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der Viruskrankheiten

Dynamic and kinetic of HCV viral load under the new treatment with direct acting antivirals in a patient cohort of the University Clinic Tuebingen 2014-2017

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Contents

Abbreviation

Abbreviation	Full name
AEs	Adverse events
ALT	Alanine-aminotransferase
ALP	Alkaline phosphatase
Bili	Bilirubin
CHC	Chronic hepatitis C
CI	Confidence interval
Crea	Creatinine
DAA	Direct-acting antivirals
DAC	Daclatasvir
EASL	European Association for the Study of the Liver
EOT	End of treatment response
EBR	Elbasvir
Epclusa	Sofosbuvir /Velpatasvir
EIA	Enzyme Immunoassay
EVR	Early virological response
FAD	US Food and Drug Administration
GT	Genotype
GZR	Grazoprevir
GZR/EBR	Grazoprevir /Elbasvir **(**Zepatier)
Harvoni	Sofosbuvir /Ledipasvir
HB	Hemoglobin
HCC	Hepatocellular carcinoma
IFN	Interferon
IFN-free	Interferon-free

INR	International normalized ratio
LC	Liver cirrhosis
LDV	Ledipasvir
LTx	Liver transplantation
NS	nonstructural
NTx	Nephrectomy
PCR	Polymerase chain reaction
PEG IFN	Pegylated interferon
Peg-IFN-α	Peginterferon alfa-2a
PI	Protease inhibitor
PLT	Platelet, Thrombocytes
RAS	resistance-associated variant
RBV	Ribavirin
RDTs	Rapid diagnostic tests
RVR	Rapid virological response
SOF	Sofosbuvir
SOF/DAC	Sofosbuvir/ Daclatasvir
SOF/EBR	Sofosbuvir/Elbasvir (Zepatier®)
SOF/LDV	Sofosbuvie/Ledipasvir (Harvoni®)
SOF/SMP	Sofosbuvir /Simeprevir
SOF/VEL	Sofosbuvir/Velpatasvir (Epclusa®)
SMP	Simeprevir
SVR	Sustained virological response
TBIL	Total bilirubin
VEL	Velpatasvir
WBC	White blood cell, Leukocytes

1. Introduction

1.1. HCV molecular biology

1.1.1. Hepatitis C virus structure

Hepatitis C virus was first discovered in 1989 and before this kind of hepatitis was called "non-A, non-B" hepatitis (NANBH). Hepatitis C virus is a number of the Flaviviridae family (Bennett et al., 2014). The virus size is 40-50 nm diameter, including 9600 nucleotides, and it is positive sense single-strand RNA (Bennett et al., 2014, Neumann et al., 1998). The hepatitis C virus includes the core and lipid envelope. Glycoproteins (E1 and E2) are embedded in the lipid envelope. The core consists of the RNA genome which surrounding by nucleocapsid.

Hepatitis C virus protein consists of structural proteins and nonstructural proteins. Structural proteins include core protein, glycoproteins (E1 and E2) and p7 protein; Nonstructural proteins include NS2, NS3, NS4A, NS4B, NS5A, NS5B (Ashfaq et al., 2011).

HCV core protein consists of 191 amino acids which can be divided into three domains according to hydrophobicity (Bukh et al., 1994). HCV envelope glycoproteins are composed of two kinds of proteins, that is E1 and E2, which highly glycosylated, and play a critical role in entering cell process. The function of E1 is to fuse subunit, and E2 is the receptor of subunit binding in the envelope (Drummer et al., 2003). P7 protein is located between E2 and NS2, it is a polypeptide including 63-amino acids. The function of the P7 protein is to build ion channels which play an important role in virus infection process (Griffin et al., 2003), and it is also necessary for virus assembling and infectious virions releasing in a special genotype (Griffin et al., 2003). NS2 is a transmembrane protein which is essential for the viral replication process in vivo and in vitro (Pietschmann et al., 2006). The NS3 protein has multifunctional activity, N-terminal of NS3 with serine protease activity, and a C-terminal of NS3 has NTPase/helicase activity (Gallinari et al., 1998). NS3 protease also plays a role in the division between NS3/4A, 4A/4B, 4B/5A and 5A/5B

(Bartenschlager et al., 1993). NS4A protein is a cofactor of NS3 protein. The interaction between NS4A and NS3 can activate the NS3 active site and promote protease cleavage (Kim et al., 1996). NS4B is a hydrophobic protein contains four transmembrane domains, which play a critical role in other viral proteins recruitment. NS4B can interact with NS4A directly and interact with NS3 and NS5A indirectly (Lin et al., 1997). NS5A is a hydrophilic phosphoprotein without transmembrane domains. NS5A plays a critical role in viral replication, it also acts on interferon response and cell signaling pathways modulation (Macdonald et al., 2004, Reed et al., 1997), and it is related to lipid droplets. NS5A Mutations are essential for viral replication and cell line replicons (Lohmann et al., 1999). NS5B structural is 'right hand' shape polymerase with finger and palm, and thumb sub domains surrounding encircled active site (Lesburg et al., 1999). NS5B is an RNA dependent polymerase and plays a critical role in new RNA genome synthesis, and also is essential for polymerase activity (Behrens et al., 1996). As a key component of the HCV replication, NS5B has become a major antiviral target (Njoroge et al., 2008).

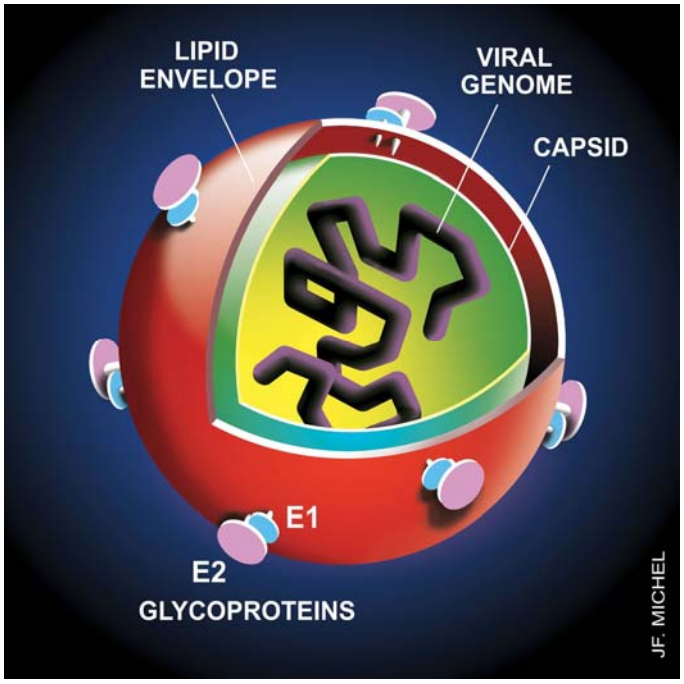

Fig. 1. Hepatitis C virus structure (Perrault and Pécheur, 2009)

1.1.2 . HCV Genotypes

HVB is divided into seven genotypes and multiple subtypes (Nakano et al., 2012). However, patients are usually only infected with a single genotype, in samples of HCV infected patients, a mixture of closely related viruses referred to as quasispecies. This so called quasispecies constantly change the genomic sequence during the course of the infection. These changes make the development of a vaccine difficult.

Epidemiological studies of hepatitis C patients from 98 countries worldwide demonstrated genotype 1 is the most common (46%), followed by genotype 3 (22%), genotype 2 (13%), genotype 4 (13%), genotype 6 (2%), and genotype 5 (1%) (Gower et al., 2014). The percentage of combination or undefined genotypes is 3%, among them, the most common subtype is genotype 1 (22%) (Gower et al., 2014).

Genotype has significant regional differences. The main genotype in Europe, North America, and Latin America is genotype 1 (62–71%), Genotype 1b accounting for 26%-71%. However, Genotype 4 proportion is large in the Middle East and North Africa (71%). In Asian countries，Genotype 3 proportion is 39%, followed by Genotype 1 (36%) (Gower et al., 2014).

Vermehren et al. screened 20,809 hepatitis C infected patients in two cities of Germany (Berlin and Frankfurt), found that the genotype 1 account for 66%, followed by G3 (19%) and G2 (5%).(Vermehren et al., 2012)

1.1.3. HCV Life cycle

Replication of Hepatitis C virus involves several complex processes and mainly take place in liver hepatocytes. Each infected cell generates about 50 virus particles per day, and a total of one trillion virions can be synthesized (Bartenschlager and Lohmann, 2000). The virus also can replicate in mononuclear cells of peripheral blood, this may influence the immunological disorders of chronical HCV infected patients. Many kinds of virus variants are produced by the high mutation rate and lead to wide variety of mutates and genotypes of HCV (Bartenschlager and Lohmann, 2000).

The Hepatitis C life cycle includes: entry, uncoating, translation and replication, assembly and release (Dubuisson and Cosset, 2014).

