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Hepatitis C virus-related mixed cryoglobulinemic endocapillary proliferative glomerulonephritis and B-cell non-Hodgkin lymphoma: a case report and literature review

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Abstract. – OBJECTIVE: Chronic HCV (hepatitis C virus) infection is recognized as the major cause of mixed cryoglobulinemia. Cryoglobulins continually precipitate and form deposits on the vascular endothelium of small to medium-sized blood vessels, which may progress to vasculitic syndrome.

CASE REPORT: A 44-year-old female patient with chronic HCV infection presented with purpuras, edema and proteinuria. Her renal findings included microscopic hematuria, moderate proteinuria and endocapillary proliferative glomerulonephritis (EnPGN) on renal biopsy. Serum cryoglobulins comprised mixed monoclonal cryoglobulins characterized by IgM kappa. The serum protein electrophoresis revealed a monoclonal M protein (9.0%). CD19 and CD20-positive B-cell oligo-monoclonal expansion in the bone marrow was revealed. Rapid relief of the clinical symptoms, the disappearance of proteinuria and a sharp decrease in the HCV viral load were observed in our case after one year of interferon therapy.

CONCLUSIONS: HCV infection-associated extrahepatic manifestations are diverse, which may lead to misdiagnosis. This is the first report of HCV-associated cryoglobulinemic EnPGN and B-NHL, which rapidly responded to interferon.

Key Words:
Mixed cryoglobulinemia, Glomerulonephritis, Hepatitis C virus, Non-Hodgkin lymphoma.

Introduction

Approximately 130-170 million people were infected with hepatitis C virus (HCV) worldwide, which has become a major cause of liver diseases[1]. Chronic HCV infection is recognized as the major cause of mixed cryoglobulinemia, which is considered to be an immune complex-mediated vasculitis that involves small to medium-sized blood vessels and leads to a symptomatological clinical triad characterized by purpura, weakness and arthralgias[2]. Kidney involvement during the course of HCV-related mixed cryoglobulinemia has been shown in approximately 30% of cases[2,3]. Membranoproliferative glomerulonephritis (MPGN) with cryoglobulinemia is the most common renal manifestation of HCV infection[4]. However, HCV-associated cryoglobulinemic endocapillary proliferative glomerulonephritis (EnPGN) has rarely been described. The prevalence of HCV infection complicated with non-Hodgkin lymphoma has been reported to range from 0.5-25%[5,6]. However, the mechanisms of HCV-induced renal damage differs from that of HCV-driven lymphoproliferation. Herein, we have described a rare case of cryoglobulinemic EnPGN and B-cell non-Hodgkin lymphoma (B-NHL) that developed in a 44-year-old female with HCV infection.

Case Report

A 44-year-old woman was referred to our hospital due to proteinuria for half a month. The patient complained of recurrent episodes of purpura and edema for 3 years. Henoch-Schönlein purpura (HSP) and Henoch-Schönlein purpura nephritis (HSPN) were diagnosed prior to the renal biopsy. The patient had been infected with HCV after a blood transfusion for 15 years prior without any treatment. Upon admission, her heart rate, respiratory rate and temperature were normal, and her blood pressure was 137/93 mmHg (1 mmHg = 0.1333 kPa). Physical examination revealed multiple palpable purpuras on both arms

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and legs. The serum creatinine level was 62.6 μmol/L (normal: 37.0-110.0 μmol/L). The serum albumin level was 39.5 g/L (normal: 40.0-55.0 g/L), and the level of ALT (alanine aminotransferase) was 43 IU/L (normal: < 40 IU/L), which was slightly elevated. The serum C4 (complement 4, 0.0317 g/L) level was almost undetectable (normal: 0.145-0.360 g/L), whereas the level of C3 (0.5040 g/L, normal: 0.785-1.520 g/L) was moderately reduced. The level of proteinuria was 1.79 g/d, and 93 red blood cells were detected per high-power field during urinary sediment analysis. Serum cryoglobulins showed mixed monoclonal cryoglobulins characterized by IgM kappa. Rheumatoid factors were high and measured 4900 IU/ml (normal: < 20 IU/ml). During the past 3 years, persistently elevated rheumatoid factor (range from 3150 IU/ml to 4900 IU/ml) were displayed. Serum protein electrophoresis revealed a monoclonal M protein (9.0%). Immunofixation electrophoresis of the serum revealed a high level of IgM (7220.0 mg/L, normal: 700-2200 mg/L) and an abnormal ratio of serum kappa/lambda (3.23, normal: 1.50-2.56). The serum anti-HCV antibody was positive using a third-generation enzyme-linked immunosorbent assay (ELISA), and the HCV-RNA was $7.0*10^5$ IU/ml with a 1b genotype. Abdominal ultrasonography found no hepatomegaly and normal bilateral renal morphology.

