

Glomerular disease and hepatitis C. Fifteen years' experience

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ABSTRACT

Introduction: Renal injury secondary to hepatitis C virus (HCV) infection was initially observed in autopsy studies and later in kidney biopsies. This study reviews the glomerular lesions found in patients with HCV infection at a single centre. It then compares the clinical and histological features of the most frequent lesion, membranoproliferative glomerulonephritis (MPGN), with those found in patients without HCV infection.

Patients and Methods: We performed a retrospective study, reviewing all native kidney biopsies of patients with positive HCV serology performed in the Pathology Department of the Complejo Hospitalario Universitario, A Coruña, Spain, between January 1995 and December 2009.

Results: Over a 15-year period, 1043 native kidney biopsies were evaluated. HCV infection was present in 31 cases and was associated with three types of glomerular lesions: MPGN (n=12), IgA nephropathy (n=2) and membranous nephropathy (n=2). Clinical and histological features of the MPGN cases (n=12) were compared with those in cases of MPGN in patients without HCV infection (n=12). Renal failure (75% of the cases in both groups) and nephrotic syndrome (75% in the HCV group vs. 91.7% in the non-HCV group; $p=0.273$) were the main clinical manifestations. The histological features were also similar in both groups: lobular pattern (33.3% vs. 58.3%; $p=0.219$), cellular crescents (41.7% vs. 25.0%; $p=0.386$), hyaline thrombi (41.7% vs. 25.0%;

$p=0.386$), exudation (33.3% vs. 41.7%; $p=0.673$) and arteriolar hyalinosis (16.7% vs. 8.3%; $p=0.537$). The immunofluorescence pattern was also similar. The histological findings in cryoglobulinaemic and non-cryoglobulinaemic cases were compared. Cellular crescents were present only in non-cryoglobulinaemic MPGN (62.5% vs. 0%; $p=0.038$).

Conclusions: The most frequent glomerular lesion associated with HCV infection was MPGN. We found no significant differences in the clinical and histological presentations of MPGN in patients with and without HCV infection; there were also no significant differences between cryoglobulinaemic and non-cryoglobulinaemic cases, except that cellular crescents were found only in the non-cryoglobulinaemic cases.

Key-Words:

Glomerular disease; hepatitis C virus infection; membranoproliferative glomerulonephritis.

INTRODUCTION

Hepatitis C virus (HCV) infects more than 170 million people worldwide and is responsible for over 1 million deaths from cirrhosis and primary liver cancers¹. Renal disease is one of the extrahepatic manifestations of HCV infection. The association of renal and hepatic disease in HCV infection was initially observed in autopsy studies and subsequently in kidney biopsies.

Many types of glomerular disease have been reported in association with HCV infection, including membranoproliferative glomerulonephritis (MPGN), membranous nephropathy, IgA nephropathy, focal and segmental glomerulosclerosis, fibrillary and immunotactoid glomerulopathy, thrombotic microangiopathies and postinfectious glomerulonephritis^{2,3}.

The kidney injury can be caused by immunological and nonimmunological mechanisms. The immune injury may be mediated by cryoglobulins, immune-complex and amyloid deposition, binding of HCV to cell surface receptors such as CD81 and SR-B1 in the renal parenchyma with direct injury, and increased expression of toll-like receptors (TLR3) preferentially in mesangial cells leading to induction of an immune response².

Nonimmunological mechanisms include the intrarenal production of IGF-1 and TGF- β caused by the insulin resistance and hyperinsulinaemia that also is responsible for the higher prevalence of diabetes in HCV infection⁴. The excess IGF-1 and TGF- β upregulates the expression of angiotensin II type 1 receptors in mesangial cells leading to excess local production of endothelin-1 and reduced synthesis of nitric oxide⁵.