During entry and uncoating process, the hepatitis C virus combined with lipoproteins form lipo Viro particles. The viral life cycle starts by binding with SRB1 and GAGs (glycosaminoglycans) The virus follows a series of complicated process, this involves multiple cell entry factors, such as CD81, SRB1, CLDN1, OCLN, NPC1L1, EGFR, TfR (transferrin receptor), tight-junction proteins and signaling proteins. After binding to the host cell, the virus is internalized by endocytosis of clathrin, the fusion starts at this stage (Dubuisson and Cosset, 2014). HCV glycoproteins（E1 and E2）are the critical determinants of viral entry, which act as receptors between the host cell and viral envelope in the fusion process (Douam et al., 2014).

When the polyprotein cleavage, the virus nonstructural proteins combined with cellular factors form the replication complex, this causes the formation of the membranous web (double membrane vesicles), where replication occurs. After cleavage virus C-terminus, core protein is loaded upon LDs. The site of the virus assembly is at the junction between ER membranes and LDs. NS5A and NS3/4A assist new replicated viral genomes transfer to the assembly sites. The replication complexes and core proteins are connected to the glycoproteins by P7 and NS2. NS5B is the essential enzyme for RNA synthesis, translating RNA genome to produce new proteins, act as new RNA replication templates and assemble to form the new viruses (Dubuisson and Cosset, 2014, Scheel and Rice, 2013).

Virus budding is assisted by the endosomal sorting complexes required for transport (ESCRT) (Ariumi et al., 2011). The ESCRT machinery assists in vesicles budding and release from the cytoplasm, and is involved in the multivesicular bodies formation (Welsch et al., 2007). After assembly and budding in the endoplasmic reticulum, viral particles are released from cells by the secretory pathway (Coller et al., 2012). The virions acquire low buoyant density characteristic during this process (Gastaminza et al., 2008, Gastaminza et al., 2006). Meanwhile, the glycans which are associated with the envelope glycoproteins are modified (Vieyres et al., 2010).

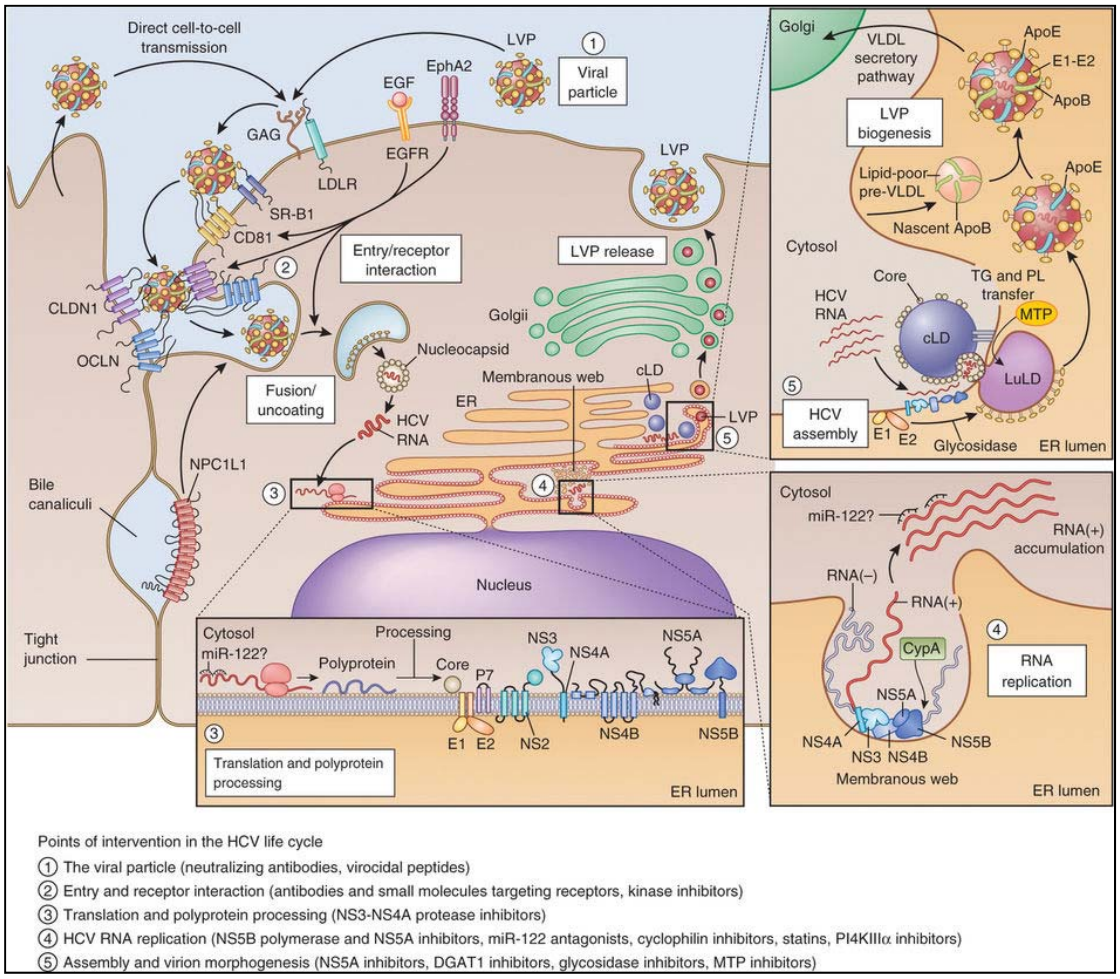

Fig. 2. HCV life cycle (Scheel and Rice, 2013)

1.2. Prevalence of Hepatitis C infection

Hepatitis C is one of the main cause leading to liver cirrhosis, liver failure, cancer and death in liver disease patients. A total of 185 million people are infected with hepatitis C worldwide. In the past decade, the seroprevalence as a whole is estimated around to 2.8% (Mohd Hanafiah et al., 2013). The latest data from the World Health Organization (WHO) show that hepatitis C infection mortality continues to increase in recent years. Only in one year like 2015, there are 1.75 million newly infected patients with hepatitis C (WHO, 2017). Epidemics of European countries data shows 15 million people (1.6% of the population) are infected with Hepatitis C (WHO, 2017). Globally, the largest HCV population live in China, which up to 29.8 million HCV infected people. The highest HCV prevalence could be detected in Egypt (>10%)

(WHO, 2017). The number of HCV patients in Asian countries accounts for half of the world's HCV population (Hajarizadeh et al., 2013). Vermehren et al. statistical analysis from the hepatitis C screening in two representative cities of Germany, showed that HCV seropositivity was up to 2.6% (Berlin 2.4%, Frankfurt 3.5%) (Vermehren et al., 2012).

1.3. Epidemiology of HCV infection

Hepatitis C infection is mainly through the blood transmission, the proportion of sexual transmission and vertical transmission are not significant (Shepard et al., 2005). In comparison to hepatitis B virus sexual or perinatal transmission of HCV is much more infrequent. The risk to acquire HCV from breast feeding is negligible and spread within families is rare.

Drug addicts and blood transfusions are high-risk groups population (Shepard et al., 2005). Cross-infection in hospitals, tattoos, acupuncture are also part of the transmission route (Alter, 2011).

1.4. Symptoms of hepatitis C infected patients

Thepatitis C incubation period is two weeks to six months. At the initial stage of the infection, about 80% of patients do not have any symptoms or symptoms are not obvious. Patients with acute symptoms may have ~~a~~ fever, fatigue, loss of appetite, nausea, vomiting, abdominal pain, dark urine, joint pain, yellow skin. When hepatitis C infection develops to liver cirrhosis, the patient symptoms become more obvious. At the end-stage of hepatitis C patient, the symptoms may be with gastrointestinal bleeding, weakness, wasting and jaundice (Hoofnagle, 1997).

1.5. Diagnosis of Hepatitis C Virus Infection

The real-time PCR is a sensitive method of quantifying HCV RNA levels from 10 IU/mL to 100 million IU/mL. The unit of HCV RNA is IU/mL which was standardized by the World Health Organization (Saldanha et al., 2005), The HCV PCR assay is faster and more accurate than the other techniques (Barbeau et al., 2004).

HCV RNA level more than 800 000 IU/mL is defined as high viral load, and a low viral load is defined as less than 800 000 IU/mL (Pawlotsky et al., 2000). A clinical study found that patients with more than 2,000,000 IU/mL baseline viral load had a lower sustained virological response rate than those with less than 2,000,000 IU/mL (McHutchison et al., 1998).

In addition to real-time PCR, other methods apply ~~to~~ for screening and diagnosing hepatitis C infection. The basic diagnostic test for HCV infection is anti-HCV antibodies. Anti-Hepatitis C Virus IgG, immunoblot assays, immunoassays（EIA）are used to identify hepatitis C infections (Kamili et al., 2012).

A confirmed positive HCV antibody test, however, cannot distinguish between acute, chronic or past infection. Assays for HCV-lgM antibody detection have not proved to be useful.

