**財團法人明日醫學基金會補助專題研究計畫**

**☑成果報告 □期中進度報告**

計畫名稱：

以干擾素或者不含干擾素抗病毒藥物廓清C型肝炎病毒和血中自體免疫抗體的關聯性

Association between Circulatory Autoantibodies and Eradication of Hepatitis C Virus by Antiviral Therapy with or without Interferon

計畫類別：**** 個別型計畫　　□ 整合型計畫  
  
執行期間： 109 年 1 月 1 日起 至 109 年 12 月 31 日止

計畫主持人： **許耀峻**  
共同主持人：  
計畫參與人員：

成果報告類型：□精簡報告 ****完整報告

處理方式：□可公開查詢

執行單位：義守大學醫學系及義大醫院肝病中心

**Association between Circulatory Autoantibodies and Eradication of Hepatitis C Virus by Antiviral Therapy with or without Interferon**

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This study will be presented at the annual meeting of the American Association for the Study of Liver Diseases (AASLD)

**Background:** Chronic hepatitis C virus (HCV) infection is associated with autoimmune manifestations including increased production of autoantibodies such as rheumatoid factor (RF). It remains unclear whether eradication of HCV infection was associated with reduction in autoantibodies and whether the results would differ between interferon-based and interferon-free regimens.

**Aim:** We aimed to investigate changes in serum RF following viral eradication in patients with chronic HCV infection who were treated with either pegylated interferon (IFN) plus ribavirin or all-oral direct-acting antiviral agents (DAAs).

**Methods:** This is a retrospective cohort study of adult HCV-infected patients treated at a teaching hospital in Taiwan. All patients had detectable HCV RNA at baseline and achieved sustained virological response (SVR) documented 12 or 24 weeks after antiviral treatment. Serum level of IgG RF was measured using latex immunoassay with a detection limit ranging from 0.9 to 2,000 IU/mL. A measurement of 15 IU/mL or above defined RF seropositivity. The changes from baseline to SVR were analyzed and the results were compared between patients treated with IFN-based regimens and those with IFN-free DAAs.

**Results:** The study population was composed of 297 patients (female 48.5%, median age 59 years, cirrhosis 16.8%). At baseline, 78 (26.3%) patients were serologically positive for RF. The proportion of RF-positive patients significantly dropped to 16.5% (n=49) at SVR (*P*<0.001). The median serum level of RF also significantly decreased from 1.6 (interquartile range [IQR], undetectable-15.8) IU/mL to an undetectable level (IQR, undetectable-6.6 IU/mL) (*P*<0.001). Significant reduction in serum RF was consistently observed in both IFN-based and IFN-free DAA groups. The proportion of RF-positive patients decreased from 24.3% to 15.4% (*P*=0.001) in IFN-free DAA-treated patients (n=214), and also from 31.3% to 19.3% (*P*=0.006) in IFN-treated patients (n=83). The changes in RF activity did not differ between patients treated with DAA and those with IFN (*P*=0.40).

**Conclusions:** We found that serum level of IgG-RF significantly dropped after eradication of HCV infection and the result was similar with either DAAs or IFN-based regimens. These findings suggest that effective antiviral treatment may decrease the excessive production of autoantibodies and thus ameliorate autoimmunity in patients with chronic HCV infection.

**Keywords:** chronic viral hepatitis C; rheumatoid factor; antiviral therapy; autoimmunity **Introduction**

Hepatitis C virus (HCV) infection is associated with extrahepatic manifestations (EHMs) in up to two thirds of infected patients1, and can include autoimmune and rheumatological complications ranging from arthritis and sicca syndrome2 to vasculitis and glomerulonephritis3. Serological testing also reveals elevated levels of autoantibodies, such as rheumatoid factor (RF), in patients with chronic hepatitis C (CHC)4, providing further evidence of the association between autoimmunity and HCV.