Bone marrow puncture and biopsy revealed actively proliferative hematopoietic tissue in the bone marrow with aggregation of reactive lymphocytes. Immunohistochemistry indicated that the lymphocytes were focally positive for CD20, CD3ε and CD5. Cloned B-cell lymphoma accounted for 12% of the nucleated cells by flow cytometry. CD19 and CD20-positive B-cell oligo-monoclonal expansion in the BM was found (Figure 1). The morphology and immunohistochemistry suggested B-NHL.

A percutaneous renal biopsy demonstrated marked endocapillary hypercellularity characterized by mesangial matrix and endothelial cell expansion with little neutrophil infiltration and pronounced lobulation of the glomeruli (Figure 2). There was a percentage of 5-8% renal tubular atrophy and amount of protein cast deposited in the tubular lumen. Immunofluorescence revealed glomerular staining for C3 (+ to ++), IgM (+ to ++), and C1q (± to +), but no staining for IgG, IgA, C4 or FN. Several glomeruli stained positive for CD68 (PGM-1) and CD68 (KP-1) (Figure 3). Both κ and λ were non-specifically detected in a few capillary endothelial cells. Electron microscopy revealed diffuse mesangial cell proliferation with a few deposits in the mesangial area, subendothelial area and paramesangial area (Figure 4 A, B). Electron microscopy also demonstrated amorphous sediment particle-like

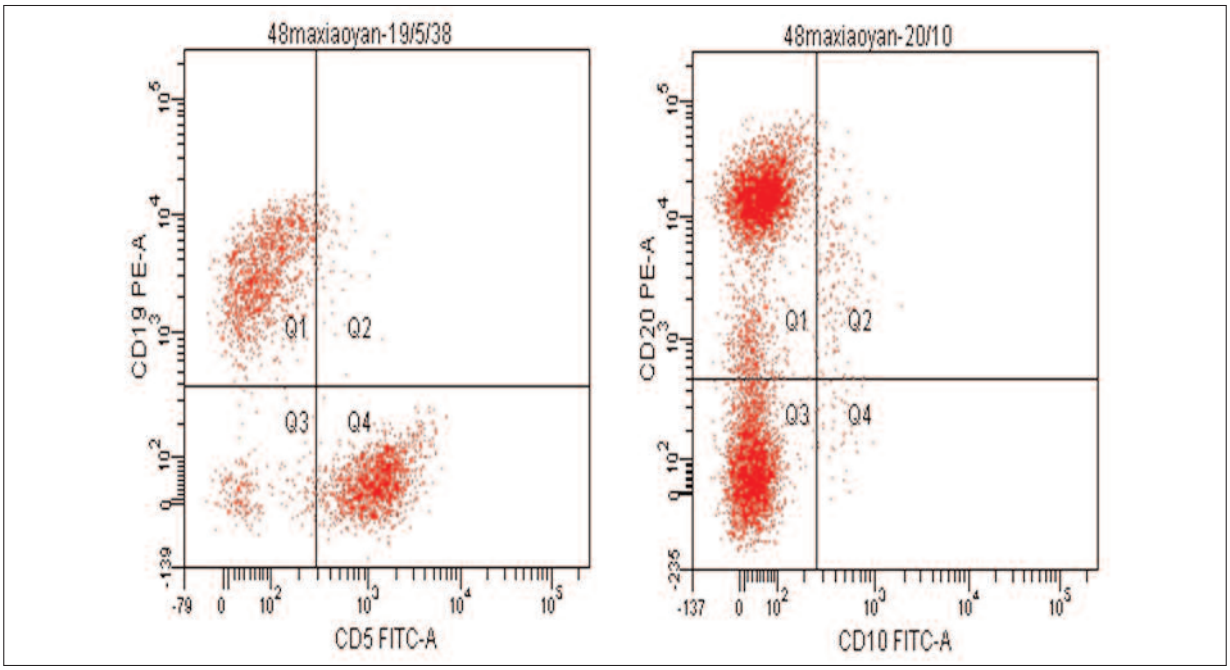

Figure 1. Flow cytometry: CD 19 and CD20-positive B-cell oligo-monoclonal expansion in the bone marrow.

