Role of different direct-acting antiviral drugs on hepatitis C virus-associated mixed cryoglobulinemia in Egyptian patients

Tarek A. Elshazly[a], Amr M. Elrabat[a], Sahar A.M. Ali[b], Muhammed Diasty[c], Mohamed El-Desoky[d]

[a]Assistant Professor of Internal Medicine Department, Faculty of Medicine, Mansoura University, [b]Assistant Professor of Microbiology and Immunology Faculty of Medicine, Menofia University, [c]Assistant Professor of Tropical Medicne Department, Mansoura University, Egypt, [d]College of Applied Medical Sciences, Jubail Imam abdulrahman bin Faisal University

Correspondence to Amr Elrabat, Assistant Professor of Internal Medicine, Mansoura University, Egypt. Tel: 00201118253050; e-mail: amr.rabat@yahoo.com

Received 11 March 2019
Accepted 5 May 2019
Published: 18 August 2020

The Egyptian Journal of Internal Medicine
2019, 31:458–464

Background
Several extrahepatic manifestations have been reported and mixed cryoglobulinemia is a clonal disorder of B cells which is strongly associated with hepatitis C virus (HCV).
New regimens of direct-acting antiviral agents (DAAs) have recently been approved for the treatment of genotype 4 HCV which offer improved results of sustained virologic response (SVR) in the treatment-naive and previously treated patients.
Aim
To assess the prevalence of cryoglobulinemia for evaluating the efficacy of DAA therapy on it.
Patients and methods
Patients received one of the following regimens: sofosbuvir, daclatasvir±ribavirin, or sofosbuvir plus ledipasvir±ribavirin follow-up for 24 weeks after treatment.
Results
A total of 132 patients were involved in our study: 65 men and 67 women. Cryocrit-positive patients clinically presented with clinical manifestations in 32 (53.3%) patients out of the 60 patients. After 12 weeks of DAAs treatment, there was a significant reduction in cryoprecipitate level and rheumatoid factor (RF) level with improvement of glomerular filtration rate, basal C4, and clinical improvement of purpura, Raynaud's phenomenon, and this was the same when 38 patients continued treatment for 24 weeks. However, comparing 12 and 24-week therapy showed significant improved difference in cryoprecipitate level but improved glomerular filtration rate, basal C4, and decreased level of RF, proteinuria, purpura, and peripheral neuropathy although it did not reach statistical significance
Conclusion
Cryocrit-positive patients were old age, cirrhotics with long duration of HCV. There is significant improvement of both laboratory and clinical parameters of cryoglobulinemia after SVR12 and more significant after SVR24. There is no significant difference in using different DDA regimen with or without ribavirin on the SVR at either 12 or 24 weeks. So, we advise basal laboratory and clinical parameters of mixed cryoglobulinemia before designing treatment regimens of HCV patients in Egypt.

Keywords:
cryoglobulin, direct-acting antiviral drugs, hepatitis C virus

Egypt J Intern Med 31:458–464

1110-7782

Introduction

About 170 million people were reported to have chronic hepatitis C virus (HCV) infection worldwide [1]. Egypt has the highest prevalence with 90% of the infections being of genotype 4 [2].

Several extrahepatic manifestations have been reported at the natural history of HCV infection; 40–74% of patients infected with HCV at least have one extrahepatic manifestation through the disease course [3].

Mixed cryoglobulinemia (MC) is a clonal disorder of B cells; it stimulates unregulated B-cell proliferation which leads to immune complex formation and deposition, which is strongly associated with HCV [4].

The intrinsic mechanism through which HCV promotes cryoglobulin production is unidentified. Virus persistence may represent a continuous stimulus for the host immune system. Cryoglobulins may represent the product of virus–host interactions at HCV-infected patients [5].

 DOI: 10.4103/ejim.ejim_41_19

Therapy for genotype 4 HCV has been pegylated interferon with ribavirin for 24 or 48 weeks, depending on the virologic response but treatment-naive patients who received this regimen had sustained virologic response (SVR) rates of 43–70% [6]. Difficulty in administration and poor tolerability associated with this treatment has been low; thus most patients with HCV in Egypt are untreated [7].