The development of autoimmune EHMs has been theorized to result from the lymphotropic potential of HCV5. Chronic infection with HCV may lead to polyclonal B cell expansion and the production of autoantibodies such as RF and cryoglobulins6. Nevertheless, it remains unclear whether eradication of HCV infection can effectively attenuate autoimmunity, reduce production of autoantibodies, or improve clinical outcomes of autoimmune disorders. Although data from patients with symptomatic mixed cryoglobulinemia were encouraging7, available evidence did not support the effects of viral clearance in reducing incidences of autoimmune catastrophes, systemic lupus erythematosus, rheumatoid arthritis, or autoimmune hepatitis.8-10

Previous studies, however, were limited by the lack of laboratory measurements and low statistical power. Because autoimmune diseases may affect any organ system and typically require a combination of clinical signs and symptoms, as well as specific biomarkers to diagnose, it is conceivably difficult to rely on a few events as the study outcome to investigate the effects of HCV eradication on auto-immunogenicity. Conversely, measurement of the changes in representative autoantibody may serve to gauge the treatment effects. In addition, findings from patients treated with interferon-based regimens may not be extrapolated in the current era of all-oral direct antiviral agents (DAAs)11-13, given that interferon is known to aggravate or induce autoimmune disorders14.

In order to elucidate how eradication of HCV infection may attenuate auto-immunogenicity and whether the results may differ between interferon-based and interferon-free regimens, we conducted this study to specifically measure the changes in serum RF following antiviral therapy using either pegylated interferon plus ribavirin or all-oral DAAs without interferon.

**METHODS and MATERIALS**

***Study setting and participants***

This is a retrospective cohort study conducted in the E-Da Hospital and E-Da Cancer (Kaohsiung, Taiwan). There were 297 adult HCV-infected patients (female 48.5%, median age 59 years, cirrhosis 16.8%) treated with eradicative antiviral therapies using either DAAs (n=214) or Peg-IFN (n=83) at E-Da Hospital and E-Da Cancer Hospital.

***Antiviral treatment***

Among them, 83 patients were treated using Peg-IFN and Ribavirin combination therapy. The dosage of ribavirin was adjusted according to the patient’s weight, and the treatment duration was determined depending on the HCV genotype of each patient. The definition of SVR is the absence of HCV 24 weeks after discontinuing IFN treatment. For the 297 patients in the DAA group, 10 drug combinations were used as follows:

1. Viekirax 2# QD，Exviera 1# BID
2. Viekirax 2# QD，Exviera 1# BID，Ribavirin 800~1000mg
3. Asunaprevir 100mg 1#BID，Daclatasvir 60mg 1#QD
4. Zepatier 1#QD(Elbasvir+Grazoprevir)
5. Zepatier 1#QD，Ribavirin 800~1000mg
6. Harvoni 1#QD
7. Harvoni 1#QD+Ribavirin 800~1000mg
8. Sovaldi 1#QD+Ribavirin 800~1000mg
9. Sovaldi 1#QD+Daclatasvir 60mg 1#QD
10. Glecaprevir+Pibrentasvir 3# QD (Maviret)

Treatment regimens and duration was determined according to the HCV genotype and presence or absence of cirrhosis and hepatic decompensation. SVR was defined as absence of HCV 12 weeks after discontinuing treatment. All patients had detectable HCV RNA at baseline and achieved sustained virological response (SVR) documented 12-24 weeks after antiviral treatment. Exclusion criteria include co-infection with HBV or HIV, a history of hepatocellular carcinoma (HCC) or other malignancies, pregnancy, previous treatment involving disease-modifying antirheumatic drugs (DMARDs, both biologic and conventional) or long-term use of oral corticosteroids, or pregnancy.

***Methods of Measurements***

This study utilizes the frozen archived serum of 297 patients . IgM-RF is measured using latex immunoassay (FUJIFILM, Hitachi Labospect Series 008AS). The detection range is 0.9-2,000 IU/mL, and a measurement above 15 IU/mL defined RF positivity. Antinuclear antibody (ANA) is measured using indirect fluorescent antibody technique (DiaSorin, ANA-FAST). Any measurement above 1:80X is considered to be a positive result. Anti-smooth muscle antibody (ASMA) is measured with indirect fluorescent antibody technique (ImmuGlo COMVI Mouse Kidney/Stomach IFA kit), with any result above 1:20x being considered as positive.

***Statistical analysis***

All statistical analyses were carried out using commercial software (SPSS, version 22.0, IBM Corp., Armonk, NY, USA). Continuous and categorical variables were summarized using the median and interquartile range (IQR) and proportion with exact numbers, respectively. The comparison of baseline and SVR RF positivity was calculated using McNemar test, and for RF value, the Wilcoxon signed rank test. Fisher’s exact test is used to calculate the p-value for RF activity. The level of significance was set as α=0.05 two-tailed.