Nucleic Acid Amplification Testing (NAT) is the method to detect the presence of HCV RNA. Nucleic acid tests can be performed qualitatively and quantitatively. The qualitative polymerase chain reaction (PCR) and transcription-mediated amplification (TMA) are qualitative tests. The real-time PCR, bDNA (branched DNA) and quantitative reverse transcription–PCR are classified to quantitative tests. NATs are most common qualitative tests which use reverse transcription PCR to detect viral HCV RNA. The NATs are usually applied to screen blood donations and confirm viremia (Ferreira-Gonzalez and Shiffman, 2004, Morishima and Gretch, 1999).

If the patient cannot afford RNA PCR detection, HCV core antigen detection also can be used instead of RNA PCR (EASL, 2017).The suspected acute hepatitis C or immunocompromised patients can apply HCV RNA testing as the initial evaluation. The HCV RNA sensitive molecular method should be used if anti-HCV antibodies are detectable. HCV antibody positive patients should be retested confirmed by HCV PCR (EASL, 2017).

1.6. Treatment of HCV

1.6.1. The previous HCV treatment with interferon and ribavirin

Since 1998, the combination of ribavirin and interferon alfa was approved by the

FDA (U.S. Food and Drug Administration) for clinical application of hepatitis C infection. From 2001, The HCV treatment with Peg-IFN-α (Peg-interferon α-2a) and Ribavirin. In these previous treatments, the treatment duration was for 24 to 48 weeks, and the virologic cure rate was about 50% (Feld and Hoofnagle, 2005). The mechanisms of IFN-α and ribavirin include T helper type 1 immune responses, induce IFN-stimulated genes (ISGs), inhibit inosine monophosphate dehydrogenase (Feld and Hoofnagle, 2005). This previous treatment was common with severe side effects, including flu-like symptoms, autoimmune diseases, neuropsychiatric symptoms, and hemolytic anemia (Manns et al., 2006). Among younger patients and females, the response rates were higher. Those patients infected with genotype 2 or 3 could be treated more successfully than compared to genotype 1.

1.6.2. The goals and endpoints of HCV treatment

The endpoints of HCV treatment is that HCV RNA cannot be detected at 12 and 24 weeks after treatment, that is, Sustained virologic response SVR12 and SVR24 were negative, and the lower limit of RNA PCR detection was less than 15 IU/ml (Martinot‐Peignoux et al., 2010). The goal of treatment is to cure HCV infection and prevent the HCV-related liver complications, such as fibrosis, cirrhosis, decompensation of cirrhosis, hepatic necroinflammation, HCC (EASL, 2017).

1.6.3. The development of direct-acting antivirals (DAAs)

The development of direct-acting antivirals (DAA) was based on the research of HCV virus structure, intended to inhibit HCV protease, such as NS3 protease, NS5A protein, NS5B polymerase, which play critical roles in the viral replication process (Pawlotsky, 2014). The first concept of HCV protease inhibitor was proposed in 2002, this is a turning point in HCV drug discovery (Lamarre et al., 2003). Since 2014, Several direct-acting antivirals (DAA) have been developed and approved for clinical applications in many countries. These compounds are principally targeted at the non-structural proteins and NS3-4 protease, the NS5A protein and the RNA-dependent polymerase and NS5B. These new viral inhibitors are very potent

and effective for all HCV genotypes. The application of these drugs has tremendously improved HCV cure rates, which now exceed clearly 90% in the tested patient population groups.

The clinical results of the new IFN-free DAAs treatment were significantly better than the previous treatment proposal with IFN. The treatment duration of direct-acting antivirals is much shorter, the viral load quickly drops to negative within 8 weeks or 12 weeks. In addition, there are fewer side effects compared with previous treatment proposals (Asselah et al., 2016).

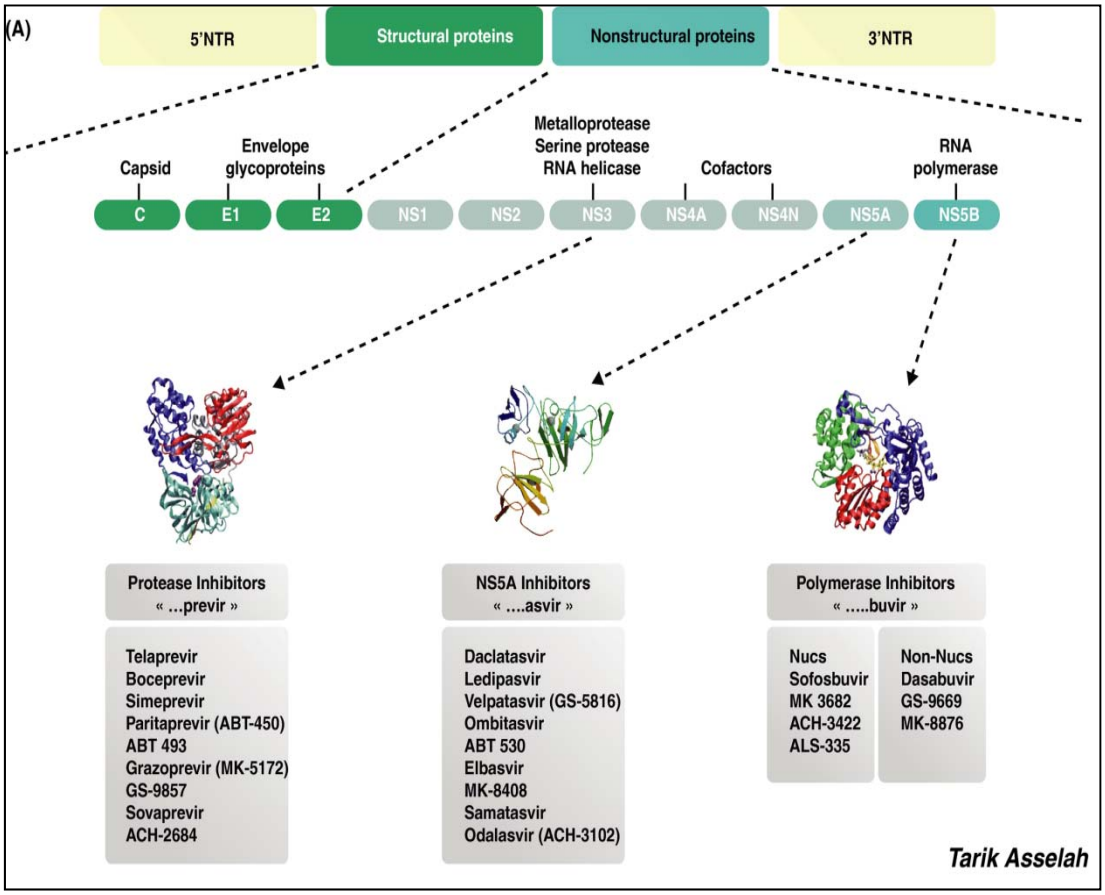

Fig. 3. Direct-acting antivirals (DAAs) for HCV treatment (Asselah et al., 2016)

1.7. HCV DAAs drugs approved in Europe in 2016

The HCV DAAs drugs available in European countries in 2016 include sofosbuvir; sofosbuvir and ledipasvir (Harvoni); sofosbuvir and velpatasvir (Epclusa); grazoprevir and elbasvir (Zepatier); daclatasvir; simeprevir; ribavirin etc. The

presentation and posology of these drugs shown in the table 1 (EASL, 2017).

Product	Presentation	Posology
Sofosbuvir	Tablets containing 400 mg of sofosbuvir	One tablet once daily (morning)
Sofosbuvir/ledipasvir	Tablets containing 400 mg of sofosbuvir and 90 mg of ledipasvir	One tablet once daily (morning)
Sofosbuvir/velpatasvir	Tablets containing 400 mg of sofosbuvir and 100 mg of velpatasvir	One tablet once daily (morning)
Paritaprevir/ombitasvir/ ritonavir	Tablets containing 75 mg of paritaprevir, 12.5 mg of ombitasvir and 50 mg of ritonavir	Two tablets once daily (morning)
Dasabuvir	Tablets containing 250 mg of dasabuvir	One tablet twice daily (morning and evening)
Grazoprevir/elbasvir	Tablets containing 100 mg of grazoprevir and 50 mg of elbasvir	One tablet once daily (morning)
Daclatasvir	Tablets containing 30 or 60 mg of daclatasvir	One tablet once daily (morning)
Simeprevir	Capsules containing 150 mg of simeprevir	One capsule once daily (morning)
Ribavirin	Capsules containing 200 mg of ribavirin	Two capsules in the morning and 3 in the evening if body weight <75 kg or Three capsules in the morning and 3 in the evening if body weight ≥75 kg (or less if dose reduction needed)

Table 1. Approved HCV DAAs in Europe in 2016 (EASL, 2017)

Sofosbuvir

The dose of sofosbuvir is 400 mg per tablet, one tablet once daily at morning. The toleration of sofosbuvir is over 12 to 24 weeks. The main excretion of the drug is by renal (80%). The common side effects of sofosbuvir combines with ribavirin were fatigue and headache (>20%). Contraindications of drug-drug interactions include: P-gh inducers (rifampin, phenytoin, carbamazepine, St. john's wort), rifapentine, modafinil, rifabutin, anti-arrhythmic (amiodarone) (EASL, 2017).