New regimens of direct-acting antiviral agents (DAAs) have recently been approved for the treatment of genotype 4 HCV which offer improved results of SVR in treatment-naive and previously treated patients [8]. So far, a few studies have reported promising results of the efficacy and tolerability of DAA therapy in patients with HCV-associated MC [9].

Aim

To assess the prevalence of cryoglobulinemia associated with hepatitis C virus infection and for evaluating the efficacy of different DAA-based combination therapies on it.

Study design

This is a cohort, prospective study. It was conducted from March 2017 till March 2018 at the outpatient clinic of the Specialized Medical Hospital, Mansoura University, Egypt, on patients with chronic HCV infection who were eligible for sofosbuvir-based therapy either naive or treatment-experienced patients [10]

Patients with cirrhosis but no esophageal varices, not ascetic, and no history of hepatic encephalopathy, platelets less than 100×10^3/l, bilirubin less than 2 mg/dl, albumin more than 3.5 g/dl, and international normalized ratio less than 1.7 were included in the study [11].

Patients with decompensated cirrhosis (Child–Pugh C), HIV or hepatitis B virus infection, chronic liver disease of non-HCV etiology, bilirubin of more than or equal to 2 mg/dl times the upper limit of normal, alanine and aspartate aminotransferase more than 10 times (upper limit of normal), platelets less than 50 000/μl, hemoglobinA1c more than 10%, and creatinine clearance less than 30 ml/min were excluded.

The study was conducted according to the Declaration of Helsinki and International Conference on Harmonization guidelines and was approved by the institution's review board before initiation. Informed written consent was taken from all patients before undertaking any study-related procedures

Antiviral therapy

All patients received one of the following regimens: sofosbuvir, daclatasvir±ribavirin, or sofosbuvir plus ledipasvir±ribavirin. Treatment was stratified on the presence or absence of cirrhosis and prior treatment experience. Patients underwent follow-up for 24 weeks after treatment [10].

Laboratory investigations

(1) Serum sample was collected from the patient for determination of liver function test including alanine transaminase, aspartate transaminase, total bilirubin, albumin using Hitachi 7600 DDP modular chemistry analyzer (Hitachi High-Technologies, Tokyo, Japan) in addition to routine complete blood count, international normalized ratio, and α-fetoprotein.
(2) ELISA test was done for determination of HBsAg, HCV, and HIV antibodies using BEB III (Dade Behring, Germany) and the positive cases for HCV was confirmed by HCV RIBA 3.0 Strip Immunoblot assay (ChironCorp., Emeryville, California, USA).
(3) Serum HCV RNA was determined using the Roche COBAS TaqMan HCV Test version 2.0 (Roche Molecular Systems, Pleasanton, California, USA) lower limit of quantitation of 25 IU/ml according to the manufacturer's instructions. It was done at baseline, at 4, 12 weeks of treatment for patients plus at week 24 for patients receiving a 24-week therapy and post-treatment weeks 4, 12, and 24.
(4) Cryoglobulin determination: cryoglobulins were precipitated from serum stored for up to 15 days at 4°C. On the 15th day of cold incubation, samples were centrifuged at 2500*g* for 10 min at 4°C. The precipitates were washed five times by 0.15 mol/l NaCl. A fraction of washed cryoglobulins was later on diluted in 0.1 mol/l NaOH, and the concentration was measured by reading the absorbance at 280 nm. Values less than 50 mg/l were considered negative.
(5) The serum IgG, IgM, rheumatoid factor, and C3 and C4 complement fraction levels were assessed using standard nephelometry according to the manufacturer's specifications (Dade Behring) and was measured in IU per milliliter. The upper limit of the normal levels of the rheumatoid factor was 60 IU/ml [12].

Other investigations

(1) Abdominal ultrasonography for hepatic scanning and evaluation of presence and/or severity of ascites and for assurance of inclusion and exclusion criteria.
(2) Upper EGD for determination of esophagogastric varices.
(3) Computed tomography for those patients with suspected focal lesions or high α-fetoprotein.

