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Neutropenia and viral load decline during treatment of hepatitis C virus genotype-4

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Original Article

ABSTRACT

Background/Aims: Neutrophil count and magnitude of decrease from baseline are not correlated with infection rate in recipients of interferon-based therapy for hepatitis C (HCV). The association of neutropenia with viral response raises the potential dilemma of trying to maintain patients on therapy despite adverse events. We studied the relationship between early viral clearance in response to treatment, and neutrophil count and fall in neutrophils in HCV-genotype 4 patients.

Materials and Methods: Two-hundred and one patients with HCV-genotype 4 were enrolled.

Results: Rapid and early virological responses (RVR and EVR) were achieved in 33.3% and 61.5% respectively. None of the patients developed symptomatic infection regardless of the degree of neutrophil decline. Neutrophil decline at week 2 significantly correlated with viral load at week 12 (r=0.40, p=0.042), and neutrophil decline at week 4 significantly correlated with viral load decline at week 12 (r=0.21, p=0.006). Using logistic regression, pretreatment neutrophil count significantly predicted RVR and EVR, such that individuals who achieved RVR and EVR had higher pretreatment neutrophils compared to non-responders (X2= 4.94, p=0.026; X2=7.67, p=0.005 respectively). Adjusting for age, sex, grade, fibrosis, and pretreatment neutropenia; decline in neutrophil count was significantly associated with lower viral load over time (t=-2.27, p=0.027) and higher viral load decline over time (t=2.73, p=0.009) and achieving EVR (t=2.04, p=0.044).

Conclusion: In genotype 4 patients, neutropenia can be a predictor of response. Neutropenia may reflect disappearance of genomic hepatitis C viral RNA from mononuclear cells. The relationship between neutropenia and response is confined to the first 12 weeks of therapy.

Keywords: HCV, genotype 4, neutropenia, pegylated interferon

INTRODUCTION

Neutropenia is a major adverse effect of interferon-α (IFN-α) therapy. The relationship between infectious complications and neutropenia was evaluated in recipients of interferon-based therapy for hepatitis C. The rates of total, fungal, viral, and bacterial infections did not correlate with the nadir neutrophil count or the magnitude of decrease from baseline. Thus neutrophil count was not correlated with infection rates in recipients of interferon-based therapy for hepatitis C (1). Suppressed granulocyte colony-stimulating factor (G-CSF) production in the presence of IFN-α may contribute to IFN-α-induced neutropenia (2). Neutropenia can be effectively treated using recombinant G-CSF (3); which is safe, well-tolerated, significantly improves patient quality of life, and is effective for preventing neutropenia and bacterial infections (4,5).

Hepatitis C virus (HCV) replication intermediates are found in extrahepatic tissues suggesting active viral replication in these cells. Recent reports suggested that peripheral blood mononuclear cells (PBMC) not only provide a reservoir, but also extrahepatic replication sites for HCV (6,7). Detectable HCV RNA in PMNC could be a factor influencing the natural history of the disease and patients with this finding have are more likely to be

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cirrhotic (8). The persistence of small quantities of HCV RNA in aviremic patients who have apparently resolved the infection could have important implications for viral transmission (9).The detection of HCV RNA in PBMC reservoirs might have important implications for effective treatment. One possible mechanism of relapse is that PBMC could serve as a reservoir for virus resistant to IFN (10). It was demonstrated that clearance of HCV RNA in PBMC at the end of IFN treatment was a predictor of durable response to antiviral therapy in patients with chronic hepatitis (11). On the contrary, Bernardin et al. reported that PBMC are unlikely to serve as a long-term reservoir of HCV in aviremic subjects (12).

The association of neutropenia with viral response raises the potential dilemma of trying to maintain patients on therapy despite the occurrence of adverse events and raises questions of whether there is correlation between response to treatment and neutropenia; and if the rate of neutrophil reduction could be an indicator of response to treatment.

