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ORIGINAL ARTICLE

A retrospective analysis of treatment outcomes in patients with hepatitis C related systemic vasculitis receiving intravenous methylprednisolone and cyclophosphamide

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Abstract The aim of this work is to describe the outcome of a series of patients with hepatitis C virus (HCV)-related vasculitis who were treated with corticosteroids and I.V. cyclophosphamide without receiving any antiviral therapy. The data of 16 patients with HCV infection and vasculitis were retrospectively analyzed for the treatment outcome in the present study. Eleven patients were females (68.8%) with a mean age of 49.6±10.0 years. Nine patients (56.2%) had medium-sized vessel vasculitis (group A) and seven patients (43.8%) had small vessel vasculitis (group B). Disease activity was assessed using the Birmingham Vasculitis Activity Score (BVAS 2003) and organ damage was assessed by the Vasculitis Damage Index (VDI). HCV infection was confirmed in all patients by the detection of antibodies to HCV in serum by ELISA and HCV RNA using qualitative PCR. Quantitative PCR was done using the branched DNA technique. None of our study patients had received antiviral therapy, but they all received I.V.-pulsed cyclophosphamide monthly for 6 months, then every 3 months for six times if needed, preceded by I.V. methylprednisolone. Twelve patients (75%) had undetectable viral load by the quantitative technique. The drop in mean BVAS recorded at different intervals was highly significant. Although there was a drop in the VDI mean between the first and second reading, it was not statistically significant. All patients responded to treatment. Seven patients (43.8%) had relapse. Two patients died (12.5%). One patient died from renal failure (group B) and another died from sepsis (group A). The treatment outcomes were not statistically significant between the two vasculitis groups. A subset of patients with HCV-related vasculitis and with low levels of viremia can be safely treated with corticosteroids and cyclophosphamide alone. Despite successful treatment, a significant proportion of patients relapse and some develop severe complications and death.

Keywords Corticosteroids · Cyclophosphamide · HCV vasculitis · Medium size vessel vasculitis · Small size vessel vasculitis

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Introduction

Hepatitis C virus (HCV) infects an estimated 170 to 200 million people worldwide, making this disease a clear and significant health issue [1]. In Egypt, the epidemiological situation differs from western countries. HCV prevalence is very high, estimated to be 10% to 20% in urban and rural areas, respectively [2].

Almost 50% to 60% of individuals with acute infection develop chronic hepatitis that can lead to cirrhosis and hepatocellular carcinoma [3]. Numerous extrahepatic manifestations have been associated with HCV, including mixed cryoglobulinemia, glomerulonephritis, porphyria cutanea tarda, and sicca syndrome [4].

Mixed cryoglobulinemia (MC) is a systemic vasculitis characterized by the proliferation of B cell clones producing pathogenic IgM with rheumatoid factor (RF) activity. The pathological hallmark of MC is a leukocytoclastic vasculitis, involving small and medium-sized vessels responsible for cutaneous and visceral organ involvement [5].

Data regarding the optimal therapeutic regimen of HCV-related vasculitis remains controversial. There is debate whether the treatment should be targeted at the viral trigger as the use of interferon-α (IFN-α) and/or the downstream pathogenic events by means of less specific approaches such as corticosteroids, immunosuppressives, or plasmapheresis [6].

Treatment of HCV-related vasculitis with IFN-α monotherapy is associated with a relatively poor response and high relapse rate especially in severe cases. A virologic response, that is, negative or significant decrease in serum HCV RNA level, has been reported in 15–60% of patients receiving 2–3 million IU, three times weekly for 6–12 months. Combination therapy with IFN-α plus ribavirin enhanced efficacy on the main HCV-related vasculitic manifestations. A clinical response was noted in all the patients with skin involvement but in only half the patients with nervous or renal involvement [7]. Clinical response is correlated with virologic response and generally requires a prolonged period of antiviral therapy (18–24 months) to obtain efficacy and avoid vasculitis relapse [8].