Definitions

(1) HCV-MCS were defined as having HCV RNA more than 1000 IU/ml at baseline and circulating cryoglobulin associated with purpura, arthralgia's, Raynaud's phenomenon, sicca syndrome, vasculitis, cutaneous ulcers, neurologic involvement (peripheral neuropathy and/or central nervous system), or renal involvement [13].
(2) Renal involvement was defined by the presence of at least two of the following clinical signs: proteinuria, hematuria, and reduced estimated glomerular filtration rate (GFR) less than 60 ml/min/1.73 m^2 without other etiology for chronic kidney disease [14].
(3) Sustained virologic response was defined as undetectable HCV RNA levels 12 weeks after treatment cessation (SVR12) and at 24 weeks (SVR24) [10].

Safety

Adverse reactions were determined by evaluating all clinical notes and laboratory results from the time of treatment initiation until 4 weeks after treatment termination.

Statistical methods

Data were collected was analyzed using SPSS version 17 computer statistical software package. The results were expressed as mean±SD. The paired *t*-test was used to determine significant difference between test and control subjects. Statistical significance level was put at *P* value less than 0.05.

Results

A total of 132 HCV RNA-positive patients were involved in our study 65 men and 67 women.

Sixty (45.4%) patients were cryocrit positive with a high significant value ($P<0.0001$) regarding disease duration, degree of cirrhosis, IgM, IgG, rheumatoid factor (RF), and C4 in comparison to the cryocrit-negative patients (Table 1).

Table 1 Baseline epidemiological, clinical, and laboratory characteristics in cryocrit-positive and cryocrit-negative hepatitis C virus patients

	Cryocrit positive (*n*=60)	Cryocrit negative (*n*=72)	*P* value
Age (mean±SD) (years)	44.5±10.8	43.6±9.8	0.616
Female/male	38/22	30/42	0.013
BMI <30 kg/m^2 [*n* (%)]	19 (31.6)	24 (33.3)	0.836
Disease duration (years)	15±5.2	9±2.6	<0.0001
Child score (A/B)	12/23	33/20	0.001
Cirrhosis [*n* (%)]	29 (48.3)	10 (13.8)	<0.0001
Platelets (<100×10^3/l) [*n* (%)]	13 (21.6)	2 (2.7)	0.0007
HCV RNA, log10 IU/ml (mean±SD)	5.4±0.96	5.2±0.88	0.214
Cryoglobulin concentration (mg/l)	320±40	0	–
RF levels (>60 IU/ml)	65.4±35.5	12.3±4.7	<0.0001
IgG (mg/dl)	2467±622	1933±570	<0.0001
IgM (mg/dl)	344±231	233±225	0.006
C4 (mg/dl)	15±2	20±8	<0.0001
SVR12	53 (88.3)	70 (97.2.3)	0.08
SVR12 (12-week therapy)	35 (92)	56 (100)	0.06
SVR12 (24 week therapy)	18 (82)	14 (88)	0. 69

HCV, hepatitis C virus; RF, rheumatoid factor; SVR, sustained virologic response. $P<0.05$, significant.

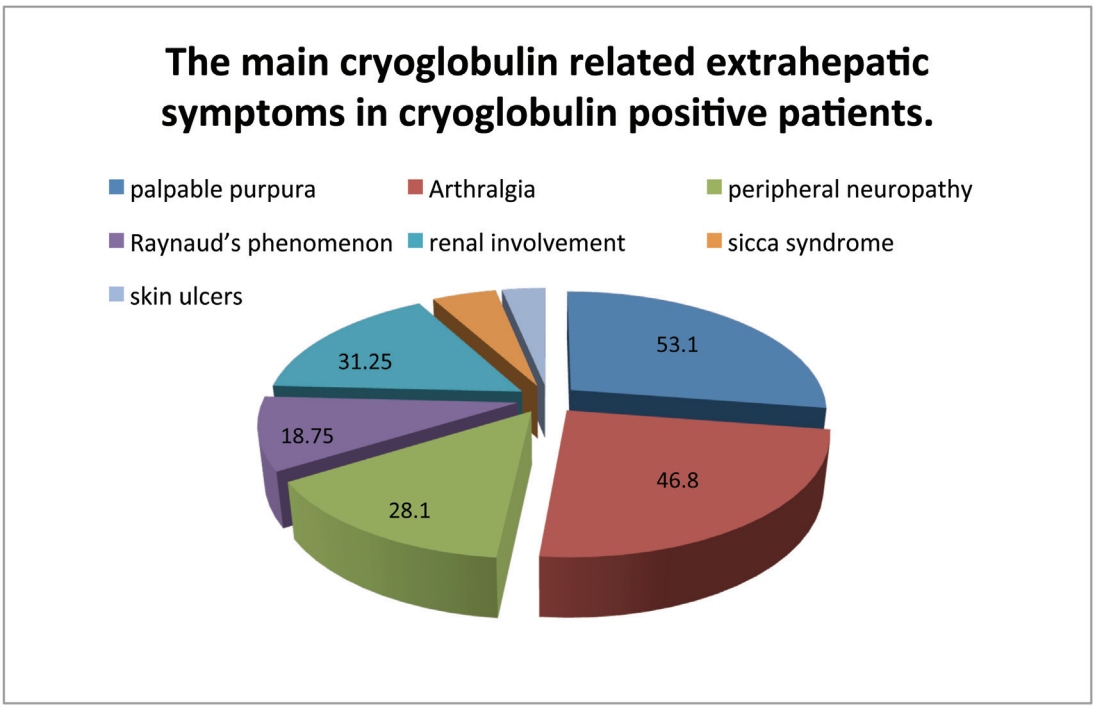
Cryocrit-positive patients presented with clinical manifestations in 32 (53.3%) patients out of the 60 (with overlap manifestations) (Fig. 1).

