incubated with anti-IgA, anti-IgG, anti-IgM, anti-C3, anti-C1q, anti-kappa, anti-lambda, and anti-fibrinogen conjugated sera.

2.4. Immunohistochemical studies

The immunohistochemical studies were performed using a murine monoclonal antibody to an NH₂-terminal synthetic form of α -SM actin (DAKO Corporation, Glostrup, Denmark). Four- μ m sections of biopsy tissue embedded in paraffin were processed by the indirect immunoperoxidase technique as previously described [3]. Sections were incubated overnight at 4°C with 1:1000 α -SM actin monoclonal antibody. The avidin-biotin-peroxidase complex procedure (Vectastain, Vector Laboratories, Burlingame, CA, USA) was employed as the detection system. Chromogen development was performed with 3,3'-diaminobenzidine (DAB) (Sigma Chemical Co., St Louis, Mo, USA), and the material was counterstained with methyl green, dehydrated, and mounted. Negative controls consisted of the omission of primary antibody in the reaction and sections obtained from normal kidneys. Glomerular expression of α -SM actin was graded semi-quantitatively according to the scale of Alpers et al. [1]: 0, no staining; 1, trace mesangial staining; 2, weak segmental mesangial staining, usually involving a small minority of the glomeruli present; 3, strong segmental mesangial staining, usually involving a majority of the glomeruli present; and 4, strong diffuse mesangial staining, usually involving all glomeruli present. The α -SM actin immunoreaction in the tubulointerstitium of the renal cortex was scored as follows: 0, absent staining; +, weak staining with focal distribution; ++, moderate

Table 1

Clinical course of 41 BD patients as a function of α -SM actin score in glomeruli

Clinical course	α -SM actin score					Total
	0	1	2	3	4	
CI	2	2	2	3	0	9
CW	1	5	2	1	0	9
CRI (ESRD)	1(3)	1(4)	8(5)	1(0)	0(0)	23
Total	7	12	17	5	0	41

Legend: BD, Berger's Disease; CI, clinical improvement, CW, clinical worsening; CRI, renal chronic insufficiency; ESRD, end stage renal disease.

Fisher exact test, $p = 1.000$, comparing favorable clinical course (CI) to unfavorable clinical course (CW plus CRI) versus 0 plus 1, and 2 plus 3 plus 4, for α -SM actin scores.

Table 2

Clinical course of 41 BD patients as a function of α -SM actin score in tubulointerstitial area

Clinical course	α -SM actin score					Total
	0	+	++	+++	++++	
CI	4	1	1	2	1	9
CW	1	5	3	0	0	9
CRI(ESRD)	1(3)	2(3)	6(3)	2(1)	1(1)	23
Total	9	11	13	5	3	41

Legend : BD, Berger's Disease; CI, clinical improvement, CW, clinical worsening; CRI, renal chronic insufficiency; ESRD, end stage renal disease.

Fisher exact test, $p = 0.719$, comparing favorable clinical course (CI) to unfavorable clinical course (CW plus CRI) versus low α -SM actin score (0 plus +), and high α -SM actin score (++ plus +++ plus ++++) as high α -SM actin score.

staining with focal distribution; + + +, strong staining with focal distribution or weak and diffuse; and + + + +, strong and diffuse. We considered 0 plus (+) as low α -SM actin score, and (++) plus (++) plus (++) as high α -SM actin score.

2.5. Statistical analysis

For statistical analysis, an electronic data bank was elaborated using the Microsoft Excel® software and the information was analyzed with the Sigma Stat® software, version 2.0. Values were expressed as mean $\pm$ standard error. Proportions were determined using the Fisher's exact test. Significance difference was accepted if $p < 0.05$.

3. Results

Forty-three patients were studied, 26 men (60.4%), 17 women (39.6%) and the mean age of onset of BD was 23.6 ± 11.4 years. Forty-one patients were white

Fig. 1. α -SM actin expression in renal cortex. A: control positivity in arterial wall ($\times 200$); B : Berger's Disease, glomerular score 1 ($\times 800$); C : Berger's Disease, glomerular score 2 ($\times 800$); D : Berger's Disease, glomerular score 3 ($\times 800$).