HCV infection also has other important implications for nephrology. Haemodialysis patients are at potential risk of blood-borne infections related to blood transfusions and the risks inherent in haemodialysis treatment. The prevalence of HCV infection is decreasing in many dialysis units in developed countries, but still ranges from 3% to 20% in Western Europe⁶⁻⁹. This falling prevalence is mainly due to the use of erythropoiesis stimulating agents, which has reduced the need for blood transfusions, and to routine serologic screening for HCV infection. A detrimental effect of HCV on dialysis and transplant patients has been shown in many studies¹⁰, with HCV infection being associated with higher mortality in the dialysis population⁹ and with lower patient and graft survival in renal transplant patients¹¹. However, this negative impact after renal transplantation is not a consistent finding¹². HCV infection has also been implicated in the pathogenesis of acute or chronic glomerulopathy in the allograft (recurrent or *de novo*), a higher rate of chronic allograft nephropathy^{13,14} and new-onset diabetes after transplantation (NODAT)¹⁵. These serious consequences represent an indication for continued investigation of

the pathophysiological mechanisms and implications of HCV infection in the kidney.

This study reviews the glomerular lesions in HCV-positive patients at a single centre and compares clinical and histological features of MPGN in HCV-positive and negative patients.

PATIENTS AND METHODS

We performed a retrospective study, reviewing all native biopsies of patients with positive HCV serology (n=31) performed in the Pathology Department of the Complejo Hospitalario Universitario, A Coruña, Spain, between January 1995 and December 2009. The results were analysed and demographic (age, gender), clinical (presence of renal failure, nephrotic syndrome, hypertension, microscopic haematuria, nephritic syndrome, purpura, other viral co-infections), laboratory (serum creatinine, proteinuria, cryoglobulins, hypocomplementaemia) and histological features (at light microscopy and immunofluorescence) of the patients with MPGN were evaluated. The results were then compared with the characteristics of patients with the same glomerular disease but without HCV infection (n=12). Quantitative variables were compared using Student t-test and categorical variables using χ^2 .

RESULTS

Over the 15 year period, 1617 kidney biopsies (1043 native, 574 allograft biopsies) were evaluated in our pathology department. Of the native renal biopsies, 31 were from patients known to have HCV infection. A review of these 31 renal biopsies showed three types of glomerular lesions: MPGN (12 cases; 37.5% of all MPGN cases), IgA nephropathy (2 cases; 1.8% of all IgA nephropathy cases) and membranous nephropathy (2 cases; 1.7% of all membranous nephropathy cases). Both patients with IgA nephropathy had a history of chronic liver disease. The demographic, clinical, laboratory and histological features of the patients with HCV-associated MPGN (12/31) were compared with the group of patients with non-HCV-associated MPGN (20/32). We were not able to review the samples in 8 cases of non-HCV-associated

Table I

Demographic, clinical, laboratorial and histological features in HCV and non-HCV associated MPGN

Demographic features			
	HCV (n=12)	Non-HCV (n=12)	P
Age (yr), mean±SD [min-max]	49±16 [28-77]	44±22 [15-79]	0.483
Male gender (n)	9 (75.0%)	8 (66.7%)	0.653
Clinical features			
Renal failure* (n)	9 (75%)	9 (75%)	
Nephrotic syndrome (n)	9 (75%)	11 (91.7%)	0.273
Hypertension (n)	8 (66.7%)	7 (58.3%)	0.674
Microscopic haematuria (n)	8 (66.7%)	7 (58.3%)	0.674
Nephritic syndrome (n)	2 (16.7%)	0 (0%)	0.139
Purpura (n)	2 (16.7%)	0 (0%)	0.139
HBV/HIV infection (n)	3/1	1/1	
Laboratory features			
Creatinine (mg/dl), mean±SD	1.8±0.9	3.1±3.6	0.231
Proteinuria (g/day), mean±SD	5.6±3.0	8.9±4.9	0.106
Positive cryoglobulins (n)	4 (33.3%)	0 (0%)	0.028
Hypocomplementaemia (n)	8 (66.7%)	5 (41.7%)	0.219
Histological features			
Lobular pattern	4 (33.3%)	7 (58.3%)	0.219
Cellular crescents	5 (41.7%)	3 (25.0%)	0.386
Hyaline thrombi	5 (41.7%)	3 (25.0%)	0.386
Fibrinoid necrosis	0 (0%)	0 (0%)	
Exudation	4 (33.3%)	5 (41.7%)	0.673
Arteriolar hyalinosis	2 (16.7%)	1 (8.3%)	0.537

* Acute (defined as a rise in serum creatinine ≥ 1.5 fold from the reference value) or chronic (defined as decreased glomerular filtration rate of less than 60 mL/min/1.73 m² for 3 or more months).