Sofosbuvir and ledipasvir (Harvoni®)

The product name of sofosbuvir and ledipasvir combination is Harvoni®. The single tablet combines 400 mg sofosbuvir and 90 mg ledipasvir. One tablet once daily at morning. The excretion route of these two components are different, the main excretion of the sofosbuvir is by renal, but the major route of ledipasvir is biliary excretion. The common side effects of sofosbuvir and ledipasvir combination were fatigue and headache. Contraindications of drug-drug interactions include: P-gp substrates (dabigatran, digoxin), amlodipine, aliskiren, carvedilol, buprenorphine,

cyclosporine, amiodarone, rosuvastatin, tenofovir, tenofovir alafenamide (TAF). Especially need to be careful about amiodarone which can cause a serious risk or even fatal asystole or bradycardia (EASL, 2017).

Sofosbuvir and velpatasvir (Epclusa®)

The product name of sofosbuvir and velpatasvir combination is Epclusa®. The single tablet combines 400 mg sofosbuvir and 100 mg velpatasvir. One tablet once daily at morning. The excretion route of these two components are different, the main excretion of the sofosbuvir is by renal, but the major route of velpatasvir is biliary excretion. The common side effects of sofosbuvir and velpatasvir combination were fatigue, headache and nausea. Contraindications of drug-drug interactions include P-gp or CYP inducers (rifabutin, rifampicin, Phenobarbital, carbamazepine, phenytoin, St John's wort), modaifnil, proton pump inhibitor; efavirenz, nevirapine and etravirine in HCV-HIV coinfection patients (EASL, 2017).

Grazoprevir and elbasvir (Zepatier®)

The product name of grazoprevir and elbasvir combination is Zepatier ®. The single tablet combines 100 mg grazoprevir and 50 mg elbasvir. One tablet once daily at morning. The main route of excretion is faecal and biliary. The common side effects of grazoprevir and elbasvir combination were fatigue and headache. Contraindications of drug-drug interactions include CYP3A or P-gp inhibitors which decrease plasma exposure of drugs, such as azole antifungals, boosted protease inhibitors, etravirine, efavirenz, phenytoin, bosentan, carbamazepine, St John's wort, modafinil, etc.(EASL, 2017).

Daclatasvir

The dose of daclatasvir is 60 mg or 30 mg per tablet, one tablet once daily. The

main excretion of the drug is by faeces. The common side effects of daclatasvir were fatigue, headache and nausea. Contraindications of drug-drug interactions include P-gh and CYP3A4 inducers, such as antimycobacterial (rifapentine, rifampicin, rifabutin), anticonvulsants (carbamazepine, oxcarbazepine, phenytoin, phenobarbital), St John's wort and systemic dexamethasone etc. (EASL, 2017).

Simeprevir

The dose of simeprevir is 150 mg per tablet, one tablet once daily. Simeprevir widely bound to albumin in plasma proteins (>99.9%). The main excretion of the simeprevir is by biliary. The common side effects of simeprevir are pruritus, rash and nausea. Contraindications of drug-drug interactions include CYP3A4 inducers, antibiotics (clarithromycin, erythromycin, telithromycin), anticonvulsants (oxcarbazepine, carbamazepine, phenytoin, phenobarbital), antifungals (ketoconazole, fluconazole, itraconazole, voriconazole), antimycobacterial (rifabutin, rifapentine, rifampin), St John's wort, dexamethasone, cisapride et (EASL, 2017).

1.8. The treatment regimens for different genotype

Combination regimen	Genotype 1	Genotype 2	Genotype 3	Genotype 4	Genotypes 5 and 6
Sofosbuvir + ribavirin	No	Suboptimal	Suboptimal	No	No
Sofosbuvir/ledipasvir ± ribavirin	Yes	No	No	Yes	Yes
Sofosbuvir/velpatasvir ± ribavirin	Yes	Yes	Yes	Yes	Yes
Ombitasvir/paritaprevir/ritonavir + dasabuvir ± ribavirin	Yes	No	No	No	No
Ombitasvir/paritaprevir/ritonavir ± ribavirin	No	No	No	Yes	No
Grazoprevir/elbasvir ± ribavirin	Yes	No	No	Yes	No
Sofosbuvir + daclatasvir ± ribavirin	Yes	Yes	Yes	Yes	Yes
Sofosbuvir + simeprevir ± ribavirin	Suboptimal	No	No	Yes	No

Table 2. Treatment regimens recommendation for each HCV genotype

(EASL, 2017)

The treatment proposal for each genotype of chronic hepatitis C patients according to European Association for the study of the liver shown in the table 3 (EASL, 2017).

Genotype	**Treatment options**
Genotype 1	1 Sofosbuvir and ledipasvir 2 Sofosbuvir and velpatasvir 3 Ritonavir boosted partitaprevir, ombitasvir and dasabuvir 4 Greazoprevir and elbasvir 5 Sofosbuvir and daclatasvir
Genotype 2	1 Sofosbuvir and velpatasvir 2 Sofosbuvir and daclatasvir
Genotype 3	1 Sofosbuvir and velpatasvir 2 Sofosbuvir and daclatasvir
Genotype 4	1 Sofosbuvir and ledipasvir 2 Sofosbuvir and velpatasvir 3 Ombitasvir, paritaprevir and ritonavir 4 Grazoprevir and elbasvir 5 Sofosbuvir and daclatasvir 6 Sofosbuvir and simeprevir
Genotype 5 Genotype 6	1 Sofosbuvir and ledipasvir 2 Sofosbuvir and velpatasvir 3 Sofosbuvir and daclatasvir

Table 3. Treatment propotion for different genotype of hepatitis C patients

1.9. Treatment regimens used in this study

Abbreviation of regimen	Full name (Components)	Genotype	Inhibitor
SOF	Sofosbuvir	1, 2, 3, 4, 5, 6	NS5B
Harvoni	Sofosbuvir Ledipasvir	1, 4, 5, 6	NS5B NS5A
SOF DAC	Sofosbuvir Daclatasvir	3	NS5B NS5A
SOF SMP	Sofosbuvir Simeprevir	HCV 1, 4 HIV	NS5B NS3/4A
Exviera+Viekirax	Exviera Viekirax	1b, 4	NS5A， NS3/4A
Epclusa	Sofosbuvir Velpatasvir	2, 3	NS5A
Zepatier	Elbasvir Grazoprevir	1, 4	NS5A

Table 4. Treatment regimens used in this study

1.10. Summary of introduction

There are approximately 185 million HCV infected people all over the world. A serious problem of HCV infection is the high chronicity rate of about 80% HCV-infected patients may develop liver cirrhosis or hepatocellular carcinoma. The HCV standard therapy with interferon and ribavirin was used since1990s, the success rate of this treatment was only about 50%, and it was HCV genotype dependent. This changed significantly since the direct-acting antivirals (DAAs) became available from 2014 onwards. The success rate is much higher and can be achieved in a very short time.

2. Materials and Methods

2.1. Patient population and data collection

A total of 446 hepatitis C patients were treated with the direct acting antivirals (DAA) in the Department of Internal Medicine I, University Hospital Tuebingen, in the period from January 2014 to 31 April 2017. Nine patients had to be deleted due to incomplete data. The final 437 patients with hepatitis C were included in the statistical study analysis. The data of 437 patients were taken from the Institute of Medical Virology University clinic of Tuebingen. The virological database of SwissLab program was used. All data of the patients were tabulated and categorized in Microsoft Excel databases.

This retrospective observational analysis approved by the institutional review board of the Medical Faculty of the University of Tuebingen. The informed consent was exempted because of anonymous assessment of medical records.

2.2. Characteristics of study cohort patients

The total number of patients is 437, consists of 265 males and 172 females. Patients age range from 19 to 87 years old, the average age is 52.6±12.9 (Mean ± SD). The Genotype of the patients include genotype 1a (134, 30.66%), genotype 1b (173, 39.59%), genotype 1 unclassified (3, 0.69%), genotype 2a/b (12, 2.75%), genotype 3a/b (89, 20.37%), genotype 4 (21, 4.81%), genotype 5 (2, 0.46%), 6 (3, 0.69%).

2.3. Special patients population

Special patients population include patients with liver cirrhosis (123, 28.15%), liver transplantation (22, 5.03%), nephrectomy (6, 1.38%), pre-treated (212, 48.51%), coinfeciton with HIV (6, 1.37%), coinfeciton with HBV (2, 0.46%), relapse (15, 3.43%), patients ≥ 60 yr (119, 27.23%), patients ≥ 80 yr (6, 1.37%). The clinical characteristics of our cohort are presented in table 5,6.