(95.3%), one were black and one asian. Median follow-up time was 53.3 ± 40.3 months. Histological examination showed the following pictures: 2 patients with mesangiopathic histological picture, where the glomeruli are essentially normal at light microscopy, (4.6%), 4 patients with diffuse mesangial hypercellularity (9.3%), 35 patients with focal and segmental glomerulonephritis (81.3%), 24 without and 11 with focal cellular crescents. There were 2 patients without glomeruli under common light microscopy. The clinical course of BD was distributed as follows: 9 patients with CI, 9 patients with CW, and 25 patients with CRI, 12 of which with ESRD. There were no patients with CR or UN clinical course.

α -SM actin expression in glomeruli and tubulointerstitial area are showed in Tables 1 and 2 and Figs 1 and 2. Table 1 shows the relationship between histological scores for α -SM actin expression in glomeruli, and clinical course of 41 BD patients. Comparing favorable clinical course (CI) to unfavorable clinical course (CW plus CRI) versus 0 plus 1, and 2 plus 3 plus 4, for α -SM actin scores, no significant correlation was observed ($p = 1.000$).

Table 2 shows the relationship between histological scores for α -SM actin expression in the tubulointerstitial area, and clinical course of 41 BD patients. Comparing favorable clinical course (CI) to unfavorable clinical course (CW plus CRI) versus low α -SM actin score (0 plus +), and high α -SM actin score (++ plus +++ plus ++++), no significant correlation was observed ($p = 0.719$).

4. Discussion

BD, first described in 1968 by Jean Berger & Nicole Hinglais, is the most common primary glomerulopathy in various countries [18]. Its most frequent clinical manifestation is recurrent macroscopic hematuria, with or without proteinuria. Its predominant histological manifestation is focal and segmental glomerulonephritis. However, both the clinical picture and the histological findings involve an enormous gamut of variation.

According to the literature, BD has a benign course, especially among children, with most patients surviving without renal failure for 15 to 20 years after the onset of the disease, and with only a few cases progressing to chronic renal insufficiency [18]. Five to 15% of the patients develop terminal chronic renal failure within 5 years, 10 to 20% within 10 years, 15 to 30% within 15 years, and 50% within 20 years after the onset of BD [21]. In the present study, 79.0% of patients developed a progressive reduction of renal function, with 73.5% developing chronic renal failure, 48% of them terminal, during a mean follow-up period of 53.3 ± 50.3 months. This rate is much higher than those reported in the literature due to the fact that in our service no renal biopsy is indicated for patients with microscopic hematuria without proteinuria or with asymptomatic proteinuria. On this basis, no real "benign" cases of BD were present in our series. According to Berger, Yaneva & Crosnier [26], the systematic examination of urine would lead to the detection of a larger number of BD cases than currently reported in the literature.

Fig. 2. α -SM actin expression in renal cortex. A: Berger's Disease, tubulointerstitial score + ($\times 200$); B: Berger's Disease, tubulointerstitial score ++ ($\times 800$); C: Berger's Disease, tubulointerstitial score +++ ($\times 200$); D: Berger's Disease, tubulointerstitial score ++++ ($\times 200$).

Alpers et al [1] showed that glomerular expression of α -SM actin was correlated with proliferating cell nuclear antigen (PCNA) in several types of human glomerulonephritis. Thus expression of α -SM actin has been assumed to reflect the activation and proliferation of mesangial cells and the consequent increase in collagen content. These investigators, in a study of 9 patients with proliferative diffuse lupus nephritis, showed that the glomerular score of α -SM actin expression was 0 in no case, 1 in 1 case, 2 in one case, 3 in 4 cases, and 4 in 3 cases. Mesangial and interstitial myofibroblasts represent a key step in renal fibrogenesis, have been associated with a poor prognosis in experimental and clinical glomerulonephritis and are the strongest predictors of progressive renal insufficiency [2,28,29, 31].