**Results**

***Characteristics of the study cohorts***

This study analyzed a total of 297 CHC patients who were successfully treated using either interferon-based or interferon-free regimens. Serum samples were collected from all participants at baseline, before initiating treatment, and SVR. The DAA group is predominantly female (n=123, 57.5%) with a median age of 62 (IQR=54-69) and the IFN group is predominantly male (n=62, 74.7%) with a median age of 49 (IQR=41-58). Baseline ANA positivity is 9.3% and 1.2% for DAA and IFN groups, respectively. Demographic and clinical features of the study population at baseline are outlined in **Table 1**.

At baseline, 78 (26.3%) patients were serologically positive for RF. The proportion of RF-positive patients significantly dropped to 16.5% (n=49) at SVR (P<0.001). The median serum level of RF also significantly decreased from 1.6 (interquartile range [IQR], undetectable-15.8) IU/mL to an undetectable level (IQR, undetectable-6.6 IU/mL) (P<0.001). Significant reduction in serum RF was consistently observed in both IFN-based and IFN-free DAA groups (**Table 2**). The proportion of RF-positive patients decreased from 24.3% to 15.4% (*P*=0.001) in IFN-free DAA-treated patients, and also from 31.3% to 19.3% (*P*=0.006) in IFN-treated patients (**Figure 1**). The changes in RF activity did not differ between patients treated with DAA and those with IFN (*P*=0.40) (**Figure 2**).

**Discussion**

We found that serum level of IgG-RF significantly dropped after eradication of HCV infection, and that the results were similar with either DAAs or IFN-based regimens. Though interferon treatment is contraindicated in viral hepatitis patients with autoimmune diseases, our findings did not show a statistically significant difference between autoantibody levels in these two groups.

These findings suggest that effective antiviral treatment may decrease the excessive production of autoantibodies and thus ameliorate autoimmunity in patients with chronic HCV infection, regardless of the treatment regimen chosen. In this study, we chose to measure serum IgM-RF because of it can be either type 2 or type 3 cryoglobulin, both of which is associated with longstanding HCV infection and the development of extrahepatic autoimmune manifestations, particularly cryoglobulinemic vasculitis. Past research has shown that the decrease of such autoantibodies after successful anti-tumor necrosis factor alpha therapy using infliximab is associated with clinical improvement in patients with rheumatoid arthritis15, therefore we extrapolate that a similar trend may also reflect improvement of clinical outcomes in CHC patients with autoimmune EHMs. Since both DAA and IFN-treated groups have shown a statistically significant decrease of serum RF levels, we infer that successful eradication of HCV using either method may be equally effective in the treatment of HCV-related autoimmune diseases and should be considered in all patients.

In clinical practice, patients with longstanding HCV infection often present with signs and symptoms of systemic autoimmune diseases. Proteinuria, vasculitis, and arthritis are all extra-hepatic features of CHC that may be shared with other autoimmune diseases such as systemic lupus erythematosus, making it at times difficult to ascertain the cause of such symptoms and initiate appropriate treatment. Our research has shown that effective HCV treatment leads to a significant decrease in serum RF, which may play a vital role in the pathogenesis of such EHMs, and also serve to illustrate the benefits of diagnosing and treating CHC early on in the disease process. These findings may be potentially useful to differentiate between different etiologies and serve as biomarkers to assess treatment effectiveness in the context of autoimmunity. Future research may help elucidate whether the decrease of autoantibody levels as observed in this study is truly associated with clinical improvement of autoimmune sequelae of CHC.

This study is limited in several ways. The small sample size may not fully reflect the entire clinical spectrum of HCV-related autoimmunity, and may affect the statistical power of the results. In addition, patients from the IFN cohort are mostly males treated for HCV at a substance abuse clinic. Since females are statistically more likely to suffer from autoimmune diseases and have higher baseline levels of autoantibodies compared to men, results from this cohort may be skewed . However, it is difficult to adjust the results of our research to account for this gender difference, as there are many factors that contribute to this gender difference and no single test to measure autoimmunity.

Though the risk of developing autoimmune EHMs may decrease after successful HCV eradication, it should be noted that the development of autoimmune disease involves a wide variety of pathological processes aside from autoantibody-mediated mechanisms. In addition, there are multiple other different autoantibodies, such as anti-LKM1, anti-thyroglobulin, and antiphospholipid antibodies, that have been associated with longstanding HCV infection. Lastly, RF and ANA are considered to only be screening tests for autoimmune diseases , which must be diagnosed by a clinician based on a combination of laboratory data, patient history, and clinical findings. A positive result without accompanying symptoms and signs is not pathognomonic of any autoimmune diseases, and spontaneous clearance of these biomarkers is also possible. Therefore, it cannot be directly ascertained from this study that the incidence and severity of clinical autoimmune diseases will decrease after successful HCV eradication.