Patients Characteristics	n N=437	Proportion
Gender	**n**	**%**
Male	265	60.6%
Female	172	39.4%
Total	437	
Age	**n**	**%**
Mean ± SD	52.6 ± 12.9	
Min - Max	19 - 87	
Patients ≥ 60 yr	119	27.23%
Patients ≥ 80 yr	6	1.37%
Genotype	**n**	**%**
1a	134	30.66%
1b	173	39.59%
1 unclassified	3	0.69%
2a/b	12	2.75%
3a/b	89	20.37%
4	21	4.81%
5	2	0.46%
6	3	0.69%

Table 5. Characteristics of study cohort patients

Special patients	Number	Proportion
Liver cirrhosis	123	28.15%
Liver transplantation	22	5.03%
Nephrectomy	6	1.38%
Pre-treated	212	48.51%
Replapse	15	3.43%
Coinfeciton HIV	6	1.37%
Coinfeciton HBV	2	0.46%

Table 6. Special patients population

2.4. Treatment regimens

All 437 patients were treated with direct-acting antivirals (DAAs) at Department of Internal Medicine, University Hospital Tübingen. The treatment regiments include SOF (68, 15.56%), SOF/DAC (106, 24.26%), SOF/SMP (34, 7.78%), SOF/LDV (Harvoni) (160, 36.61%), Exviera/Viekirax (49, 11.21%), SOF/VEL (Epclusa) (16, 3.66%), GZR/EBR (Zepatier) (4, 0.92%). The proportions of different treatment regimens are shown in Table 7.

Treatment regimens	Number	Proportion
SOF	68	15.56%
SOF/DAC	106	24.26%
SOF/SMP	34	7.78%
SOF/LDV (Harvoni)	160	36.61%
Exviera/Viekirax	49	11.21%
SOF/VEL (Epclusa)	16	3.66%
GZR/EBR (Zepatier)	4	0.92%

Table 7. Distribution of Treatment regimens

Abbreviation name	Full name	Product name	Pharmaceutical company	Country
SOF	Sofosbuvir	SOVALDI®	Gilead Sciences, Inc.	America
SOF/LDV (Harvoni)	Sofosbuvir Ledipasvir	HARVONI®	Gilead Sciences, Inc.	America
SOF/DAC	Sofosbuvir Daclatasvir	SOVALDI® DAKLINZA™	Gilead Sciences	America
SOF/SMP	Sofosbuvir Simeprevir	SOVALDI® OLYSIO®	Gilead Sciences, Medivir AB Janssen Pharmaceutica	America Swedish Belgium
Exviera/ Viekirax	Exviera Viekirax	EXVIERA® VIEKIRAX®	AbbVie	Switzerland
SOF/VEL (Epclusa)	Sofosbuvir Velpatasvir	EPCLUSA®	Gilead Sciences, Inc.	America
GZR/EBR (Zepatier)	Grazoprevir Elbasvir	ZEPATIER®	Merck & Co, Inc	America

Table 8. DAAs treatment regiments components and manufacturer

Drugs Abbreviation	Component and dosage	Posology
SOF	Sofosbuvir 400mg	once daily
LDV/SOF (Harvoni)	Single tablet containing: Sofosbuvir 400mg Ledipasvir 90mg	once daily
SOF/DAC	Sofosbuvir 400mg Daclatasvir 60mg	once daily
SOF/SMP	Sofosbuvir 400mg Simeprevir 150mg	once daily
Exviera/Viekirax	Exviera 250 mg Viekirax 12.5/50 /75 mg	once daily
SOF/VEL (Epclusa)	Single tablet containing: Sofosbuvir 400 mg Velpatasvir 100 mg	once daily
GZR/EBR (Zepatier)	Single tablet containing: Grazoprevir 100mg Elbasvir 50mg	once daily

Table 9. Treatment regiments dose and duration (EASL, 2017)

2.5. Parameters and observation periods

PCR tests and recording periods:

Real-time PCR was used to detect the hepatitis C viral load from peripheral blood of the patients, The PCR (Polymerase chain reaction) in cell culture was detected quantitatively.

For virological analyses, Roche CobasAmpliprep/Roche Cobas TaqMan (Roche Diagnostics GmbH, Mannheim, Germany) was used. The lower limit of quantification (LLOQ) is 15 IU/mL.

HCV RNA levels in serum or plasma are typically measured at 0 (Baseline viral load), 2, 4 weeks (rapid virologic response, RVR); 8, and 12 weeks (early virologic response: EVR) of therapy; end of treatment (8, 12, or 24 weeks depending on HCV genotype and treatment response); and 12, 24, 48 weeks post-treatment (sustained virologic response: SVR12, SVR12, SVR48).

2.6. Interpretation of reference values

The quantitative result expressed in IU/mL or log IU/mL, which means the degree of active HCV viral replication in the blood. The CobasTaqMan has result values range from 15 to 100 million IU/mL (1.18 - 8.00 log IU/mL) for quantification of hepatitis C virus.

The result value of <15 IU/mL indicates that HCV RNA is detected, but this level of HCV RNA cannot be quantified accurately.

The result value of >100 million IU/mL (>8.00 log IU/mL) indicates the active HCV viral replication. The viral load quantification cannot be quantified accurately if the HCV RNA level above this upper limit.

2.7. Laboratory and Clinical parameters

The following qualitative parameters were collected:

1 Biometric data:

- Age
- Gender
- Genotype of HCV
- Main and secondary diagnoses

2 Clinical data

- Liver cirrhosis
- Liver transplantation
- Nephrectomy
- Pre-treated
- Relapse
- Coinfection with HIV
- Coinfection with HBV
- Start time of treatment
- End time of treatment
- Duration of treatment
- Completion of treatment

3 Laboratory parameters:

- Viral load (HCV RNA copies/ml of blood)

- ALT (Alanine transaminase) (U/L)
- Hemoglobin (mg/dl)
- PLT (Platelets) (10^9/L)
- Bilirubin (µmol/L)
- Creatinine (mg/dl)
- WBC (leukocytes) (10^9/L)

4 Viral load measuring time

- PCR 0 (Baseline viral load)
- PCR 2 (2 weeks during treatment)
- PCR 4 (4 weeks during treatment)
- PCR 8 (8 weeks during treatment)
- PCR 12 (12 weeks during treatment)
- PCR 24 (24 weeks during treatment)
- **SVR** (Sustained virologic response)
- SVR 12 (12 weeks post treatment)
- SVR 24 (24 weeks post treatment)
- SVR 48 (48 weeks post treatment)

5 Comorbidity and complications:

- Liver cirrhosis
- Liver failure
- Liver transplantation
- Liver cancer
- Death during or after treatment

2.8. Statistical analysis

437 patients were included for statistical analysis. All collected data and parameters were stored in a database of the Microsoft Office Excel 2010®. The data were statistically analyzed using JMP13.0 software. Multiple of statistical analysis methods were used in this study, including student's t-test, normal distribution test, distribution analysis, bivariate analysis, one-way analysis, logistic fit analysis and contingency analysis, etc. The SVR 12 rate, relapse patients, coinfection with HBV or HIV, and liver cirrhosis patients were enrolled in the multi-factor analysis.

3. Results

3.1. Characteristics of study cohort patients

Patients characteristics	n, % N=437
Gender	
Male	265 (60.6%)
Female	172 (39.4%)
Age	
Mean±SD	52.6±12.9 yr
(Min-Max)	19 – 87 yr
Patients ≥ 60 yr	119 (27.23%)
Patients ≥ 80 yr	6 (1.37%)

Table 10. Characteristics of cohort hepatitis C patients

3.1.1. Gender

The patient population consisted of 265 males and 172 females, and the proportion of male patients (60.6%) was obviously more than that of female patients (39.4%), with statistically significant differences (Fig.4).

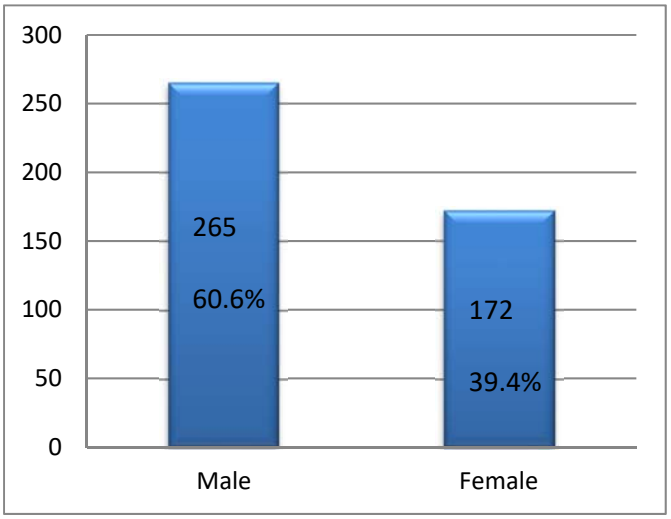

Fig. 4. Gender proportion of 437 hepatitis C patients

3.1.2. Age

Patients age range from 19 to 87 years old, the average age is 52.59±12.98 yr(Mean±SD). The frequency distribution pattern of age is shown in Fig， 50-60 age accounted for the largest proportion, followed by the 60-65 age group and 45-50 age group, 15-25 age group and 80-90 age group accounted for the smallest proportion.