The use of polyethylene glycol-conjugated (pegylated) IFN- α-2b and ribavirin can achieve clinical response in most patients with HCV-mixed cryoglobulinemic vasculitis. This clinical response correlated with the eradication of HCV and a shorter duration (14 months) of treatment [5, 9]. There have been cases reported where complete clinical responders had viral clearance long after clinical remission while others remained in clinical remission despite the persistent viremia [10, 11]. There have been reports of discordance between viral response and cryoglobulinemia as well as between vasculitis and cryoglobulinemia [12, 13]. There is a possibility that treatment with interferon therapy results in cryoglobulinemia [14]. It has been reported that some of the manifestations of HCV-related vasculitis may worsen with IFN therapy as peripheral neuropathy and skin ulcers [15–17].

Italians have reported on the efficacy of anti-CD20 monoclonal antibody (rituximab) treatment in patients with HCV cryoglobulinemic vasculitis resistant or intolerant to IFN-α monotherapy. This uses monoclonal antibodies directed to CD20 antigen, a transmembrane protein expressed on pre-B lymphocytes and mature B lymphocytes. Rituximab proved effective on skin vasculitis, peripheral neuropathy, arthralgia, and low-grade B cell lymphoma. Most clinical responders had a decrease in serum cryoglobulins and an increase in serum C4 levels; however, there were concerns regarding the propensity of rituximab to worsen HCV viremia which may lead to more severe HCV-induced lesions and/or cryoglobulinemic relapses in subsequent years [18].

Vasculitis relapses usually present with the same vasculitis manifestations that were noted at presentation. These clinical relapses are usually associated with relapsing HCV viremia [19]. Some of the relapses of HCV-associated mixed cryoglobulinemic vasculitis, however, have been reported to occur despite repeated negative results for HCV by PCR analysis. These authors found that two out of eight patients had an underlying B cell lymphoproliferative disorder and one Sjogren's syndrome emphasizing the importance of screening them in cases of HCV–MC vasculitis relapse where a sustained viral response is achieved [20].

Mortality occurs in non-responders after prolonged vasculitis course, and it is usually related to sepsis due to the underlying disease or to the therapy. There are reports of mortality rates ranging between 8% and 15% [8, 9, 12, 21–23].

The aim of this work is to describe the outcome of a series of patients with HCV-related vasculitis who were treated with corticosteroids and IV cyclophosphamide without receiving antiviral therapy.

Patients and methods

The data of 16 patients with HCV infection and vasculitis attending the department of Rheumatology and Rehabilitation, Kasr El-Eini Hospital, College of Medicine, Cairo University were retrospectively analyzed in the present study. The protocol of the research project has been approved by the institution within which the work was undertaken and it conforms to the provisions of the world medical association's Declaration of Helsinki.

Eleven patients were females (68.8%) and five were males (31.2%). The mean age of all patients was 49.6± 10.0 years. HCV infection was confirmed in all patients by detection of HCV RNA using polymerase chain reaction (PCR qualitative). Quantification of the viral load was done by the branched DNA technique. By this technique, some patients with positive HCV–RNA by PCR qualitative were undetectable according to the sensitivity of the test used at that time. The viral load was considered weak if it is between the undetectable level and 2×10^5, moderate if is between 2×10^5 and 5×10^5, high if it is between 5×10^5 and 10^6, and very high if it is higher than 10^6 genome/ml. Quantitative PCR was carried out in all patients before and at the intervals of 6 months of cyclophosphamide (CYC) pulses, and it was redone at any relapse of the manifestations. Detection of HCV antibodies (HCVAb) by an enzyme-linked immunosorbent assay (ELISA) was also carried out.

None of our study patients ever received antiviral therapy (in the form of IFN-α), but they all received I.V.-pulsed CYC monthly for 6 months (0.75 mg/cm^2), then every 3 months for six times if needed. It was administered in 500 ml of 5% glucose solution at a rate of 100 ml/h. It was preceded by I.V. methylprednisolone, then oral steroids

0.5 mg/kg/day (maximum 40 mg/day) tapered gradually, according to the patient's condition to 5–10 mg/day over 6 months.