MATERIALS AND METHODS

Patients selection

This analysis includes data from 241 patients with chronic HCV genotype 4 infection, who were treated and followed at the Hamad Medical Corporation outpatient clinic in the state of Qatar. Patients were considered to have chronic HCV infection if they had persistent increase in alanine aminotransferase (ALT), positive HCV serology, detection of HCV-RNA, and histological pattern of chronic active hepatitis. The study was approved by the ethics research committee of Hamad Medical Corporation and was performed according to the applicable regulations and ethical principles defined in the Declaration of Helsinki. All patients provided written informed consent to participate in the study. All patients were treated with a peginterferon/ ribavirin regimen: 180 µg of subcutaneous peginterferon-2α (Pegasys®, Hoffmann-LaRoche, Basel, Switzerland) once weekly and ribavirin (COPEGUS®; La Roche) given at an oral dose of 1000 mg (body weight ≤75kg) or 1200mg (body weight ≥75mg) for 48 weeks. Rapid viral response (RVR) was defined as loss of detectable serum HCV RNA at week 4 of treatment. Early viral response (EVR) was defined as loss of detectable serum HCV RNA at week 12 of treatment. Patients were excluded from receiving treatment if they had active alcohol consumption over 80 g/ day, concurrent hepatitis B virus infection, immunodeficiency viruses, autoimmune hepatitis, hemochromatosis, or were on antiviral or corticosteroid therapy.

Biochemical assay

After an overnight fast, blood samples were drawn and analysed for various metabolites and hormones. Aliquots of these samples were used for biochemical analysis immediately. White blood cell and differential including neutrophil count were obtained using the Automated Sysmex XE-2100 analyser (Sysmex, Kobe, Japan).

Virology

Hepatitis C virus genotype was determined by the Inno LiPA HCV II assay (Innogenetics Inc., Alpharetta, GA, USA). Serum levels of HCV-RNA were measured using RT-PCR (Amplicor Molecular System, version 2.0 Hoffmann-La Roche, Basel, Switzerland). The detection limit was 50 IU/ml. Anti-HCV was tested using a commercial ELISA kit (Axsym HCV version 3.0; Abbott Laboratories, Chicago, IL, USA).

Liver histology

At the time of enrolment, needle biopsy of the liver was done on each patient for histological analysis. Each biopsy specimen was examined independently two pathologists. Necroinflammation and fibrosis were assigned a Scheuer score from 0 to 4. Patients were further subdivided into mild fibrosis (stages I and II) and cirrhosis (stages III and IV).

Statistical methods and analysis

Statistical analyses were conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC).

We explored data for outliers and normality, and transformed as necessary if the normality assumption was violated. We used Spearman's correlation coefficients to assess the relationship between variables. We used the Chi-square tests to assess for differences in proportions (Fisher's exact test for sparse data). Logistic regression was used to assess the association of EVR and neutrophil count at individual time points. Repeated measurement regression analysis was employed to test the significance of the association between change in neutrophil count and HCV viral load over time using random mixed models.

RESULTS

Two-hundred and one HCV-genotype 4 patients were enrolled in the study.

Approximately 88% were men with a median age of 46 years (range 40-52.5). RVR and EVR were achieved in 33.3% and 61.5%, respectively in the current study.

Baseline neutrophil counts significantly positively correlated with neutrophil decline and neutrophil count at week 2 ($r=0.38$, $p=0.005$ and $r=0.52$, $p<0.0001$, respectively), neutrophil count and decline at week 4 ($r=0.76$, $p<0.0001$ and $r=0.62$, $p<0.0001$, respectively), neutrophil count and decline at week 12 ($r=0.70$, $p<0.0001$ and $r = 0.46$, $p< 0.0001$, respectively), viral load decline at week 4 ($r=0.22$, $p=0.044$), and viral load decline at week 12 ($r=0.28$, $p<0.0001$); and negatively correlated with viral load at week 12 ($r=-0.24$, $p=0.001$). Neutrophil count at week 2 significantly positively correlated with neutrophil count at week 4 ($r=0.66$, $p<0.0001$). Neutrophil decline at week 2 significantly correlated with viral load at week 12 ($r=0.40$, $p=0.042$).