Different DAAs regimens were used on different patients (Table 2), 123 (93.2%) patients achieved SVR12 and nine (6.8%) patients did not respond to therapy who were significantly ($P<0.0001$) of old age 62±4.9. All of them were cirrhotics and seven were cryocrit positive, eight of them received a 12-week therapy (Table 3).

After 12 weeks of DAAS treatment, there was a significant reduction in the cryoprecipitate level and RF level with improvement of GFR, basal C4, and clinical improvement of purpura, Raynaud's phenomenon (Table 4) and this was the same when 38 patients continued treatment for 24 weeks (Table 5).

However, comparing 12 and 24-week therapy showed significant improved difference in cryoprecipitate level but improved GFR, basal C4, and decreased level of RF, proteinuria, purpura, and peripheral neuropathy although it did not reach statistical significance (Table 6).

Figure 1



Main cryoglobulin-related extrahepatic symptoms in cryoglobulin-positive patients.

Table 2 Demographic data and medical history of the study

Variables	All patients (*n*=132)
Age (years)	43 (35, 53)
Sex	
Male	65 (49.2)
Female	67 (50.8)
Presence of cirrhosis	41 (31.1)
Class of cirrhosis (*n*=41)	
A	17 (42)
B	24 (58)
Duration of illness (years)	6 (5, 8)
Cryocrit positive	60 (45.4)
Treatment history	
Naive	111 (84)
Interferon	8 (6)
Sofusbuvir or daclatasvir	13 (10)
12 weeks sustained response	
Responders	123 (93)
Nonresponders	9 (7)
Treatment regimen	
Sofusbuvir+daclatasvir for 12 weeks	50 (38)
Sofusbuvir+daclatasvir+ribavirin for 12 weeks	22 (17)
Sofusbuvir+ledipasvir for 12 weeks	17 (13)
Sofusbuvir+ledipasvir+ribavirin for 12 weeks	5 (4)
Sofusbuvir+daclatasvir for 24 weeks	19 (14)
Sofusbuvir+daclatasvir+ribavirin for 24 weeks	9 (7)
Sofusbuvir+ledipasvir for 24 weeks	6 (5)
Sofusbuvir+ledipasvir+ribavirin for 24 weeks	4 (3)

Table 3 Demographic data and medical history of responders and nonresponders

Variables	Responders (*n*=123)	Nonresponders (*n*=9)	*P* value
Age (years)	42±10.6	62±4.9	<0.0001
Sex			
Male	60 (49)	5 (56)	0.7
Female	63 (51)	4 (44)	
Presence of cirrhosis	32 (26)	9 (100)	<0.0001
Class of cirrhosis (*n*=41)			
A	15 (47)	2 (22)	0.19
B	17 (53)	7 (78)	
Duration of illness (years)	7.2±3.5	8.1±3.7	0.5

P<0.05, significant.

