



Treatment Related Hematologic Changes in a Population of Iranian Patients with Chronic Hepatitis C Infection from 2009 to 2014

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Abstract

Background: We aimed to assess the frequency of hematologic changes, response to treatment, rate of discontinuation and dose reduction in Hepatitis C positive patients, treated with Interferon and ribavirin from Apr 2009 to Mar 2014.

Methods: In this cross-sectional study, out of registered patients, 554 were assessed and 150 patients with positive HCV PCR, regular patient visits to clinic and complete records were included. HCV viral load, complete blood count and liver enzyme levels were measured before initiation of treatment and monthly. Exclusion criteria's were other types of hepatitis and HIV infection, autoimmune or blood diseases, illegal drug use and treatment with bone marrow suppressors. The data was analyzed using SPSS.

Results: Out of 150 patients, 135 (90%) were male. Mean age was 39.7 ± 10.7 (range 23-74) yr old. Forty-six patients (30.7%) had genotype 1 and 99 (66%) genotypes 2 and 3. Treatment regimens were prescribed as Pegafron+Ribavirin in 125 (83.3%), Interfron+Ribavirin 9 (6%) and Pegintron+Ribavirin in 16 (10.7%) of patients... The prevalence of anemia in genotype 1 patients was higher ($P=0.044$). There was no association between sex and leukocyte changes. Thirty-four (22.7%) patients had moderate and severe thrombocytopenia. Six patients had severe anemia and Ribavirin dose was adjusted.

Conclusion: The hematological changes are common side effects of conventional hepatitis C treatment regimens. Although drug dose adjustment is not usually necessary, due to severe anemia in genotype 1 patients, we should treat high-risk patients cautiously and made the appropriate changes in drug dosage at the right time.

Keywords: Interferon, Ribavirin, Leukopenia, Anemia, Thrombocytopenia, Hepatitis C, Genotype

Introduction

Hepatitis C is a chronic inflammatory disease of the liver caused by hepatitis C virus (HCV), an RNA virus and a member of the family flaviviridae. The most common route of transmission of this disease is via contaminated blood (1).

Currently, about 170 million people are infected with HCV worldwide (2). Hepatitis C is the most common cause of chronic liver diseases after al-

coholic hepatitis. Number of people with positive hepatitis C is less than 1% in different cities of Iran (1). The disease is chronic in the majority of patients and leads to cirrhosis and liver cancer and the patient needs to liver transplantation (1, 3). Hence, treatment of hepatitis C and prevention of progress to cirrhosis is important and eradication of HCV is the goal of the treatment.

During the past decade, there has been great improvement in the treatment of HCV-positive patients. Available treatments for hepatitis C in Iran are combination of conventional or pegylated interferon (PEG-IFN) and ribavirin (RBV) for 24 to 48 wk, according to virus genotype, which resulted in about 50% SVR (Sustained virologic response) for genotype 1 and 80% for genotype 2 and 3 (1, 4).

Two types of interferon are approved for treatment of hepatitis C includes PEG-IFN alfa-2a (Pegasys or Pegaferon) and peg-IFN alfa-2b (Pegintron). Standard duration of treatment for PEG IFN plus RBV is about 48 wk for genotype 1 or 4 and 24 wk for genotype 2 or 3.

Combination therapy can always be accompanied by a series of adverse effects, including Flu like syndrome, depression, autoimmune disorders, and hematologic adverse effects such as anemia, neutropenia, and thrombocytopenia that occur in about 32% of patients (5).

Hematologic adverse effects are well tolerated in most cases and only in rare cases lead to a dose reduction. In this case, there is no accurate data and statistics. In this study, we aimed to assess the frequency of hematologic changes and the effects of them on the response to treatment, and discontinuation or dose reduction of treatment regimens.

Materials and Methods

In a cross-sectional study, all patients with positive hepatitis C referred to Hepatitis Clinic of Imam Khomeini Hospital Complex, Tehran, Iran, were eligible for treatment of chronic hepatitis from Apr 2009 to Mar 2014 after exclusion criteria applied. With convenient sampling, out of registered documents in our center, 554 patients were assessed and 150 patients were included (Fig. 1).