Neutrophil count at week 4 significantly positively correlated with neutrophil count at week 12 ($r=0.78$, $p<0.0001$). Neutro-

Table 1. Spearman correlations of viral loads and neutrophils count

r P value N	Viral load week 0	Viral load week 2	Viral load week 4	Viral load week 12	Viral load decline w2	Viral load decline w4	Viral load decline w12	Neutro w0	Neutro w2	Neutro w4	Neutrow 12	Neutrodecline w2	Neutrodecline w4
Viral load week 2	0.565												
	0.012												
	19												
Viral load week 4	0.267	0.649											
	0.012	0.004											
	88	18											
Viral load week 12	0.139	0.436	0.566										
	0.055	0.070	<.0001										
	190	18	89										
Viral load decline w2	-0.189	-0.897	-0.660	-0.351									
	0.437	<.0001	0.003	0.154									
	19	19	18	18									
Viral load decline w4	0.050	-0.134	-0.935	-0.510	0.470								
	0.644	0.597	<.0001	<.0001	0.049								
	88	18	88	86	18								
Viral load decline w12	0.366	0.295	-0.311	-0.845	0.057	0.497							
	<.0001	0.235	0.004	<.0001	0.823	<.0001							
	190	18	86	190	18	86							
Neutrophils week 0	0.112	0.042	-0.135	-0.237	0.015	0.219	0.276						
	0.117	0.877	0.210	0.001	0.957	0.044	0.000						
	198	16	88	190	16	85	187						
Neutrophils week 2	-0.071	-0.146	-0.007	-0.373	0.135	0.037	0.163	0.519					
	0.720	0.562	0.974	0.055	0.592	0.854	0.427	<.0001					
	28	18	28	27	18	27	26	52					
Neutrophils week 4	0.121	0.012	-0.216	-0.117	0.051	0.249	0.165	0.762	0.663				
	0.090	0.961	0.041	0.109	0.842	0.020	0.025	<.0001	<.0001				
	197	18	90	189	18	87	186	209	40				
Neutrophils week 12	0.074	0.515	-0.166	-0.091	-0.495	0.164	0.104	0.703	0.285	0.779			
	0.380	0.024	0.122	0.283	0.031	0.134	0.225	<.0001	0.149	<.0001			
	142	19	88	140	19	85	137	142	27	144			
Neutrophils decline w2	0.285	0.259	-0.054	0.402	-0.147	0.125	0.015	0.381	-0.521	-0.278	-0.005		
	0.158	0.332	0.795	0.042	0.587	0.553	0.945	0.005	<.0001	0.091	0.980		
	26	16	26	26	16	25	25	52	52	38	25		
Neutrophils decline w4	0.023	0.000	0.088	-0.179	0.046	0.010	0.205	0.616	-0.127	0.119	0.132	0.631	
	0.749	1.000	0.419	0.015	0.870	0.930	0.006	<.0001	0.448	0.086	0.118	<.0001	
	193	15	87	186	15	84	183	209	38	209	141	38	
Neutrophils decline w12	0.045	-0.449	0.185	-0.101	0.444	-0.102	0.156	0.458	0.270	0.154	-0.062	-0.102	0.633
	0.599	0.081	0.091	0.240	0.085	0.363	0.071	<.0001	0.191	0.069	0.460	0.626	<.0001
	139	16	85	138	16	82	135	142	25	141	142	25	141

phil decline at week 4 significantly correlated with viral load decline at week 12 (r=0.21, p=0.006), neutrophil decline at week 2 (r=0.62, p<0.0001), and neutrophil decline at week 12 (r=0.63, p<0.0001); but negatively correlated with viral load at week 12 (r=-0.179, p=0.015).

Neutrophil count at week 12 significantly correlated with viral load at week 2 (r=0.52, p=0.024), and significantly negatively correlated with viral load decline at week 2 (r= 0.50, p=0.031; Table 1).