Discussion

Infection with HCV has been involved in a variety of adverse extrahepatic consequences, with its role convincingly established in the pathogenesis of MCS, in which underlying HCV infection is almost present [15] The antiviral therapy of HCV infected patients has changed dramatically with the new DAAs [16]. Limited evidence is currently available researching the treatment with DAAs in patients with HCV-related MC [17].

In all, 132 patients were enrolled in our study with the prevalence of cryoglobulinemia being 45.4% (60) and this is in accordance with Roccatello *et al.* [18].

Table 4 Basal laboratory investigations and clinical signs compared with those after 12 weeks of end of antiviral therapy

Variables	Basal values (*n*=132)	Values after 12 weeks (*n*=132)	*P* value
Cryoprecipitate	1.13±1.79	0.125±0.48	<0.0001
Glomerular filtration rate	78.99 ±15.57	82.8±11.83	<0.0001
Low basal C4	76 (57.6)	124 (93.9)	<0.0001
Presence of rheumatoid factor	56 (42.4)	14 (10.6)	<0.0001
Presence of proteinuria	7 (5.3)	3 (2.3)	0.13
Presence of hematuria	4 (3)	0	0.13
Presence of purpura	19 (14.4)	4 (2.3)	<0.0001
Presence of peripheral neuropathy	10 (7.6)	7 (5.3)	0.38
Presence of Raynaud's phenomena	17 (12.9)	1 (0.8)	<0.0001
Presence of arthritis	3 (2.3)	3 (2.3)	1
Presence of sicca syndrome	1 (0.8)	1 (0.8)	1
Presence of skin ulcer	1 (0.8)	1 (0.8)	1

P<0.05, significant.

Table 5 Basal laboratory investigations and clinical signs compared with those after 24 weeks of sustained antiviral response of 24-week therapy

Variables	Basal values (*n*=38)	After 24 weeks values (*n*=38)	*P* value
Cryoprecipitate	2.3±2.4	0.303±0.65	<0.0001
Glomerular filtration rate	72.5±17.8	78.3±14	<0.0001
Low basal C4	15 (40)	31 (82)	<0.0001
Presence of rheumatoid factor	23 (61)	10 (26)	<0.0001
Presence of proteinuria	5 (13)	2 (5)	0.25
Presence of hematuria	4 (11)	0	0.13
Presence of purpura	11 (29)	3 (8)	0.0082
Presence of peripheral neuropathy	8 (21)	6 (16)	0.63
Presence of Raynaud's phenomena	11 (29)	1 (3)	0.002
Presence of arthritis	2 (5)	2 (8)	1
Presence of sicca syndrome	1 (3)	1 (3)	1
Presence of skin ulcer	1 (3)	1 (3)	1

P<0.05, significant.

Symptomatic cryoglobulinemia was observed in 32 (53.3%) patients out of the 60 sixty patients and this in contrast to a study done by Emery *et al.* [19] who reported that of the 83 treated patients 18 (21%) had symptomatic cryoglobulinemia, but this may be attributed to different genotypes and limited number of patients.

Table 6 Effect of treatment duration on cryoglobulinemic symptoms and laboratory findings

Variables	12 weeks (*n*=94)	24 weeks (*n*=38)	*P* value
Cryoprecipitate difference	0.59±1.02	2.04±2.03	0.001
Glomerular filtration rate difference	3±5.9	5.8±7.5	0.035
Low basal C4 difference (*n*=48)	32 (34)*	16 (42)*	0.38
Difference of rheumatoid factor (*n*=42)	29 (31)*	13 (34)*	0.71
Difference of proteinuria (*n*=4)	1 (1)	3 (8)	0.038
Difference of purpura (*n*=15)	7 (7)	8 (21)*	0.026
Difference of peripheral neuropathy (*n*=3)	1 (1)	2 (5)	0.032

P<0.05, significant. *Number of patients like 34 patients out of 94.

Both cryocrit-positive and cryocrit-negative patients were involved to evaluate the role of DAAS on different laboratory and clinical parameters and this is in contrast to a study by Hassan *et al.* [20] who studied only 63 out of 120 patients who were only cryopositive.