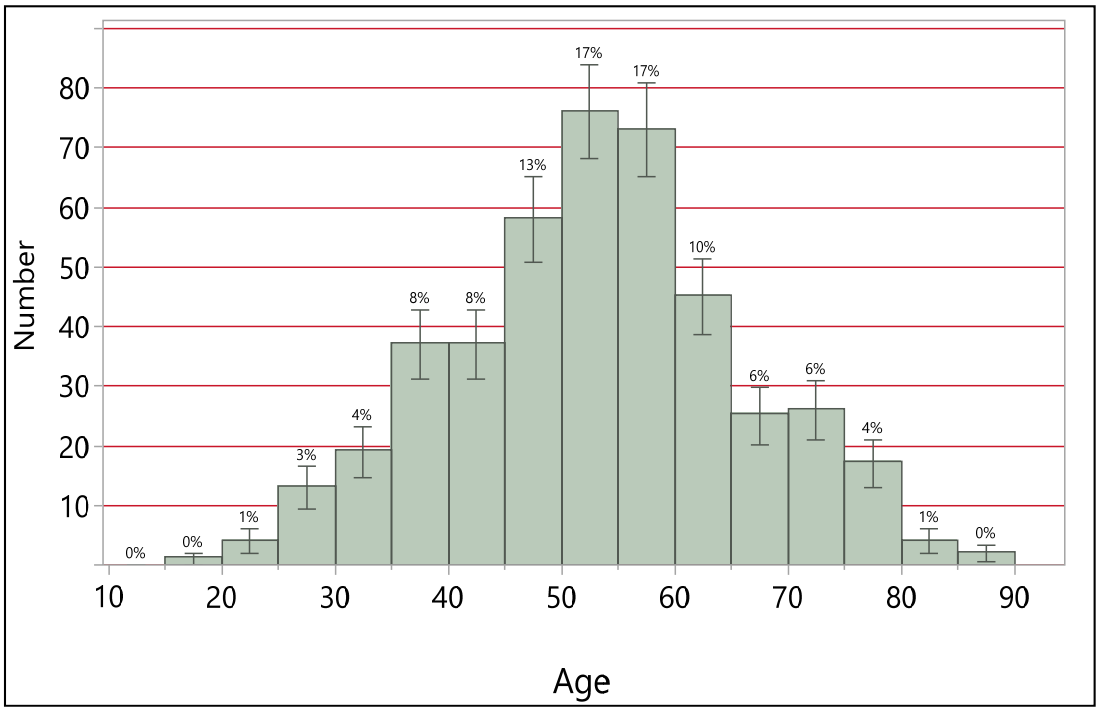

Fig. 5. Age distribution of hepatitis C patients

3.1.3. Genotypes of HCV

The Genotype of the patients include genotype 1a (134, 30.66%), genotype 1b (173, 39.59%), genotype 1 unclassified (3, 0.69%), genotype 2a/b (12, 2.75%), genotype 3a/b (89, 20.37%), genotype 4 (21, 4.81%), genotype 5 (2,0.46%), 6 (3, 0.69%).

It is clear that the proportion of patients with genotype 1a/b is the largest group, followed by genotype 3 group, genotype 5 and genotype 6, the lowest number of patients.

Genotype	N	%
1a	134	（30.66%）
1b	173	（39.59%）
1 unclassified	3	（0.69%）
2a/b	12	（2.75%）
3a/b	89	（20.37%）
4	21	（4.81%）
5	2	（0.46%）
6	3	（0.69%）

Table 11. The proportion of different genotypes of HCV

3.1.4. Treatment duration

There were three different types of treatment duration: 8 weeks（48, 10.98%）, 12 weeks（313, 71.62%）, 24 weeks (75, 17.16%). Most of Hepatitis C patients had a 12-weeks duration of treatment (Fig. 12).

Treatment duration	n	% N=437
24 weeks	75	17.16%
12 weeks	313	71.62%
8 weeks	48	10.98%

Table 12. The proportion of treatment duration

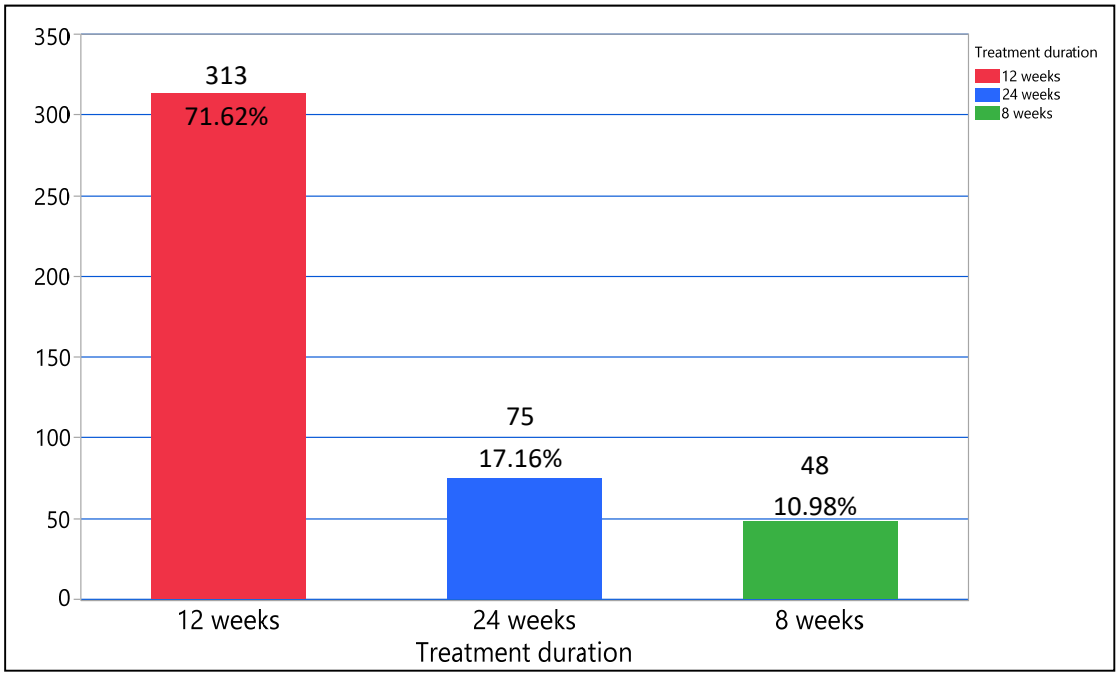

Fig. 6. Treatment duration of hepatitis C patients

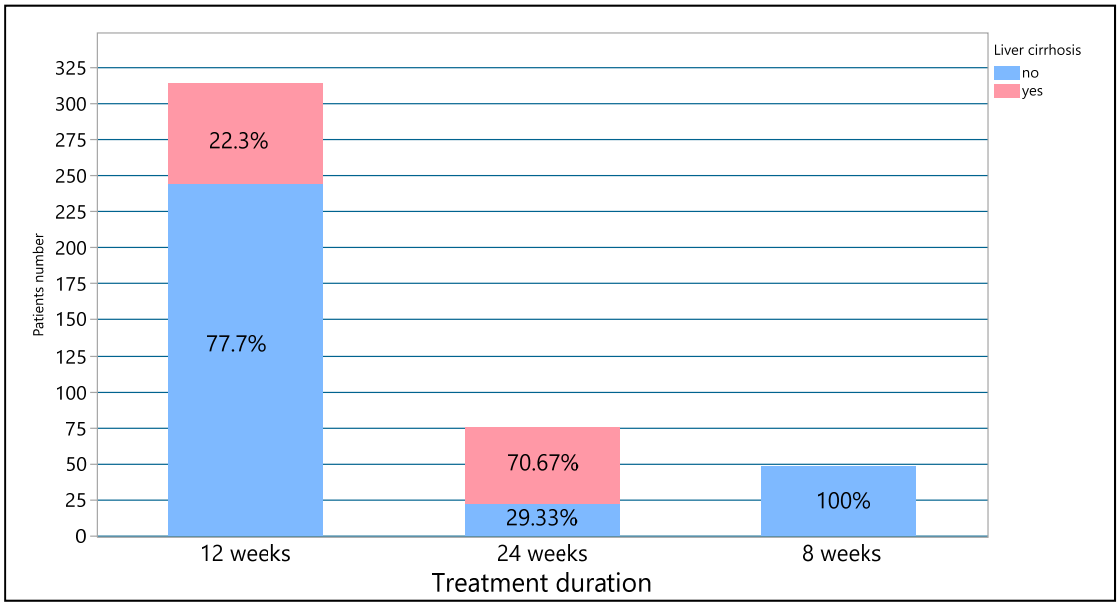

Fig. 7. Treatment duration of with/without liver cirrhosis patients

3.1.5. Treatment regimens

All 437 patients were treated with direct-acting antivirals (DAAs) in the Department of Internal MedicineI, University Hospital Tuebingen. The treatment regimens include SOF (68, 15.56%), SOF/DAC (106, 24.26%), SOF/SMP (34, 7.78%), LDV/SOF (Harvoni) (160, 36.61%), Exviera/Viekirax (160, 36.61%), SOF/VEL (Epclusa) (16, 3.66%), GZR/EBR (Zepatier) (4, 0.92%). Among these treatment Regimens, the number of LDV/SOF (Harvoni)is the largest, followed by SOF/DAC, the least use is Epclusa and Zepatier. The proportions of different treatment regimens are shown in table 13 and figure 8.