HBV: Hepatitis B virus; HIV: Human immunodeficiency virus

MPGN because of poor preservation, so the comparison was made with the remaining 12 patients (Table I).

The mean age was similar in both groups (49±16 years in the HCV group and 44±22 years in the non-HCV group), and there was a predominance of males (75% and 66.7%). Acute or chronic renal failure, (present in 75% of patients in both groups) and nephrotic syndrome (75% in the HCV-group vs. 91.7% in the non-HCV group) were the main clinical features at disease presentation. Plasma creatinine (PCr) and proteinuria (Pr) were not significantly different in HCV vs. non-HCV patients (PCr: 1.8±0.9 mg/dl vs. 3.1±3.6 mg/dl, $p=0.231$; Pr: 5.6±3.0 g/day vs. 8.9±4.9 g/day, $p=0.106$). Prevalence of hypocomplementaemia was also similar (66.7% vs. 41.7%, $p=0.219$). Cryoglobulinaemia was present in 33.3% of the HCV group, but in none of the non-HCV cases ($p=0.028$).

The main histological features of MPGN were similar in HCV and non-HCV cases (Table I and Fig. 1): lobular

pattern (33.3% vs. 58.3%; $p=0.219$), cellular crescents (41.7% vs. 25.0%; $p=0.386$), hyaline thrombi (41.7% vs. 25.0%; $p=0.386$), exudation (33.3% vs. 41.7%; $p=0.673$) and arteriolar hyalinosis (16.7% vs. 8.3%; $p=0.537$). The histological findings in cryoglobulinaemic and non-cryoglobulinaemic HCV-associated MPGN were also compared (Table II). The presence of a lobular pattern (25.0% vs. 37.5%; $p=0.665$), hyaline thrombi (50% vs. 37.5%; $p=0.679$), exudation (50% vs. 25%; $p=0.386$) and arteriolar hyalinosis (25% vs. 12.5%; $p=0.584$) were not different between cryoglobulinaemic and non-cryoglobulinaemic HCV-associated MPGN. However, cellular crescents were only present in the non-cryoglobulinaemic cases (62.5% vs. 0%; $p=0.038$).

Immunofluorescence for IgA, IgG, IgM, C₃, C₄, C_{1q}, albumin, fibrinogen, kappa and lambda was performed in all biopsies. The most frequent deposits were of IgM, C₃, IgG and IgA. Granular glomerular deposits of IgM and C₃ were found in all cases. IgG was found in 58.3% of HCV-associated MPGN and