SVR12 showed no significant difference between cryocrit-positive and cryocrit-negative groups and this is in agreement with a study by Gragnani *et al.* [21], who reported that MC is a negative predictor factor of virologic response and clearance of HCV led to resolution of symptomatic MCS. Also, there were higher rates of reduction of symptomatic cryoglobulinemia with longer follow-up following DAA therapy [22]. In contrast, Martin and Fabrizi [23] said that there is a discrepancy between SVR and clinical improvement. However, another study indicates that inferior SVR rates in HCV patients with cryoglobulinemia have been overcome with DAA therapy [13].

In cryocrit-positive patients, we found that disease duration (15±5.2) and degree of cirrhosis (48.3%) and presence of Child B (23) were highly coexistent significant (*P*<0.0001) factors associated with high cryoglobulin level (320±40) and this is consistent with a study by Roccatello *et al.* [18] who stated that long-term HCV infection and old age represent predisposing factors for the development of MC and high degree of cirrhosis and is associated with high level of cryoglobulin in HCV patients.

Regarding our study, after treatment with different regimens, we found a significant change between basal values and after 12 weeks in all patients (132) with a significant reduction in cryoprecipitate value

($P<0.0001$) and improved GFR, basal C4, and improvement of laboratory and clinical parameters and this matches with a study by Hassan *et al.* [20] who found a significant decrease in their mean cryoglobulin level from 41.47±12.32 to 5.12±3.59 μg/ml SD and then to 5.09±3.02 μg/ml SD, 12–24 weeks post-therapy, respectively ($P<0.001$), with significant decrease in RF concentrations and elevation in C3 and C4 serum levels approaching the normal value.

We noticed in our study, a 24-week regimen gives the same significant values regarding the same parameters. Therefore, in studying both groups separately to determine the effect of drug duration between 12 weeks (94) and 24 weeks (38) we found a significant value ($P<0.0001$) in cryoprecipitate difference which increased when the patient completed 24 weeks from 0.59±1.02 to 2.04±2.0 and the GFR difference showed more improvement from 3±5.9 to 5.8±7.5 and improved number of patients with low C4 from 32 to 16 and the number of patients with RF from 24 to 13 and proteinuria, purpura, and peripheral neuropathy indicating that MC has improved with long-duration treatment (in spite of no statistically significant value).

As well, we found no difference between sofosbuvir, daclatasivir and sofosbvir, and ledipasvir regarding the effect on MC and no value as well of adding ribavirin on MC and this in agreement with Sollima *et al.* [24].

Although long-duration treatment regimens have improved clinical and laboratory outcomes of mixed cryoglobulinemia, this is against the results of Cornella *et al.* [25], who detailed a case series of five patients with chronic HCV complicated by MC who received 24 weeks' triple therapy with oral antiviral agents. Clearance of serum cryoglobulins was not found in any of these patients but this may be explained by the very small sample size.

Conclusion

We concluded from our study that the prevalence of cryoglobulinemia is 45.4% and is presented by laboratory or clinical findings. Cryocrit-positive patients were old age, cirrhotics with long duration of HCV. There is significant improvement of both laboratory and clinical parameters of cryoglobulinemia after SVR12 and is more significant after SVR24. There is no significant difference in using different DDA regimens with or without ribavirin on the SVR either 12 or 24 weeks. So, we advise basal laboratory and clinical parameters of MC before designing treatment regimens of HCV patients in Egypt.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Averhoff FM, Glass N, Holtman D. Global burden of hepatitis C considerations for healthcare providers in the United States. Clin Infect Dis 2012; 55:510–515.

2 Guerra J, Garenne M, Mohamed MK, Fontanet A. HCV burden of infection in Egypt: results from a national survey. J Viral Hepat 2012; 19:560–567.

3 Agnello V. The aetiology of mixed cryoglobulinaemia associated with hepatitis C virus infection. Scand J Immunol 1995; 42:179–184.

4 Romitelli F, Paolo Pucillo L, Basile U, Stasio E. Comparison between the traditional and a rapid screening test for cryoimmunoglobulins detection. Biomed Res Int 2015; 2015:783063.

5 Rehermann B. Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. J Clin Invest 2009; 119:1745–1754.

6 Khattab MA, Ferenci P, Hadziyannis SJ, Colombo M, Manns MP, Almasio PL, *et al.* Management of hepatitis C virus genotype 4: recommendations of an international expert panel. J Hepatol 2011; 54:1250–1262.