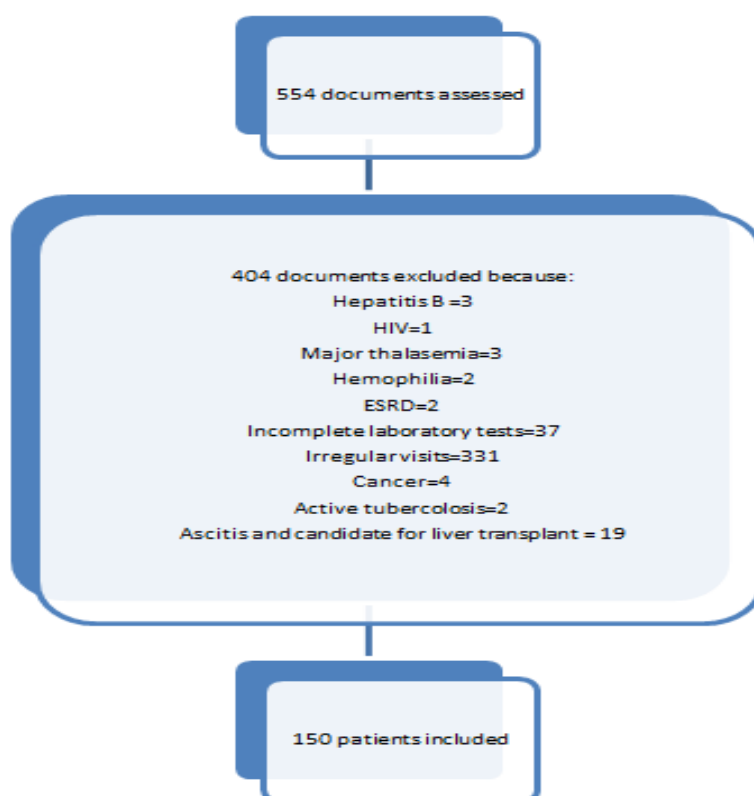


Fig. 1: Diagram for the inclusion of the patients

The study was approved ethically and methodologically by research council of infectious diseases department of Tehran University of Medical Sciences.

Hepatitis C viral load, complete blood count (CBC) and liver enzyme tests include:

(AST, ALT, Bill, PT, INR) were measured before initiation of treatment. Duration of treatment was determined depending on the genotype of the virus from 6 months to 1 year.

During the treatment period, AST, ALT, and CBC rechecked monthly. CBC performed with automated analyzer.

All of patients were visiting every month and following of any new signs or symptoms, especially those related to infections such as fever, chills, cough, and dysuria by an infectious disease specialist.

Inclusion criteria were

1. A definitive diagnosis of hepatitis C (positive HCV RNA PCR)
- 2 - Regular patient visits to clinic (at least every two months)
3. Hematologic changes recorded on a regular basis (at least every two months)

Exclusion criteria were

- 1- Other types of hepatitis (B, D) and HIV-1 infection
- 2- Autoimmune diseases, blood disorders (leukemia, lymphoma, hemophilia), renal diseases that need blood transfusion, symptomatic cardiovascular disease
- 3- Taking illegal drugs during treatment of hepatitis C
- 4- Treatment with bone marrow suppressive drugs in last six months

Definitions

Anemia was defined as hemoglobin concentration 10-12 gr/dl (mild to moderate) and <10 gr/dl (severe).

Leukopenia was defined as WBC count 2000-4000 cells/ μ L (mild to moderate), and <2000 cells/ μ L (severe).

Thrombocytopenia was defined as platelet count < 150,000/ μ L. This was further classified as mild to moderate (platelet count 100,000/ μ L-150,000/ μ L), and severe (<100,000/ μ L) thrombocytopenia.