Treatment Regimens	**Full name (Component)**	**n Proportion N=437**
LDV/SOF (Harvoni)	Sofosbuvir Ledipasvir	160, （36.61%）
SOF/DAC	Sofosbuvir Daclatasvir	106, （24.26%）
SOF	Sofosbuvir	68, （15.56%）
Exviera/Viekirax	Exviera Viekirax	49, （11.21%）
SOF/SMP	Sofosbuvir Simeprevir	34, （7.78%）
SOF/VEL (Epclusa)	Sofosbuvir Velpatasvir	16, （3.66%）
GZR/EBR (Zepatier)	Elbasvir Grazoprevir	4, （0.92%）

Table 13. Treatment Regimens of 437 Hepatitis C patients

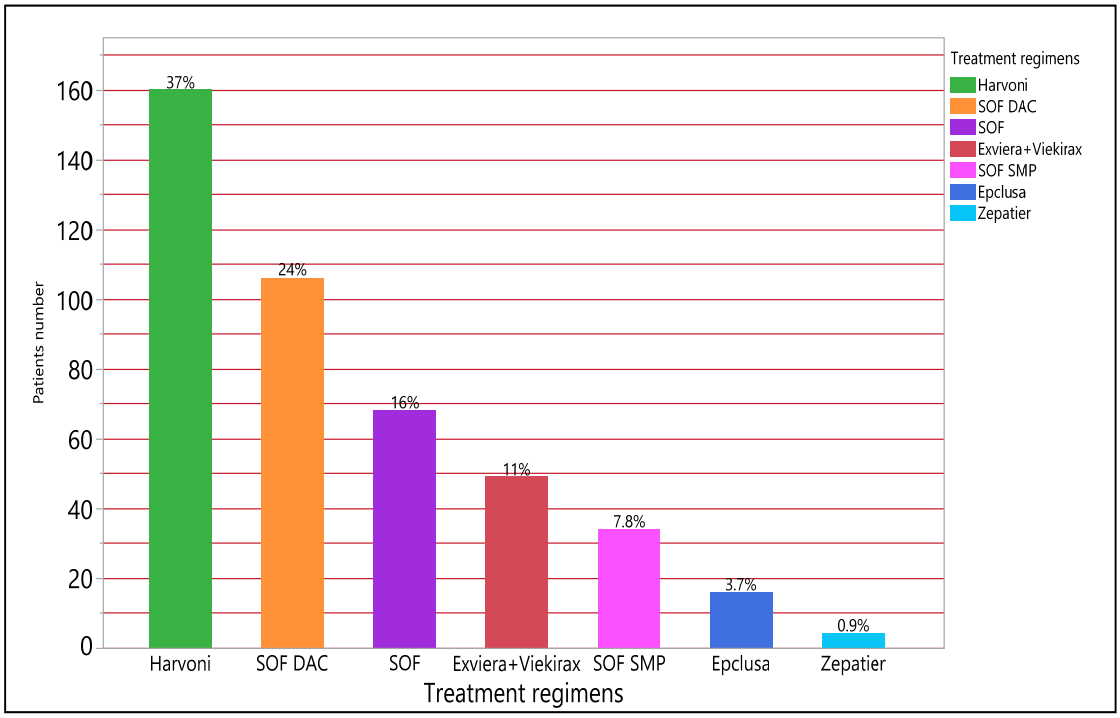

Fig. 8. Treatment regimens of hepatitis C patients

3.1.6. Baseline clinical chemistry of the 437 hepatitis C patients

Test	Unit	Mean	Std Dev	Min	Max
Hemoglobin	(mg/dL)	14.43	5.26	5.2	115
WBC	(/dL)	8169.54	37169.08	655	766660
PLT	(1000/µL)	182.47	95.28	3	1085
INR	(INR)	1.08	0.25	0.9	4.4
Creatinine	(mg/dL)	0.82	0.62	0	6.8
Bilirubin	(mg/dL)	1.42	6.31	0.1	98
ALT	(IU/1)	82.97	62.01	14	465

Table 14. Baseline clinical chemistry of 437 hepatitis C patients

3.2. Overall treatment outcome

437 Hepatitis C patients were treated with second-generation direct-acting antivirals (DAAs). All the 437 patients' viral load dropped to zero within 24 weeks during the treatment. Among them, 403 patients successfully with SVR12 HCV RNA negative; 15 patients relapsed; 2 patients died and one patients terminated, 16 patient (3.66%) lost to follow up after treatment.

Treatment outcome	Number
SVR12 negative	403
Relapse	15
Death	2
Lost to follow up	16
Termination	1
Total	437

Table 15. Overall treatment outcome of total 437 patients

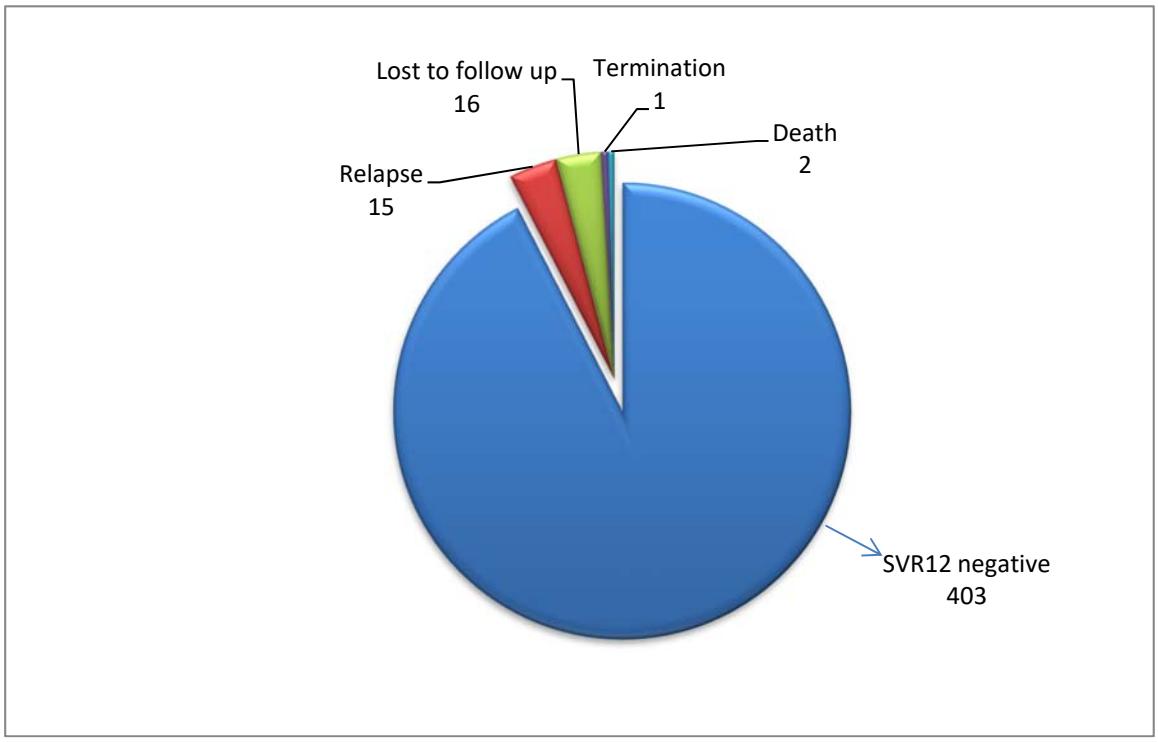

Fig. 9. Overall treatment outcome of total 437 patients

3.3. Analysis of SVR12 rate

Overall SVR12 rates

The overall SVR12 rate is 95.95%. 420/437 patients have finished the follow up after treatment and with SVR12 data available. 16/437 patients lost to follow up, and 1/437 patient terminated although his viral load has dropped to negative during treatment. These 17/ 437 patient (16 lost to follow up and 1 termination) were excluded from the analysis of SVR12 rate because without data of SVR12. Total 420/437 patients were involved in the analysis of SVR 12 rate. These 420 patients including SVR12 negative (403/420, 95.95%), relapse (15/420, 3.57%) and death (2/420, 0.47%).