7 Centers for Disease Control and Prevention (CDC). Progress toward prevention and control of hepatitis C virus infection- Egypt, 2001–2012. MMWR Morb Mortal Wkly Rep 2012; 61:545–549.

8 Muir AJ. The rapid evolution of treatment strategies for hepatitis C. Am J Gastroenterol 2014; 109:628–635.

9 Urraro T, Gragnani L, Piluso A, Fabbrizzi A, Monti M, Fognani E, *et al.* Combined treatment with antiviral therapy and rituximab in patients with mixed cryoglobulinemia: review of the literature and report of a case using direct antiviral agents-based antihepatitis C virus therapy. Case Reports Immunol 2015; 2015:816424.

10 European Association for Study of Liver. EASL 2015 recommendations on treatment of hepatitis C 2015. J Hepatol 63:199–236.

11 Shiha G, Seif S, Maher M, Etreby S, Zalata K. Comparison between transient elastography (Fibroscan) and liver biopsy for the diagnosis of hepatic fibrosis in chronic hepatitis genotype 4. Egypt Liver J 2014; 4:106–111.

12 Santagostino E, Colombo M, Cultraro D, Muc¸a-Perja M, Gringeri A, Mannucci PM. High prevalence of serum cryoglobulins in multitransfused hemophilic patients with chronic hepatitis C. Blood 1998; 92:516–519.

13 Sise ME, Bloom AK, Wisocky J, *et al.* Treatment of hepatitis C virus-associated mixed cryoglobulinemia with direct-acting antiviral agents. Hepatology 2016; 63:408–417.

14 Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, *et al.* A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150:604–612.

15 Cacoub P, Comarmond C, Domont F, *et al.* Cryoglobulinemia vasculitis. Am J Med 2015; 128:950–955.

16 Pawlotsky JM, Aghemo A, Back D, *et al.* European Association for Study of Liver. EASL recommendations on treatment of hepatitis C 2015. J Hepatol 2015; 63:199–236.

17 Saadoun D, Resche Rigon M, Pol S, Thibault V, Blanc F, Pialoux G, *et al.* PegIFNα/ribavirin/ protease inhibitor combination in severe hepatitis C virus-associated mixed cryoglobulinemia vasculitis. J Hepatol 2015; 62:24–30.

18 Roccatello D, Sciascia S, Rossi D, Solfietti l, *et al.* The challenge of treating hepatitis C virus-associated cryoglobulinemic vasculitis in the era of anti-CD20 monoclonal antibodies and direct antiviral agents. Oncotarget 2017; 8:41764–41777.

19 19Emery JS, Kuczynski M, La D, *et al.* Effi cacy and safety of direct acting antivirals for the treatment of mixed cryoglobulinemia. Am J Gastroenterol 2017; 112:1298–1308.

20 Hassan AM, Osman HA, Mahmoud HS, Hassan MH, Hashim AA, Ameen HH. Sofosbuvir-daclatasvir improves hepatitis C virus-induced mixed cryoglobulinemia: Upper Egypt experience. Infect Drug Resist 2018; 11:895–901.

21 Gragnani L, Fognani E, Piluso A, *et al.* Long-term effect of HCV eradication in patients with mixed cryoglobulinemia: a prospective, controlled, openlabel, cohort study. Hepatology 2015; 61:1145–1153.

22 Saadoun D, Pol S, Ferfar Y, *et al.* Efficacy and safety of sofosbuvir plus daclatasvir for treatment of HCV-associated cryoglobulinemia vasculitis. Gastroenterology 2017; 153:49–52.e5.

23 Martin P, Fabrizi F. Benefit of direct-acting antiviral therapy for cryoglobulinemia due to hepatitis C infection. Am J Gastroenterol 2017; 112:1309–1310.

24 Sollima S, Milazzo L, Peri AM, Torre A, Antinori S, Galli M. Persistent mixed cryoglobulinaemia vasculitis despite hepatitis C virus eradication after interferon-free antiviral therapy. Rheumatology (Oxford) 2016; 55:2084–2085.

25 Cornella SL, Stine JG, Kelly V, Caldwell SH, Shah NL. Persistence of mixed cryoglobulinemia despite cure of hepatitis C with new oral antiviral therapy including direct-acting antiviral sofosbuvir: a case series. Postgrad Med 2015; 127:413–417.