The data of full monthly examination recorded for every patient was as follows: the presence of rheumatologic manifestations, including arthralgia; arthritis; myalgia; sicca manifestations; skin manifestations (Raynaud's phenomenon, purpura, distal ulcers, gangrene); neurological (peripheral and/or central nervous system (CNS) manifestations, including impaired cognitive function and abnormal findings on magnetic resonance imaging of the brain); cardiac; pulmonary; renal; gastrointestinal (peptic ulcers, mesenteric microaneurysms); hepatic; ear, nose and throat (ENT); or ophthalmologic involvement. Histologically confirmed vasculitis was also recorded.

Electrophysiological study findings were also reported. Peripheral neuropathy observed was classified as polyneuropathy or mononeuritis multiplex which was then classified into axonal or demyelinating using the criteria for chronic inflammatory polyneuropathies of the American Academy of Neurology [24].

The Birmingham Vasculitis Activity Score (BVAS, 2003) [25], an updated version of the BVAS [26], was used for scoring disease activity at the onset of the disease, after 6 months, after 18 months, and at the relapses of the disease if any. This score is a clinical index of the degree of vasculitis activity in nine separate organ systems, namely the systemic, cutaneous, mucous membranes/eyes, ENT, chest, cardiovascular, abdominal, renal, and nervous systems. The maximum score for persistent abnormalities is 33 and for new/worse symptoms and signs, it is 63.

Vasculitis Damage Index (VDI) was used for the assessment of organ dysfunction, damage, or scarring which had been present for at least 3 months and had occurred since the onset of vasculitis [27]. The index had been scored in the study at the same intervals as the BVAS. The VDI is a tabulated list of 64 items of damage grouped into 11 organ-based systems namely musculoskeletal, skin, ENT, pulmonary cardiovascular, peripheral vascular disease, renal gastrointestinal, ophthalmic, neuropsychiatric, and other damage/drug toxicity [28].

Patients were classified into two groups according to the size of the vessel involved: group A, patients with medium-sized vessel vasculitis, there were nine patients (56.2%), four of them had small vessel vasculitis in addition; and group B, patients with small-sized vessel vasculitis, only seven patients (43.8%).

The biochemical tests were measured monthly and included complete blood count, erythrocyte sedimentation rate, liver transaminases, serum albumin, serum creatinine, and urine analysis. All were evaluated for RF using qualitative determination using latex suspension kits (Cromatest, Spain), antinuclear antibodies (ANA) by indirect immunofluorescence using mouth–stomach–kidney section as a substrate, the type of ANA specified whether homogenous, rim, speckled, or nucleolar. Anti-double stranded nucleic acid (anti-DNA) was done if ANA was positive. Antineutrophil cytoplasmic antibodies (ANCA) were performed using indirect immunofluorescence assay on ethanol-fixed neutrophils. Cryoglobulins were isolated by centrifugation of refrigerated patient's serum in Wintrobe's tubes.

Inclusion criteria for the study were: (1) signs of vasculitis in the presence of HCV infection (HCV positive by qualitative PCR), (2) treatment with methylprednisolone followed by cyclophosphamide for at least 6 months, and (3) no previous intake of antiviral therapy nor within the follow-up. Exclusion criteria included: (1) HBV patients by performing hepatitis B surface antigen (HbsAg) and hepatitis B core antibody (HbcAb) tests using the ELISA technique, (2) human immunodeficiency virus by performing anti-human immunodeficiency virus (HIV-I and -II) antibody testing by ELISA technique, (3) coexistence of autoimmune, lymphoproliferative or other infectious disease (except HCV infection), and (4) presence of any other cause of vasculitis.

The evaluation of the patients and the response to treatment included in this study were done initially, after 6 months, after 18 months, and when relapse occurred.

The patients that had an improvement in their baseline clinical manifestations whether complete or incomplete were regarded as responders. Non-responders did not have a clinical response. Relapse was defined as the reappearance of clinical signs of vasculitis whether those present at the start or the appearance of new and additive signs.

Statistical methods

The means and standard deviation (SD) were computed for the continuous variables, the difference between the means was tested by standard t, or the one-way analysis of variance (ANOVA) as appropriate. The 95% confidence was computed by ANOVA. For comparison of percentages chi-squared (χ^2) was used. Differences were considered to be significant when p value was less than 0.05.