Laboratory tests such as complete blood cell count, bleeding associated with hematologic complications, infection secondary to neutropenia, the drug dose, and the rates of discontinuation were measured.

Independent variables including: age, sex, underlying disease (Such as diabetes mellitus, chronic renal failure without the need for treatment of anemia, chronic stable and asymptomatic heart and or lung disease), duration of HCV infection, and history of HCV treatment, liver transaminase levels, genotype and viral load of virus were assessed.

The supervisor assessed the accuracy of the collected data. Data were analyzed statistically by SPSS 16.0 (Chicago, IL, USA). The level of significance was considered at $P \leq 0.05$.

Maintenance of the information of all patients at all stages of the research was a priority. There was no cost for patients. All the states of the Helsinki principles were applied.

Results

Out of 554 registered patients, 150 (135 male and 15 female) were included. Mean age was 39.7 ± 10.7 (range 23-74) yr old. Inclusion chart of patients is shown in Fig. 1.

Five patients (3.3%) had previous history of treatment with Interferon. Thirteen patients (8.7%) had underlying diseases including diabetes mellitus (3 patients), hypothyroidism (3 patients), lichenplanus (2 patients) and others (5 cases).

Forty-six patients (30.7%) had genotype 1 and 99 (66%) genotypes 2 and 3 of hepatitis C virus. Genotyping was not done for 5 (3.3%) patients.

Treatment regimens were prescribed as Pegafon+Ribavirin in 125 (83.3%), Interferon+Ribavirin 9 (6%) and Pegintron+Ribavirin in 16 (10.7%) of patients.

Hematologic changes

Out of 150 patients, hemoglobin concentrations of 72 (48%) patients were in normal ranges. Median of hemoglobin concentration was 15.3 gr/dl (IQR: 14.1-16.3) before initiation of treatment, it decreased to 13.95 gr/dl (IQR: 12.6-15.1)

after one month and 12.42(IQR:11.6-13.2) after 6 months of treatment (Fig. 2). Frequency of Hematologic changes according to gender; underlying disease, treatment regimen, genotype, age and viral load are shown in Table 1.

Table 1: Freq. of hematologic changes according to gender, underlying disease, treatment regimen, genotype, age and viral load

	Group	Gender male N(%)	Underlying disease Yes			Treatment regimen			HCV Genotype		Age mean(SD)	Viral load
			N=13 n(%)	Pegaftron+Rib N=125	Interfron+Rib N=9	Pegintron+Rib N=16	1N=46	2 and 3 N=99				
Hb changes	>12 mg/dl	70 (51.9)	2(15.4)	58(46.8)	5 (55.6)	8(50)	16(34.8)	54(54.5)	35.8(7.1)	2915849(6333916.8)		
	10 to 12 mg/dl	51 (37.8)	7(53.8)	51(41.1)	4(44.4)	6(37.5)	22(47.8)	38(38.4)	41.2(11.6)	2312805(3969429.2)		
	<10 mg/dl	14 (10.3)	4(30.8)	15(12.1)	0(0)	2(12.5)	8(17.4)	7(7.1)	51.3(11.7)	878154.3(1513051.3)		
	P-value	0.018	0.014		0.931		0.044	<0.001	0.349			
WBC changes	>4000	30(21.6)	1 (7.7)	29(22.6)	3(33.3)	2(12.5)	7(15.2)	27(27.3)	35.7(9.3)	1335053(3030322.09)		
	2000 to 4000	92(68.7)	12(92.3)	82(66.1)	6(66.7)	12(75)	34(73.9)	63(63.6)	40.3(11)	2996404(5801847.4)		
	<4000	13(9.7)	0(0)	14(11.3)	0(0)	2(12.5)	5(10.9)	9(9.1)	45.1(9.8)	1026133(1944742.6)		
	P-value	0.337	0.15		0.68		0.28	0.028	0.0161			
Platelet changes	>150000	58(42.5)	7(53.8)	52(41.1)	7(77.8)	5(31.3)	18(39.1)	46(46.5)	36.8(10.3)	1847576(3649004.8)		
	100000 to 150000	48(35.8)	5(38.5)	44(35.5)	1(11.1)	7(43.8)	18(39.1)	34(34.3)	39.5(8.9)	3124755(6771816.4)		
	<100000	29(21.6)	1(7.7)	29(23.4)	1(11.1)	4(25)	10(21.7)	19(19.2)	45.4(11.7)	2431803(4007733.02)		
	P-value	0.571	0.487		0.338		0.71	0.02	0.418			