Proliferation of mesangial cells, which acquire the characteristics of smooth muscle cells with a consequent increase in the expression of α -SM actin, has been reported in experimental models of rat glomerulonephritis induced with anti-Thy antibodies, Habu snake venom, and concanavalin/anti-concanavalin antibodies [1,19]. Alpers et al. [1], studied the glomerular compartment of 113 renal biopsies from patients with different types of glomerulonephritis using two monoclonal antibodies to specific muscle actin: HHF35 ("a pool of muscle actins") and α -SM actin (smooth muscle actin) and observed a high correlation between the increased expression of α -SM actin and cell proliferation rate. The authors concluded that most proliferative glomerular diseases are associated with an increased

expression of muscle actins, especially smooth muscle actin, which would then represent a useful prognostic marker of glomerular damage.

Similarly, Mise et al. [23] studied the glomerular expression of nonmuscle-type myosin heavy chain (SMemb) and α -SM actin in 45 patients with BD. Only mesangial expression of SMemb showed a significant correlation with mesangial matrix accumulation and this expression was elevated in the patients with poor renal prognosis. In contrast, the expression of α -SM actin showed no significant correlation with renal prognosis.

In the present study, 79.0% of the 19 patients exhibiting a score of 0 and 1 in the glomerular compartment showed an unfavorable course, and 77.3% of the 22 patients exhibiting scores of 2, 3 and 4 had an unfavorable course, with the difference being nonsignificant ($p = 1.00$). Our data showed that there was no relationship between the glomerular scores and prognosis, and they agree with those reported by Mise et al. [23] but disagree with those reported by Alpers et al. [1] and those by Utsunomiya et al. [30]. These authors suggest that macrophages recruited into the mesangium may induce phenotypic modulation of mesangial cells and that mesangial α -SM actin expression predicts a progressive decline in renal function in patients with BD.

Boukhalfa et al. [6] studied the expression of α -SM actin in the glomerular and tubulointerstitial compartment of 51 renal biopsies from patients with various forms of nephropathies and observed that during

glomerular disease the expression of α -SM actin is not correlated with the degree of endo- and extracapillary proliferation or with the presence of focal glomerular necrosis. The increased expression of α -SM actin was almost always observed in the periglomerular and/or peritubular interstitium, suggesting extraglomerular activation of smooth muscle cells and/or fibroblasts during glomerular disease. The authors thus concluded that there is a positive correlation between interstitial fibrosis and α -SM actin expression, suggesting that the activated fibroblasts are involved in interstitial fibrosis. On the other hand, they found no correlation between the intensity of glomerular damage and the expression of α -SM actin. Similarly, Ranieri et al. [25] showed that the expression of PDGF- β and α -SM actin is increased in patients with BD who have moderate or severe renal tubulointerstitial lesions compared to patients with discrete lesions.

In our series, 75.0% of the 20 patients with an interstitial score of 0 and + showed an unfavorable course as compared to 81.0% of the 21 patients with scores of ++, +++ and +++, with no significant difference between them. Our data showed that there was no relationship between the tubulointerstitial scores and prognosis. They disagree with those reported by Boukhalfa et al [6] and Ranieri et al [25]. On the other hand, they agree with those of Utsumiya et al [30] who found no significant difference between the score of interstitial α -SM actin expression and the decline of renal function in patients with BD.

Our data show that the score for α -SM actin in the glomerular and tubulointerstitial compartments is not correlated with the clinical course of primary IgA nephropathy. In the same way, recent studies in our laboratory (86 patients with lupus nephritis) showed that unfavorable clinical outcome of lupus nephritis was correlated with World Health Organization (WHO) class IV compared to the other classes, and with the chronicity index in WHO class III patients, but not with the score of α -SM actin expression in glomeruli or tubulointerstitial area [11]. Curiously, when the focus is not the clinical course but the degree of disease activity, Kaneko et al. studying 90 needle-biopsy specimens showed that glomerular α -SM actin expression was mostly correlated with histological grading and reflects the histological activity of BD [20].

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