Results

The study included 16 HCV vasculitis patients, 11 females (68.8%) and five males (31.2%) with a male to female ratio of 0.45. The mean age was 49.6±10.0 years with a range between 34 and 67 years. The mean age of onset was 42.6± 11.2 years with a range between 21 and 58 years. The mean disease duration was 6.8±7.7 years with a range between 2 and 28 years.

Table 1 The general features of all patients, group A and group B

	All patients, *N*=16	Group A, *N*=9	Group B, *N*=7	*P*
Age (years)	49.6±10.0	50.3±10.2	48.5±10.5	0.740
Duration (years)	6.8±7.7	5.6±7.0	8.6±8.8	0.458
Age of onset (years)	42.6±11.2	44.6±9.5	40.2±13.6	0.471

In 13/16 patients (81.3%), vasculitis was the presenting symptom of HCV while the remaining were presenting by other manifestations of HCV infection. HCV was attributed to blood transfusion in three cases, to previous operations in four cases, and two were medical assistants. Four patients (25%) were diabetic all of which were in group B, and eight patients (50%) were hypertensive; five in group A and three in group B. Thirteen patients (seven in group A and six in group B) had intermittently elevated liver transaminases.

There were no statistically significant differences in age, age at onset, and disease duration between the groups with medium and small-sized vasculitis (Table 1).

In Table 2, arthritis was asymmetric in eight patients (five in group A and three in group B) and symmetric in one patient (group A). Gangrene occurred in the upper limbs in five patients, and in the lower limbs in three patients. Nerve conduction velocity studies of the patients with peripheral neuropathy revealed that two patients had axonal-demyelinating lesions, one had axonal lesion, and three had demyelinating lesions. Dysarthria was found in two patients (one in group A and one in group B), memory loss in one patient (group B), personality changes in one patient (group B), and infarction with involutional brain changes in MRI in one patient (group B).Cardiac involve-

Table 2 The clinical and laboratory manifestations of all, group A and group B patients

		All patients	Group A		Group B		Significance	
			Number	Percent	Number	Percent	χ^2	*P*
Sex	Female	11	5	55.6	6	85.7		
	Male	5	4	44.4	1	14.3	1.667	0.107
Clinical manifestations	Arthralgia	7	4	44.4	3	42.9	3.883	0.049*
	Arthritis	9	6	66.7	3	42.9	1.667	0.197
	Raynaud's phenomenon	2	2	22.2	0	0.0	0.178	0.182
	Malar rash	2	1	11.1	1	14.3	0.036	0.849
	Purpura	5	0	0.0	5	71.4	9.351	0.002*
	Gangrene	6	6	66.7	0	0.0	7.467	0.006*
	Gastritis	5	2	22.2	3	42.9	0.780	0.377
	Peripheral neuropathy	9	3	33.3	6	85.7	4.390	0.036*
	Mononeuritis multiplex	3	3	33.3	0	0.0	2.782	0.090
	IPF	1	0	0.0	1	14.3	1.370	0.242
	Cardiac involvement	4	1	11.1	3	42.9	2.118	0.146
	CNS involvement	4	1	11.1	3	42.9	2.116	0.146
	Retinal vasculitis	1	0	0.0	1	14.3	1.370	0.242
	Nephritis	2	1	11.1	1	14.3	0.036	0.849
	Hepatomegaly	9	3	33.3	6	85.7	4.390	0.036*
	Chronic active hepatitis	3	1	11.1	2	28.6	0.788	0.325
Laboratory	RF	11	7	77.8	4	57.1	0.780	0.377
	ANA	5	3	33.3	2	28.6	0.042	0.838
	HCVAb	14	8	88.9	6	85.7	0.163	0.687
	Cryoglobulins	3	0	0.0	3	42.9	4.747	0.029*
	Decreased C3	3	0	0.0	3	42.9	4.747	0.029*
	Decreased C4	3	0	0.0	3	42.9	4.747	0.029*