WBC changes during treatment

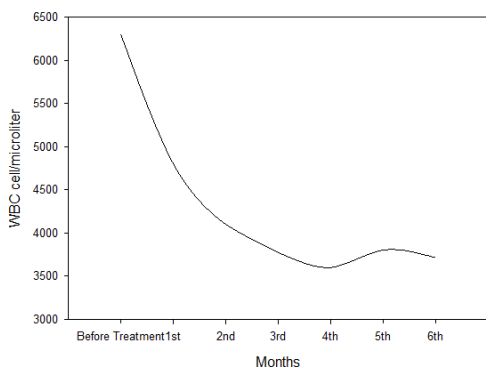


Fig. 2: Hemoglobin changes during treatment

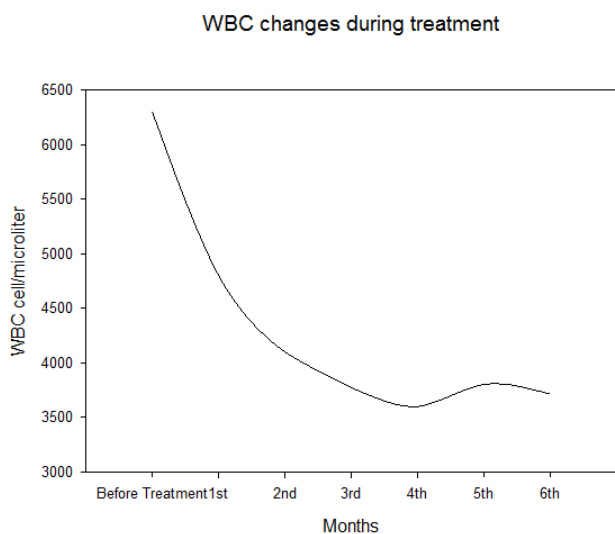


Fig. 3: WBC changes during treatment

Anemia in women was more severe than men. The age of patients with severe anemia was older than patients with mild anemia or normal hemoglobin. The prevalence of anemia in people with underlying disease was higher than those without underlying disease. A significant relation between history of previous treatment and anemia was not observed. The prevalence of anemia in patients with genotype 1 virus was higher ($P=0.044$). No relation between the type of treatment and hemoglobin changes were observed. However, there was no association between sex and leukocyte changes, the changes in leukocyte count were higher in older patients. Moreover, we found an association between leukocyte count and the history of previous treatment.

Genotype of the virus and the type of treatment had no association with leukocyte changes.

Median of WBC count was 6300 cell/microL (IQR: 4200-7787.5) before initiation of treatment, it decreased to 4800 cell/microL (IQR: 3515-5950) after one month and 3717.22 cell/microL (IQR: 3525-4100) after 6 months of treatment (Fig. 3).

Leukopenia is not significantly associated with serious infection. In our study, we just had one case of inguinal abscess that refers to surgeon for

drainage; the patient had a previous history of splenectomy.