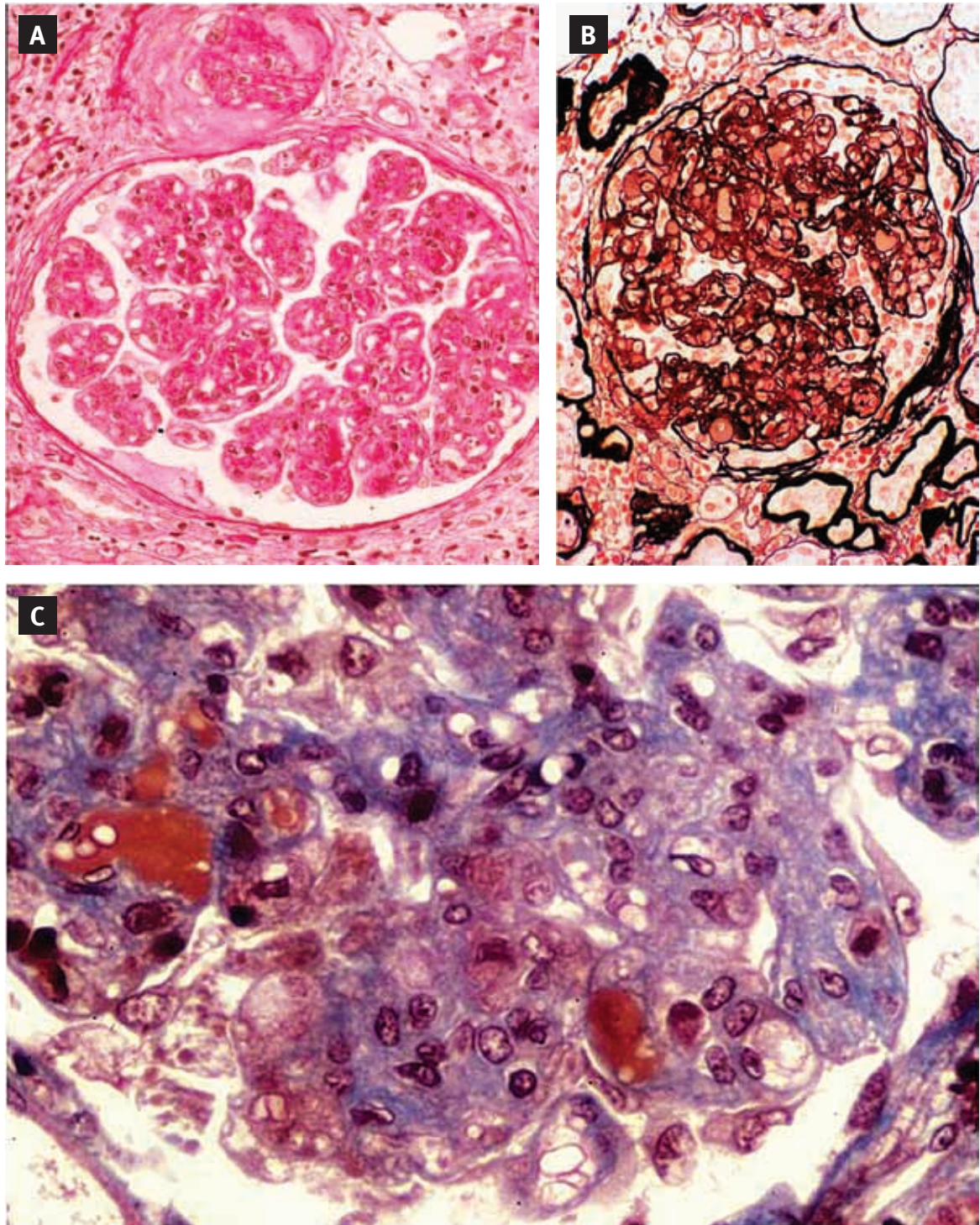


Figure 1

Membranoproliferative glomerulonephritis.

a) lobular pattern and double contours of the GBM (PAS stain $\times 400$); **b)** double contours of the GBM resembling "tram tracks" Silver stain $\times 400$; **c)** lobular pattern and multiple thrombi (Masson Trichrome stain $\times 600$).

Table II

Histological characteristics of cryoglobulinaemic and non-cryoglobulinaemic HCV-associated MPGN

Cryoglobulinaemic (4/12)			Non-cryoglobulinaemic (8/12)			p
	n	%		N	%	
Lobular pattern	1	25	Lobular pattern	3	37.5	0.665
Cellular crescents	0	0	Cellular crescents	5	62.5	0.038
Hyaline thrombi	2	50	Hyaline thrombi	3	37.5	0.679
Fibrinoid necrosis	0	0	Fibrinoid necrosis	0	0	
Exudation	2	50	Exudation	2	25	0.386
Arteriolar hyalinosis	1	25	Arteriolar hyalinosis	1	12.5	0.584

66.7% of non-HCV MPGN ($p=0.673$). IgA was found in 41.7% of the cases in both groups.

DISCUSSION

In our review, the glomerular lesions associated with HCV infection were MPGN (12 cases), IgA nephropathy (2 cases) and membranous nephropathy (2 cases). The prevalence of HCV infection was 37.5% of all patients with MPGN, 1.8% in IgA nephropathy and 1.7% in membranous nephropathy.

MPGN is the most frequently found glomerulopathy in all autopsy and kidney biopsy studies in HCV patients, with or without associated cryoglobulinaemia^{3,16}. The prevalence of HCV infection in MPGN varies between 15% and 60% in different series and rises to 96% when cryoglobulinaemia is also present³. Cryoglobulins are proteins that become insoluble at low temperatures, usually below 4° C. They are associated with HCV infection in more than 90% of cases, with non-HCV cases being mainly related to paraneoplastic phenomena and connective tissue diseases¹⁷.