IPF interstitial pulmonary fibrosis

*$p<0.05$

Table 3 The mean and change in BVAS and VDI

	First	Second	Third	Fourth	Significance	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	F ratio	*P*
BVAS	13.1±7.6	1.6±1.4	4.4±5.8	4.5±6.8		
95% confidence	9.0–17.1	0.9–2.4	1.3–7.6	0.9–8.1	11.11	0.00001
Percent change		−87.4	170.7	−1.4		
VDI	2.3±3.1	1.4±1.2	1.0±1.4	1.0±1.4		
95% confidence	0.6–3.9	0.7–2.0	0.2–1.8	2.5–1.8	1.498	0.224
Percent change		−38.7	−27.5	0.0		

ment was diagnosed in four patients. Pericarditis was detected in one patient (group B), diastolic dysfunction in three patients (one in group A and two in group B), and valvular lesions in three patients (one in group A and two in group B). Peptic ulceration occurred in two patients, one in each group. Nine patients had hepatomegaly and three had chronic active hepatitis by biopsy, but none of the patients had liver cirrhosis.

In five patients (31.2%) that had positive ANA, three had homogeneous pattern, two of which were in group A and one in group B. The speckled pattern was present in two patients, one in each group. None of the patients had anti-DNA antibodies nor ANCA. Three patients within group B (18.8%) gave positive results for cryoglobulins.

The viral load was reported to be undetectable in the majority of the cases (nine patients in group A and three in group B) by the quantitative method although positive by the qualitative method used at the time of diagnosis, mild in two patients (group B) while in one patient (group B) it was moderate, and in one patient (group B) it was very high.

The distribution of clinical manifestations among the medium and small-sized vasculitis was not different except for arthralgia, purpura, gangrene, peripheral neuropathy, hepatomegaly, cryoglobulins, decreased complement 3 (C3) and decreased complement 4 (C4) differences were significant ($p<0.05$).

The first BVAS (1) and VDI (1) were recorded initially when the patients were active before receiving any treatment. All patients remitted for a variable duration of 2–4 months after receiving the I.V. methylprednisolone and cyclophosphamide. The second reading of BVAS (2) as well as VDI (2) was taken about 6 months from the start of treatment in all the patients. BVAS (3) and VDI (3) were taken 18 months after the start of treatment.

Seven patients (43.8%) had relapse of the vasculitis after a mean duration of 19.3±14.6 months from the date of the last pulse of CYC, with a range of 3–36 months (three in group A and four in group B). The recurrence occurred with the same presenting manifestations but in three of them with added manifestations. The BVAS (4) and VDI (4) were assessed for the relapsed group only. Table 3 shows that there was a marked drop between the first and second BVAS (87.4%) denoting an improvement in response to treatment. BVAS (3) and (4) rose but not to the original values. The difference between the means of the BVAS was highly significant. Although there was a drop in the VDI mean between the first and second reading, it was not statistically significant.

The difference between the various outcomes in the medium and small-sized vasculitis was not statistically significant (Table 4). The residual disability was seen in six patients as follows: one patient had pulmonary hypertension (6.25%) which was secondary to interstitial pulmonary fibrosis, one patient had peripheral weakness (6.25%), two patients had paresthesias (12.5%), all these were in group B while the two patients with loss of fingers and gangrene (12.5%) were in group A.

From the seven patients that relapsed, three had a second remission with undetectable viremia. Two patients had persistent manifestations. One of them had a low viral load which remained low, and the other patient had a high viral load which became moderate.

Table 4 The patient outcome according to vasculitis type

		Group A		Group B		Significance	
		Number	Percent	Number	Percent	χ^2	*P*
Outcome	Initial remission	6	66.7	6	85.7	0.042	0.838
	Relapse	3	33.3	4	57.1	0.907	0.341
	Second remission	2	22.2	1	14.3	0.163	0.687
	Residual	2	22.2	4	57.1	2.049	0.152
	Death	1	11.1	1	14.3	2.938	0.086

Two out of the relapse patients died (12.5% of the whole study group). One had renal failure (group B), and he passed 14 months after the last pulse of CYC. the other patient (group A) had sepsis mostly because of the underlying disease, as she had her last pulse of CYC 10 months before she passed. Two out of the three patients with cryoglobulinemia relapsed, one of whom was the patient who died of renal failure.