**REFERENCES**

1. 1. Cacoub P, Comarmond C. New insights into HCV-related rheumatologic disorders: A review. J Adv Res 2017; 8(2): 89-97.

2. Palazzi C, D'Amico E, D'Angelo S, Gilio M, Olivieri I. Rheumatic manifestations of hepatitis C virus chronic infection: Indications for a correct diagnosis. World J Gastroenterol 2016; 22(4): 1405-10.

3. Zignego AL, Ferri C, Pileri SA, Caini P, Bianchi FB, Italian Association of the Study of Liver Commission on Extrahepatic Manifestations of HCVi. Extrahepatic manifestations of Hepatitis C Virus infection: a general overview and guidelines for a clinical approach. Dig Liver Dis 2007; 39(1): 2-17.

4. Yang DH, Ho LJ, Lai JH. Useful biomarkers for assessment of hepatitis C virus infection-associated autoimmune disorders. World J Gastroenterol 2014; 20(11): 2962-70.

5. Zignego AL, Gragnani L, Piluso A, et al. Virus-driven autoimmunity and lymphoproliferation: the example of HCV infection. Expert Rev Clin Immunol 2015; 11(1): 15-31.

6. Knight GB, Gao L, Gragnani L, et al. Detection of WA B cells in hepatitis C virus infection: a potential prognostic marker for cryoglobulinemic vasculitis and B cell malignancies. Arthritis Rheum 2010; 62(7): 2152-9.

7. Fabrizi F, Dixit V, Messa P. Antiviral therapy of symptomatic HCV-associated mixed cryoglobulinemia: meta-analysis of clinical studies. J Med Virol 2013; 85(6): 1019-27.

8. Zeng Y, Chen S, Fu Y, et al. Gut microbiota dysbiosis in patients with hepatitis B virus-induced chronic liver disease covering chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. J Viral Hepat 2020; 27(2): 143-55.

9. Hsu YC, Ho HJ, Huang YT, et al. Association between antiviral treatment and extrahepatic outcomes in patients with hepatitis C virus infection. Gut 2015; 64(3): 495-503.

10. Vento S, Cainelli F, Renzini C, Concia E. Autoimmune hepatitis type 2 induced by HCV and persisting after viral clearance. Lancet 1997; 350(9087): 1298-9.

11. European Association for the Study of the Liver. Electronic address eee, Clinical Practice Guidelines Panel C, representative EGB, Panel m. EASL recommendations on treatment of hepatitis C: Final update of the series(). J Hepatol 2020; 73(5): 1170-218.

12. Yu ML, Chen PJ, Dai CY, et al. 2020 Taiwan consensus statement on the management of hepatitis C: Part (II) special populations. J Formos Med Assoc 2020; 119(7): 1135-57.

13. AASLD/IDSA HCV guidance panel. Recommendations for testing, managing, and treating hepatitis C. Updated August 27, 2020. 2020.

14. Dumoulin FL, Leifeld L, Sauerbruch T, Spengler U. Autoimmunity induced by interferon-alpha therapy for chronic viral hepatitis. Biomed Pharmacother 1999; 53(5-6): 242-54.

15. Alessandri C, Bombardieri M, Papa N, et al. Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following anti-TNFalpha therapy (infliximab) in rheumatoid arthritis is associated with clinical improvement. Ann Rheum Dis 2004; 63(10): 1218-21.