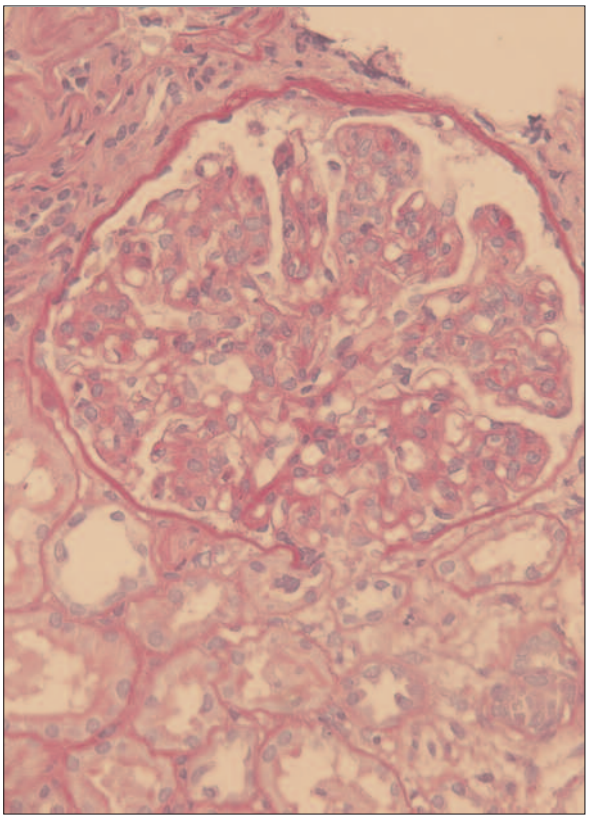

Figure 2. Renal biopsy: diffuse endocapillary hypercellularity characterized by mesangial matrix and endothelial cell expansion with few neutrophil infiltration were demonstrated. Lobulation of the glomeruli was revealed by light microscopy (PAS stain, × 400).

electron-dense deposits under the basement membrane, which were assumed to represent circulation immune complexes (Figure 4 C). These findings led to the diagnosis of cryoglobulinemic EnPGN.

The final diagnosis was HCV-infection-related cryoglobulinemic EnPGN and B-NHL. Recombinant human interferon-alfa-2b (IFN-α-2b) was administered at a dose of 3 million units every other day for 3 months, followed by pegylated INF-α-2b (Peg-Intron, Schering-Plough, USA) 30 μg weekly for 9 months. A rapid response was observed in this patient, characterized by the disappearance of proteinuria and the inactivity of the urinary sediment (Table I). There was no recurrence of renal damage or HCV antibodies during a 1-year follow-up period.

Discussion

HCV infection leads to chronic liver disease and extrahepatic manifestations. Up to 40-74% of patients infected with HCV develop at least one extrahepatic manifestation during the course of the disease, including cryoglobulinemia, lymphoproliferative disorders, renal disease, porphyria cutanea tarda, Mooren's corneal ulcer and keratoconjunctivitis sicca[7]. Cryoglobulins precipitate on the vascular endothelium of small to medium-sized blood vessels of various organs,