The expansion of HCV-reactive B-cells leads to antibody production, including cryoprecipitating antibodies¹⁸. Immuno-complex deposition in the glomerular capillary walls produces complement activation and release of proinflammatory cytokines, resulting in an MPGN, usually type I. Other types of immuno-complex mediated glomerular disease can occur, not mediated by cryoglobulins. The pattern of glomerular injury will depend on the physico-chemical characteristics of the immuno-complexes such as their size and charge, their rate of production and factors such as

phagocytic clearance and quantity of complexes reaching the kidney. The deposits may be preferentially mesangial (mesangioproliferative GN), subendothelial (membranoproliferative GN) or subepithelial (membranous GN). The secondary form of membranous nephropathy related to HCV-infection is more often associated with atypical features, such as glomerular hypercellularity and/or mesangial and subendothelial immuno-complexes, than the idiopathic disease.

In our patients with HCV-associated MPGN, renal failure and nephrotic syndrome were the main clinical features (75%), followed by hypertension and microscopic haematuria (66.7%). Nephritic syndrome and purpura each occurred in 16.7% of the cases. The clinical presentation was not different from that of patients without HCV infection, with the exception of cryoglobulinaemia, and although non-HCV patients had higher levels of plasma creatinine and proteinuria, these differences were not statistically significantly. Histological features at light microscopy were also very similar. Most of the patients in the non-HCV group probably had idiopathic MPGN, with the exception of two patients with Hepatitis B and human immunodeficiency virus (HIV) infection. Our results support the view that idiopathic and secondary MPGN are very difficult to distinguish at light microscopy.

Cryoglobulinaemia was present in 33.3% of all HCV-positive patients. Johnson *et al.*¹⁹ found similar results in a group of 35 patients with MPGN, with 31.4% having cryoglobulinaemia. The sensitivity of the detection methods and the sample handling before laboratory examination remain major problems in cryoglobulin detection, and the prevalence could actually be much higher. Histologically, there are some features that are more common in cryoglobulinaemic MPGN than in other aetiologies. The presence of hyaline thrombi in capillary loops (in almost 72% of the cases) with less endocapillary proliferation and massive leukocyte infiltration (especially monocytes), the existence of small and medium vessels fibrinoid necrosis and inflammatory cells (5-30%), even in the absence of obvious glomerular damage, are some of the distinguishing characteristics²⁰. In our review, none of these histological characteristics on light microscopy was significantly different, although cellular crescents were only present in non-cryoglobulinaemic MPGN (37.5%), probably reflecting more aggressive glomerular lesions in those cases.

On electron microscopy, the presence of tubular units (62-63 nm) in the subendothelial space (and the mesangium) are almost pathognomonic of cryoglobulinaemia. However, as we did not perform electron microscopy, we can only speculate that even more cases of cryoglobulinaemia might have been discovered had we employed it. The other glomerulopathies associated with HCV infection in our study were IgA nephropathy and membranous nephropathy. Because of the small number of patients, these glomerular lesions are not extensively described. The cases of IgA nephropathy were probably secondary to chronic hepatic disease and not directly related with the HCV infection. Fabrizi *et al.*²¹ reviewed the association of HCV infection and glomerulonephritis and found that 6% of patients with IgA nephropathy were HCV-positive, a much higher percentage than in our study. However, the number of patients with chronic hepatic disease was not reported. Regarding membranous nephropathy, our prevalence of HCV infection was lower than in other series that reported prevalences between 5.49% and 8.3%³. The possibility of a chance association of HCV infection and IgA nephropathy or membranous nephropathy must be taken into consideration.

CONCLUSIONS

The glomerular lesion most frequently associated with HCV infection was MPGN. There were no significant differences in the clinical and histological presentations of MPGN in patients with or without HCV infection, apart from the frequency of cryoglobulinaemia (present in 33.3% of HCV-positive patients with and none of the HCV-negative patients). The only significant difference in presenting features between HCV-positive patients with or without cryoglobulinaemia, was that cellular crescents were found only in those without cryoglobulinaemia.

Conflict of interest statement. None declared.

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