**Table 1**. Baseline characteristics of patients with CHC treated with DAA and IFN

|  |  |  |
| --- | --- | --- |
|  | DAA (n=214) | IFN (n=83) |
| Age | 62(54-69) | 49(41-58) |
| Male, *n* (%) | 91(42.5) | 62(74.7) |
| Cirrhosis, *n* (%) | 47(22) | 3(3.6) |
| AST, U/L | 57(36.8-97.3) | 63(45-97) |
| ALT, U/L | 65(39.8-117.3) | 95(53-137) |
| Bilirubin, mg/dL | 1.0(0.9-1.3) | 0.9(0.7-1.1) |
| White blood cell count, 103/μl | 5.7(4.7-6.9) | 6.4(5.1-7.5) |
| Hemoglobin, g/dL | 14.1(12.8-15.1) | 14.8(13.8-16) |
| Platelet count, 103/μl | 175(140-226) | 196(148-225) |
| Creatinine, mg/dL | 1.0(0.9-1.3) | 1.1(0.9-1.2) |
| HCV-RNA, IU/mL | 1904858(392420-5670998) | 2007626(97150-6260222) |
| Genotype, *n* (%) |  |  |
| 1 | 132(61.7) | 38(45.8) |
| 2 | 65(30.4) | 34(41) |
| 3 | 1(0.5) | 3(3.6) |
| 6 | 16(7.5) | 8(9.6) |
| Fibrosis 4 score | 2.7(1.7-4.4) | 1.7(1.3-2.7) |
| ANA, *n* (%) |  |  |
| <1:40 | 194(90.7) | 82(98.8) |
| 1:40-1:80 | 12(5.6) | 1(1.2) |
| 1:160-1:640 | 8(3.7) | 0 |

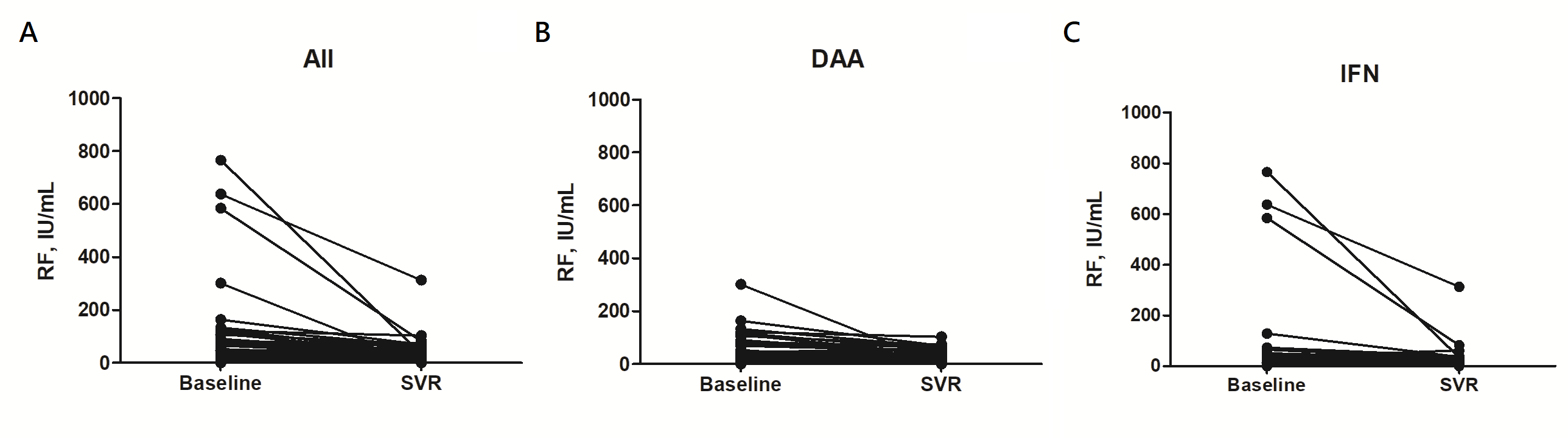
CHC, chronic hepatitis C; DAA, direct-acting antiviral agents; IFN, interferon; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ANA, antinuclear antibody

**Table 2.** The positivity and values of RF in patients with CHC at baseline and SVR

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| RF | DAA (n=214) | | | IFN (n=83) | | |
|  | Baseline | SVR | *P* value | Baseline | SVR | *P* value |
| Positivity# | 52(24.3%) | 33(15.4%) | 0.001 | 26(31.3%) | 16(19.3%) | 0.006 |
| Values, IU/mL | 1.4(0.5-13.8) | 0.5(0.5-6.6) | <0.001 | 2.1(0.5-17.7) | 0.5(0.5-5.8) | <0.001 |

Positivity# was defined as a measurement above 15 IU/mL. RF, Rheumatoid factor; CHC, chronic hepatitis C; SVR, sustained viral responseDAA, direct-acting antiviral agents; IFN, interferon

**Figure 1.** Changes in serum level of rheumatoid factor IgG following eradication of hepatitis C virus infection according to antiviral regimens



**Figure 2.** No significant differences between the regimens of antiviral therapy in changes of rheumatoid factor