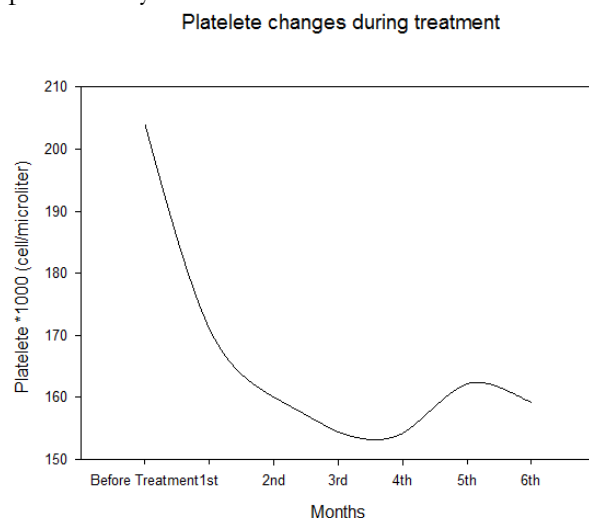


Fig. 4: Platelet changes during treatment

Due to the unavailability of differentiation count results for some of our patients, we could not report the number of absolute neutrophils counts (ANC).

Median of platelet count was 204000 cell/microL (IQR: 162250-238750) before initiation of treatment, it decreased to 171000 cell/microL (IQR: 141000-220750) after one month and 159150 cell/microL (IQR: 135250-188000) after 6 months of treatment (Fig. 4).

Fifty-two (34.6%) patients had mild and 34 (22.7%) had moderate and severe thrombocytopenia.

Older age and history of previous treatment for hepatitis C had association with thrombocytopenia. All of the patients with previous history of treatment catch thrombocytopenia. However, there was no association between the genotype of virus or treatment regimen with the count of platelet. Drug dosage was changed in 9 (6%) patients because of hematologic side effects. Six patients had severe anemia and Ribavirin dose was adjusted.

One patient had thrombocytopenia and two other patients had neutropenia then dose of interferon was adjusted.

One patient had platelet count lower than 25000 that was simultaneous with the end of treatment period.

Drug dose was changed in nine (6%) patients because of hematologic side effects (six cases of

anemia, one case of thrombocytopenia and two cases of neutropenia). Frequency of other adverse effects and actions are showed in Table 2.

Table 1: Frequency of non-hematologic treatment related side effects and actions

<i>Adverse events</i>	<i>n (%)</i> <i>N=150</i>	<i>Actions</i>
Weakness	11(7.3)	Symptomatic therapy
Syncope	-	-
Cough and sputom	2 (1.3)	Assesment for infections were negative
Inguinal abscess	1(0.6)	Surgical drainage
Dental abcess(without fever)	1(0.6)	Temporary discontinuation of treatment
Epistaxis	1(0.6)	Symptomatic therapy
GI bleeding	2 (1.3)	Admission and treatment
Vaginal bleeding	1(0.6)	Symptomatic therapy
Itching	4(2.6)	Symptomatic therapy
Itching with skin lesions	1(0.6)	discontinuation of treatment
Erythrodermia	1(0.6)	discontinuation of treatment
Facial nerve palsy	1(0.6)	discontinuation of treatment
Resistant ascites	1(0.6)	discontinuation of treatment
Total	27 (100)	-

Discussion

We found a low rate (6%) of drug dose adjustment due to hematologic changes in spite of relatively medium to high rate of anemia, leukopenia, and thrombocytopenia among 150 patients evaluated for hematological changes of hepatitis C treatment. In our study, 90% of patients were male. In other studies, the number of male patients was higher than women too (6-8).

Female gender due to several factors has a lower risk of chronic HCV infection. Allele specific gene IL28B, enhance the ability of infected person to clear hepatitis C virus from the body (9, 10). Moreover, mononuclear cells in females produce higher amount of INF-a by TLR7 stimulation thus can cause stronger innate immunity against viral infections (11).

The higher prevalence of risky behavior such as intravenous drug use among males in most regions including in our country is another explanation for higher prevalence of HCV infection in males. Therefore, based on the informa-

tion it is expected to find the most cases of chronic hepatitis among men.

The mean age of the patients in our study was about 39 yr. In other studies, most of patients were in the third to fifth decades of life and the mean age was 33 to 54 yr (6, 8).

In all studies, including our study, younger age could be a warning to policy makers and health authorities. Because the age of high risk behavior has declined in recent decades.