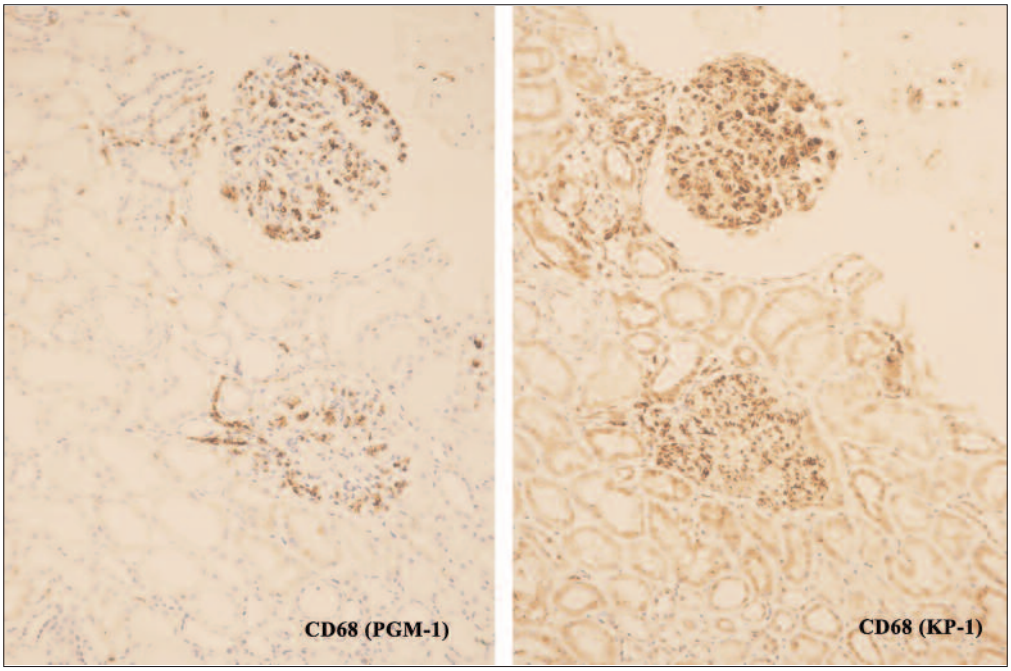

Figure 3. Renal biopsy: several glomeruli were positively staining with CD68 (PGM-1) and CD68 (KP-1) by immunofluorescence (× 400).

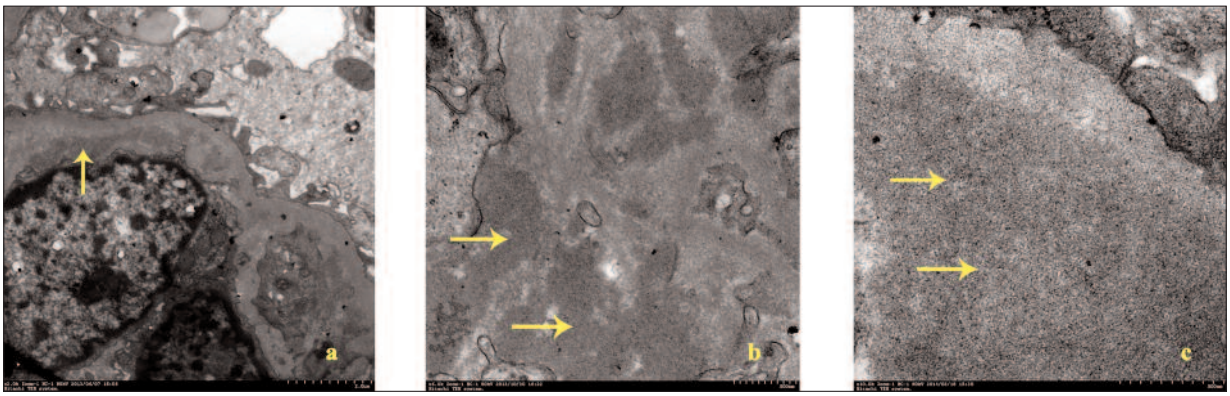

Figure 4. Renal biopsy: ***A,*** Electron microscopy showed that subendothelial electron-dense deposits (× 20,000). ***B,*** Paramesangial electron-dense deposits (× 60,000). ***C,*** Amorphous sediment particle-like electron-dense deposits under the glomerular basement membrane (× 100,000). The arrows point to the electron-dense deposits.

such as the skin, kidneys and peripheral nerves, and may cause vasculitis syndrome[8]. Common manifestations of HCV-associated cryoglobulinemic vasculitis include skin lesions (95%), arthralgia (53%), frequent peripheral nervous system involvement (66-74%) and glomerulonephritis (30-34%)[7,9,10].