Neutrophil count and viral load

Using logistic regression, pretreatment neutrophil count significantly predicted RVR and EVR, such that individuals who achieved RVR and EVR had higher pretreatment neutrophil count than individuals who did not achieve RVR and EVR (X2=4.94, p=0.026 and X2=7.67, p=0.005, respectively). Neutrophil count at week 12 significantly predicted EVR (X2= 6.20, p=0.013). We examined the association between neutrophil count, neutrophil decline over time (baseline, week 2, week 4, and week 12), and the concomitant viral load.

Adjusting for age, sex, grade, fibrosis, and prior treatment for neutropenia; neutrophil count significantly correlated with viral load (t=12.76, p<0.0001; Figure 1). In addition, adjusting for baseline neutrophil count, decline in neutrophil count was significantly associated with lower viral load over time (t=-2.27, p=0.027), higher viral load decline over time (t=2.73, p=0.009), and achieving EVR (t=2.04, p=0.044).

DISCUSSION

Identification of factors predicting response and adherence to therapy is critical in the management of hepatitis C. This study assessed the significance of neutropenia as a predictor of virological response and its relation to viral clearance.

Maximal adherence to PEG-IFN and ribavirin is essential to achieve SVR. However, neutropenia frequently causes poor treatment tolerance and PEG-IFN dose reduction.

Neutropenia can be effectively treated with recombinant G-CSF. Poor correlation between total white blood cell and neutrophil count in patients with neutropenia makes monitoring therapy with absolute neutrophil count necessary.

The aetiology of neutropenia during the course of IFN/ribavirin therapy in HCV patients is debated. PEG-IFN-dose dependently suppresses the bone marrow (13), idiosyncratic immunemediated (14), and suppression of G-CSF production by PBMCs (2), are possible explanations. No genetic determinants of PEG-IFN-induced neutropenia have been identified (15). The most striking point is that, in spite of frequently reported neutropenia during PEGIFN therapy in HCV-genotype 4, it is not associated with increased risk of infection (16). Similarly, none of our patients reported serious infection regardless of

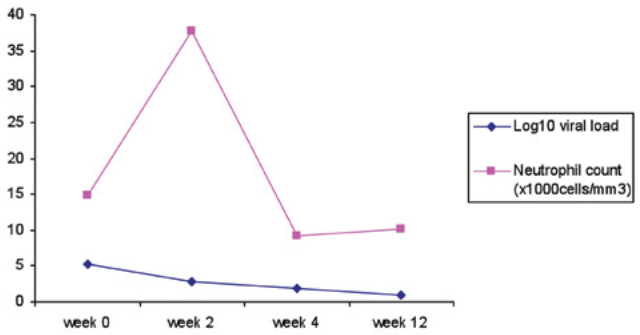

Figure 1. Neutrophil count and log10 viral load by duration of treatment.

the absolute neutrophil count or degree of neutrophil decline compared to pretreatment values. This raises the question whether the antiviral effect of interferon goes in parallel with its effect on the peripheral blood neutrophil count. In our study, there was significant correlation between pretreatment neutrophil count and response in HCV-genotype 4. In addition, the degree of neutrophil count decline correlated with viral clearance at weeks 4 and 12. According to Chung et al. (2010) (17), the correlation between neutropenia and response was confined to the first 12weeks of therapy. As previously described by Toro et al. (1999) (18), we isolated HCV-RNA from neutrophils, lymphocytes, and platelets; with similar kinetics to serum RNA, during PEG-IFN/ribavirin therapy (preliminary report). Some reports demonstrated that HCV-RNA may persist and replicate in PBMC of healthy, anti-HCV antibody-positive, serum HCV RNA-negative patients who have persistently normal ALT levels, even in occult HCV infection (19). As reported, clearance of HCV RNA in PBMC at the end of IFN treatment was a predictor of durable response to antiviral therapy in patients with chronic hepatitis C (20).

Our report suggests that pretreatment neutrophil count and the degree of decline can be useful in predicting the response to therapy in patients with HCV genotype 4. The neutropenia during PEG-IFN therapy could reflect PBMC-viral clearance rather than an adverse effect and in turn obviate the recommendation for PEG-IFN dose reduction as the primary strategy for management of treatment-related neutropenia.

Conflict of Interest: No conflict of interest was declared by the authors.

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