In treatment of chronic hepatitis C patients, combination of interferon or PEGylated interferon (PEG-IFN) plus ribavirin is the recommended form of therapy because of availability, efficacy and also costs of treatment (12, 13). This combination has some hematologic side effects, like anemia, leukopenia, and thrombocytopenia.

Anemia

The combination therapy was associated with hematological disorders. Anemia was the most important side effect because it can reduce health-related quality of life. Patients with anemia

have symptoms like weakness and fatigue that affect their function. Anemia is one of the main reasons for change in dosage or discontinuation of treatment (14). In our study, anemia had the greatest role in changing the dose of drugs.

Interferon has inhibitory effects on bone marrow, and reduced levels of blood cells, including the count of RBCs. Moreover, Ribavirin can cause RBC lysis and enhances the inhibitory effect of interferon (15). Therefore, the combination of these two drugs has synergistic effects and decrease hemoglobin concentration. This anemia is called mixed anemia (16-18).

We changed the treatment regimen in 6 (4%) patients, but in previous studies, the frequency was higher and reported from 7% to 31% (12, 16).

The incidence of anemia in non-PEGylated IFN-treated patients is higher than the PEG-IFN- α (19). Ribavirin doses greater than 800mg/day, especially higher than 1000 mg/day is accompanied by higher anemia rate. However, we did not find any significant relationship between anemia and type of treatment. Usually, hemoglobin level drops at 4-8 wk of treatment and then remained at a plateau state and after end of treatment; it will cure (14). We found older age, female gender, having underlying diseases and genotype 1 HCV infection, as risk factors for anemia.

Neutropenia

The most common side effect of combination therapy with Peg-IFN α and Ribavirin was severe neutropenia in a randomized control trial and these results were found in other studies (5, 6, 20). In a clinical trial on patients with HCV, neutropenia is the most common reason for dose reduction of PEG-INF in combination therapy regimens (14). Peg-IFN compared to the standard-IFN α has a higher risk for neutropenia and neutropenic risk may be dose-dependent (12).

In our study, frequency of severing leukopenia was 10.7% that is higher than previous reports. We did not consider to the WBC count before initiation of treatment, and this item could affect our findings. Use of Peg-IFN α and Ribavirin in most of our study patients may consider as a reason for higher prevalence of neutropenia in our

study. However, like other studies, we found that leukopenia is not significantly associated with serious infection. Older age and positive history of previous treatment were two risk factors of neutropenia in our patients.

Thrombocytopenia

Thrombocytopenia is one of side effects of interferon. Thrombocytopenia in hepatitis C patients can happen; therefore, about 22% of patients with thrombocytopenia are seropositive for HCV, as reported in a previous study (21).

We found that older patients more likely to have thrombocytopenia. Platelet count lesser than 50000/ml was found in patients that have platelet count lesser than 100000/ml before the initiation of treatment. Similar results were found (22).

Dosage of PEG-IFN α -2b plus Rib can be changed because of severe thrombocytopenia (23). Drug dosage was changed just in one of our patients (0.6%) because of thrombocytopenia.