HCV-associated cryoglobulinemic glomerulonephritis is due to the deposition of immune complexes composed of HCV antigen, anti-HCV IgG antibodies, Toll-like receptor (TLR) agonists, complement and IgM with rheumatoid factor activity[11]. The immune complexes may promote local inflammation at the site of deposition. The positive staining for C1q observed during the immunofluorescence study of our patient indicated that efficient engagement of C1q by cryoglobulins plays an important pathogenetic mechanism in cryoglobulinemic glomerulonephritis[12]. Clinically, patients with HCV infection-related cryoglobulinemic glomerulonephritis might present with various degrees of proteinuria and microscopic hematuria. Patients whose condition is serious may exhibit nephrotic syndrome and acute nephritic syndrome with rapid renal impairment[9]. During immunofluorescence studies, subendothelial deposits of IgM and IgG were detected in 91.7% of patients. Under electron microscopy, lattice or microtubule-like electron-dense deposits may be observed deposited under the subendothelial and glomerular capillary lumen[9]. The renal pathology of this patient often mimicked HSPN. However, the negative staining for IgA on this patient's renal biopsy can differentiate her presentation from that of HSPN. Further more, the level of serum C3 and C4 are near normal in patients with HSPN, whereas low levels of C3 and undetectable levels of C4 have been shown in cryoglobulinemia patients with HCV infection[13,14]. For patients with severe HSPN, glucocorticoid treatment with or without cytotoxic agents has been suggested. However,

Table I. Laboratory parameters of our case after 1 year's treatment.

Variable	On admission	3 months after treatment	12 months after treatment
Serum chemistry			
HCV-RNA (IU/ml)	7.0*105	1.39*103	1.12*103
C4 (g/L)	0.0317	0.0397	0.0466
C3 (g/L)	0.5040	0.8071	0.9130
RF (IU/ml)	4900	No data	1350
monoclonal M protein (%)	9	8.8	6.1
IgM (mg/L)	7220	6080	5440
Serum cryoglobulinemia	Positive	Positive	Positive
Urinalysis			
Proteinuria by urine routine test (g/L)	0.6 (1+)	0.2 (+/-)	0.2(+/-)
RBC by urine routine test (per high-power field)	93	15	20

our patient with HCV-associated cryoglobulinemic EnPGN responded rapidly to interferon.

Among patients infected with HCV and presenting with mixed cryoglobulinemia, approximately 37% also had B-cell lymphoma[15]. Only 10% of B-NHL cases have been attributed to HCV infection[16]. The mechanism of HCV-induced B-NHL involves the hepatitis C virus binding to the tetraspanin CD81 ligand on the surface of B lymphocytes via the HCV envelope E2 protein. This interaction could cause the aberration of Bcl2, a proto-oncogene that can inhibit apoptosis, and therefore induce B-cell lymphoproliferation[17]. The renal injury of our patient involved lymphoma. CD68 is a special biomarker of the monocyte-macrophage cell lineage, which might be expressed in various types of NHL[18]. The positive staining of both CD68 (KP-1) and CD68 (PGM-1) indicates that the kidney lesion might also be associated with lymphoma.

The diagnosis of HCV-associated glomerulonephritis was difficult to establish because HCV is an RNA virus and, therefore, susceptible to damage during fixation and prolonged paraffin embedding[19]. Cryoglobulinemic vasculitis secondary to HCV infection was the crucial mechanism of HCV-induced organ injury in this case. The treatment of HCV-related cryoglobulinemic glomerulonephritis should be individualized according to the severity of disease. For patients with mild or moderate disease activity, pegylated IFN-α combined with ribavirin (at a dose adapted for the creatinine clearance) for at least 12 months is the current standard of care for anti-HCV therapy, although more aggressive therapy (i.e., plasmapheresis and methylprednisolone) is required in patients with severe conditions[9]. For patients with low-grade B-cell lymphoma, limited available data suggest that interferon therapy might also reverse its progress.