Discussion

The treatment guidelines for HCV-associated extrahepatic features should be based not only on the pathogenic mechanisms, but also on the accurate, individual assessment of the activity/severity of both extrahepatic clinical features and the underlying liver disease [29, 30]. For patients with mild or moderate vasculitic symptoms, such as articular involvement or cutaneous vasculitis, IFN-α with or without ribavirin may suffice, but for patients with life-threatening organ involvement a combination of antiviral agents and immunosuppressive therapy is suggested [31].

Despite previous reports of the successful treatment of HCV-associated cryoglobulinemia vasculitis with antiviral therapy where the viral level determines the type of remission (whether complete or incomplete) [5, 9, 19, 32], we here introduce a subset of patients with HCV vasculitis having undetectable viremia and achieving remission with the traditional high dose steroid regimen as well as cyclophosphamide without adding antiviral therapy. We achieved the same success rate in terms of clinical and virologic response as well as the percentage of relapses as those studies that encouraged the use of early antiviral therapy [5, 9, 19, 20, 33]. The reason none of the patients received antiviral therapy was that all patients except for four had repeatedly undetectable HCV results, their liver enzymes remained unchanged on several readings and none showed any progression to liver cirrhosis.

We postulate that in our patient category the immune system kept the viral infection under control and resulted in an undetectable viremia. Reports discussing the finding of a non-specific stimulatory effect of HCV antigens on monocytes derived from patients with previous HCV infection and undetectable HCV by PCR analysis may signify that there is HCV replication below the detection limit of the PCR technique in some patients (which some refer to as occult HCV infection). This persistent low-level viremia may explain the occasional altered cytokine and immunological responses [34–36].

The absence of anti-HCV antibodies in two patients out of the 16 can be due to the low viremia not causing an immunological response and this agrees with Caudai et al. who showed that there can be a discrepancy between HCV-RNA and anti-HCV antibody results [37].

The percentage of cryoglobulins (18.75% of total patients) among our HCV patients is lower than other studies. Monia et al. in 2005 showing 40% of their 76 patients [38] and Wong and colleagues in 1996 showing 50% cryoglobulins in their HCV patient category [39]. A possible explanation is that 56.2% of our patients had medium-sized vessel vasculitis, and in this subset of patients, cryoglobulins are uncommonly detected [40]. The deterioration in one of the three cryoglobulinemic patients leading to his death was principally connected to the underlying glomerulonephritis progressing to renal failure as the condition progressed despite the low viral load. This could be explained by an autonomous process of B cell expansion inducing the deposition of immune complexes and causing the patient to reach this final state. The presence of reduced C3 and C4 levels in this patient despite the reduced viral load encourages our explanation which has also been considered by other authors [41–43].

The other mortality was due to sepsis mostly due to the underlying disease and unrelated to the drug regimen as the patient had stopped CYC 10 months before her death. Our mortality rate (12.5%) is within the rate of most reports that had rates between 8% and 15% [5, 9, 11, 19, 23].

Our relapse rate was less than the study of Landau et al. [20] who had seven out of his eight patients with HCV cryoglobulinemic vasculitis relapse despite receiving antiviral therapy and achieving a sustained viral response. Therefore, relapses are present even with effective antiviral therapy [44]. The use of antiviral therapy in our study could not have prevented the relapses because the viral load was low and even if it had been given the same percentage of relapse would have been expected. The presence of peripheral neuropathy and gangrene made us reluctant to use IFN-α especially with its known propensity to worsen these manifestations [15–17].

Some authors have demonstrated increased HCV viremia when corticosteroids are given for a short time (1–6 months) and when steroids are withdrawn the viremia reverts to previous levels [45]. Others have shown that their use neither increased HCV RNA nor worsened liver functions. The previous reports of increased mortality and morbidity with the use of these drugs were related to the severity of the underlying hepatic and autoimmune features [15, 30, 31]. From our study, the corticosteroids did not result in an increase in viremia.

Conclusion

A subset of HCV-related vasculitis patients with low levels of viremia can be safely treated with corticosteroids and cyclophosphamide alone. Despite successful treatment, a significant proportion of patients relapse and some develop

severe complications and death. Further research in this category of patients is needed to further establish the safe use of this drug regimen especially on larger series of patients.

Disclosures None

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