Conclusion

The hematological changes are common side effects of conventional hepatitis C treatment regimens. Although drug dose adjustment is not usually necessary, due to severe anemia in older patients and patients with genotype 1, we should treat high-risk patients cautiously and made the appropriate changes in drug dosage at the right time.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Khodabandehloo M, Roshani D (2014). Prevalence of hepatitis C virus genotypes in Iranian patients: a systematic review and meta-analysis. *Hepat Mon*, 4(12):e22915.
2. Li HC, Lo SY (2015). Hepatitis C virus: Virology, diagnosis and treatment. *World J Hepatol*, 7(10): 1377-89.
3. Seeff LB (2002). Natural history of chronic hepatitis C. *Hepatology*. 36: S35-46.
4. Strader DB, Wright T, Thomas DL, Seeff LB (2004). American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C. *Hepatology*, 9(4):1147-71.
5. Fried MW (2002). Side effects of therapy of hepatitis C and their management. *Hepatology*, 36:S237-244.
6. Roomer R, Hansen BE, Janssen HL, de Knegt RJ (2010). Risk factors for infection during treatment with peginterferon alfa and ribavirin for chronic hepatitis C. *Hepatology*, 52(4):1225-31.
7. Serrano-Villar S, Quereda C, Moreno A, Pérez-Eliás MJ, Casado JL, Royuela A, Dronda F, Navas E, Hermida JM, Moreno S (2013). Neutropenia during therapy with peginterferon and ribavirin in HIV-infected subjects with chronic hepatitis C and the risk of infections. *Clin Infect Dis*, 57(3):458-64.
8. Antonini MG, Babudieri S, Maida I, Baiguera C, Zanini B, Fenu L, Dettori G, Manno D, Mura MS, Carosi G, Puoti M (2008). Incidence of neutropenia and infections during combination treatment of chronic hepatitis C with pegylated interferon alfa-2a or alfa-2b plus ribavirin. *Infection*, 36(3):250-5.
9. van den Berg CH, Grady BP, Schinkel J et al (2011). Female sex and IL28B, a synergism for spontaneous viral clearance in hepatitis C virus (HCV) seroconverters from a community-based cohort. *PLoS One*, 6(11):e27555.
10. Grebely J, Page K, Sacks-Davis R et al (2014). The effects of female sex, viral genotype, and IL28B genotype on spontaneous clearance of acute hepatitis C virus infection. *Hepatology*, 59(1):109-20.
11. Berghöfer B, Frommer T, Haley G, Fink L, Bein G, Hackstein H (2006). TLR7 ligands induce higher IFN-alpha production in females. *J Immunol*, 15; 177(4):2088-96.
12. Fried MW, Shiffman ML, Reddy KR et al (2002). Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med*, 26;347(13):975-82.
13. Sun LJ, Yu JW, Kang P, Zhao YH, Yan BZ (2012). A study of the relationship between neutropenia and clinical infection risk during treatment with peginterferon alfa-2a and ribavirin for chronic hepatitis C. *Zhonghua Nei Ke Za Zhi*, 51(1):46-50.
14. Ong JP, Younossi ZM (2004). Managing the hematologic side effects of antiviral therapy for chronic hepatitis C: anemia, neutropenia, and thrombocytopenia. *Cleve Clin J Med*, 71 ; 3:S17-21.
15. Sulkowski MS (2003). Anemia in the treatment of hepatitis C virus infection. *Clin Infect Dis*, 37: S315-22.
16. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD, Ling MH, Cort S, Albrecht JK (1998). Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med*, 339(21):1485-92.
17. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C, Albrecht J (1998). Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for

- treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet*, 31:1426-32.
18. Chang CH, Chen KY, Lai MY, Chan KA (2002). Meta-analysis: ribavirin-induced haemolytic anaemia in patients with chronic hepatitis C. *Aliment Pharmacol Ther*, 16(9):1623-32.
 19. Dieterich DT, Spivak JL (2003). Hematologic disorders associated with hepatitis C virus infection and their management. *Clin Infect Dis*, 37(4):533-41.
 20. Manns MP, McHutchison JG, Gordon SC et al (2001). Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet*, 22; 358(9286):958-65.
 21. García-Suárez J, Burgaleta C, Hernanz N, Albarran F, Tobaruela P, Alvarez-Mon M (2000). HCV-associated thrombocytopenia: clinical characteristics and platelet response after recombinant alpha2b-interferon therapy. *Br J Haematol*, 110(1):98-103.
 22. Lin KH, Hsu PI, Yu HC, Lin CK, Tsai WL, Chen WC, Chan HH, Lai KH (2012). Factors linked to severe thrombocytopenia during antiviral therapy in patients with chronic hepatitis c and pretreatment low platelet counts. *BMC Gastroenterol*, 18:12:7.
 23. Kowdley KV (2005). Hematologic side effects of interferon and ribavirin therapy. *J Clin Gastroenterol*, 39:S3-8.