Conclusions

We presented a rare case of HCV-related mixed cryoglobulinemic EnPGN and B-NHL. IFN has been shown to be effective in mix cryoglobulinemia patients with renal involvement and B-NHL.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Carvalho-Filho RJ, Narciso-Schiavon JL, Tolentino LH, Schiavon LL, Ferraz ML, Silva AE. Central nervous system vasculitis and polyneuropathy as first manifestations of hepatitis C. World J Gastroenterol 2012; 18: 188-191.
2) Ko HM, Hernandez-Prera JC, Zhu H, Dikman SH, Sidhu HK, Ward SC, Thung SN. Morphologic features of extrahepatic manifestations of hepatitis C virus infection. Clin Dev Immunol 2012; 2012: 740138.
3) Alpers CE, Smith KD. Cryoglobulinemia and renal disease. Curr Opin Nephrol Hypertens 2008; 17: 243-249.
4) Lo KY, Chen CY, Lee CS. Hepatitis C virus-associated type II mixed cryoglobulinemia vasculitis complicated with membranous proliferative glomerulonephritis. Ren Fail 2009; 31: 149-152.
5) Besson C, Canioni D, Lepage E, Pol S, Morel P, Lederlin P, Van Hoof A, Tilly H, Gaulard P, Coiffier B, Gisselbrecht C, Brousse N, Reyes F, Hermine O; Groupe d'Etude des Lymphomes de l'Adulte Programs. Characteristics and outcome of diffuse large B-cell lymphoma in hepatitis C virus-positive patients in LNH 93 and LNH 98 Groupe d'Etude des Lymphomes de l'Adulte programs. J Clin Oncol 2006; 24: 953-960.
6) Ennishi D, Maeda Y, Niitsu N, Kojima M, Izutsu K, Takizawa J, Kusumoto S, Okamoto M, Yokoyama M, Takamatsu Y, Sunami K, Miyata A, Murayama K, Sakai A, Matsumoto M, Shinagawa K, Takaki A, Matsuo K, Kinoshita T, Tanimoto M. Hepatic toxicity and prognosis in hepatitis C virus-infected patients with diffuse large B-cell lymphoma treated with rituximab-containing chemotherapy regimens: a Japanese multicenter analysis. Blood 2010; 116: 5119-5125.
7) Galossi A, Guarisco R, Bellis L, Puoti C. Extrahepatic manifestations of chronic HCV infection. J Gastrointestin Liver Dis 2007; 16: 65-73.
8) Charles ED, Dustin LB. Hepatitis C virus-induced cryoglobulinemia. Kidney Int 2009; 76: 818-824.
9) Terrier B, Cacoub P. Cryoglobulinemia vasculitis: an update. Curr Opin Rheumatol 2013; 25: 10-18.
10) Apartis E, Léger JM, Musset L, Gugenheim M, Cacoub P, Lyon-Caen O, Pierrot-Deseilligny C, Hauw JJ, Bouche P. Peripheral neuropathy associated with essential mixed cryoglobulinaemia: a role for hepatitis C virus infection? J Neurol Neurosurg Psychiatry 1996; 60: 661-666.
11) Kamar N, Rostaing L, Alric L. Treatment of hepatitis C-virus-related glomerulonephritis. Kidney Int 2006; 69: 436-439.
12) Sansonno D, Tucci FA, Ghebrehiwet B, Lauletta G, Peerschke EI, Conteduca V, Russi S, Gatti P, Sansonno L, Dammacco F. Role of the receptor for the globular domain of C1q protein in the pathogenesis of hepatitis C virus-related cryoglobulin vascular damage. J Immunol 2009; 183: 6013-6020.

13) Sansonno D, Gesualdo L, Manno C, Schena FP, Dammacco F. Hepatitis C virus-related proteins in kidney tissue from hepatitis C virus-infected patients with cryoglobulinemic membranoproliferative glomerulonephritis. Hepatology 1997; 25: 1237-1244.

14) Tarantino A, Campise M, Banfi G, Confalonieri R, Bucci A, Montoli A, Colasanti G, Damilano I, D'Amico G, Minetti L, et al. Long-term predictors of survival in essential mixed cryoglobulinemic glomerulonephritis. Kidney Int 1995; 47: 618-623.

15) Nicolau A, Tanasescu R, Balanescu E, Balanescu P, Patra cu R, Tanasescu C. Hepatitis C virus-mixed cryoglobulinemia-lymphoma relationship. Rom J Intern Med 2011; 49: 3-10.

16) Negri E, Little D, Boiocchi M, La Vecchia C, Franceschi S. B-cell non-Hodgkin's lymphoma and hepatitis C virus infection: a systematic review. Int J Cancer 2004; 111: 1-8.

17) Ferri C, Zignego AL. Relation between infection and autoimmunity in mixed cryoglobulinemia. Curr Opin Rheumatol 2000; 12: 53-60.

18) Lin Z, Chen B, Xu X, Wang X, Lin G. Analysis of clinical characteristics of 516 patients with non-Hodgkin's lymphoma in Shanghai area. Hematology 2014; 19: 99-107.

19) Sabry AA, Sobh MA, Irving WL, Grabowska A, Wagner BE, Fox S, Kudesia G, El Nahas AM. A comprehensive study of the association between hepatitis C virus and glomerulopathy. Nephrol Dial Transplant 2002; 17: 239-245.