SVR12 rates of subgroups

SVR12 rate for genotype were: 96.92% for GT1a, 95.78% for GT1b, 93.02% for GT3, 100% for GT1 unclassified, GT2, GT4, GT5 and GT6.

SVR12 rate for treatment regiments were: 96.71% for Harvoni, 95.28% for SOF/DAC, 93.94% for SOF, 100% for Exviera/Viekirax, 91.18% for SOF/SMP, 100% for Epclusa, 100% for Zepatier.

SVR12 rate for comorbidity and coinfection were: 90.76% for liver cirrhosis, 90.91% for liver transplantation, 95.61% for pretreated. SVR12 rate 100% for coinfection with HIV, 50% for coinfection with HBV.

SVR12 rate for gender: 94.53% for male, 98.17% for female.

SVR12 rate for treatment duration: 95.96% for 8 weeks, 97.01% for 12 weeks, 91.89% for 24 weeks.

SVR12 rate for baseline viral load: The patients with high viral load (>800,000 IU/mL) and very high viral load (>6,000,00 IU/mL) have higher SVR12 rate compared to the low viral load (<800,000 IU/mL).

SVR12 rate statistical correlation with liver cirrhosis (P=0.0016*), liver transplantation (P=0.0166), coinfection with HBV (P=0.0004*), baseline PLT (P=0.0068*).

3.3.1. Analysis SVR12 rate of genotype

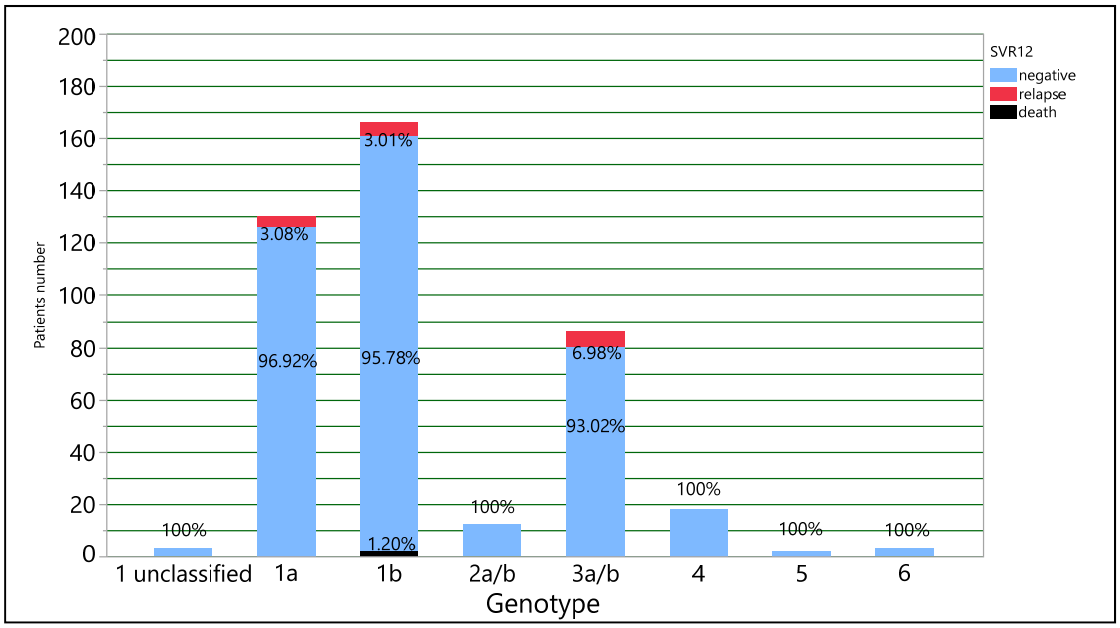

Fig. 10. Analysis of SVR12 rate by genotype

Count Row %	SVR12	Relapse	Death	Total
1 unclassified	3 100.00	0 0.00	0 0.00	3
1a	126 96.92	4 3.08	0 0.00	130
1b	159 95.78	5 3.01	2 1.20%	166
2a/b	12 100.00	0 0.00	0 0.00	12
3a/b	80 93.02	6 6.98	0 0.00	86
4	18 100.00	0 0.00	0 0.00	18
5	2 100.00	0 0.00	0 0.00	2
6	3 100.00	0 0.00	0 0.00	3
Total	403	15	2	420

Table 16. Analysis SVR 12 outcome by genotype

3.3.2. Analysis SVR12 rate of treatment regimens

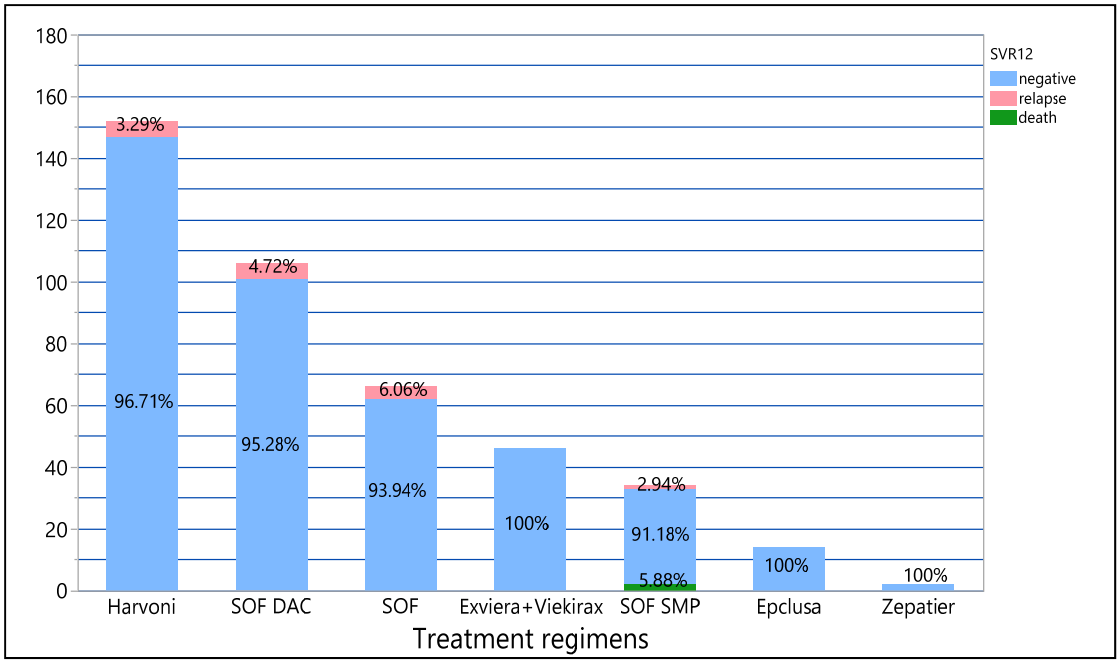

Fig. 11. Analysis SVR12 rate of treatment regimens

Count Row %	**Negative**	**Relapse**	**Death**	**Total**
Epclusa	14 100.00	0 0.00	0 0.00	14
Exviera+Viekirax	46 100.00	0 0.00	0 0.00	46
Harvoni	147 96.71	5 3.29	0 0.00	152
SOF	62 93.94	4 6.06	0 0.00	66
SOF DAC	101 95.28	5 4.72	0 0.00	106
SOF SMP	31 91.18	1 2.94	2 5.88	34
Zepatier	2 100.00	0 0.00	0 0.00	2
Total	403	15	2	420

Table 17. Analysis SVR12 rate of treatment regimens

3.3.3. Analysis treatment regimens for different genotypes

3.3.3.1 Treatment regimens for GT1a

Count Row %	**SVR12 Negative**	**Relapse**	**Total**
Exviera+Viekirax	8 100.00	0 0.00	8
Harvoni	78 98.73	1 1.27	79
SOF	11 84.62	2 15.38	13
SOF DAC	27 96.43	1 3.57	28
SOF SMP	2 100.00	0 0.00	2
Total	126	4	130

Table 18. Treatment regimens SVR12 rate of GT1a

3.3.3.2 Treatment regimens for GT1b

Count Row %	**SVR12 Negative**	**Relapse**	**Death**	**Total**
Epclusa	1 100.00	0 0.00	0 0.00	1
Exviera+Viekirax	34 100.00	0 0.00	0 0.00	34
Harvoni	61 95.31	3 4.69	0 0.00	64
SOF	11 91.67	1 8.33	0 0.00	12
SOF DAC	28 96.55	1 3.45	0 0.00	29
SOF SMP	22 91.67	0 0.00	2 8.33	24
Zepatier	2 100.00	0 0.00	0 0.00	2
Total	159	5	2	166

Table 19. Treatment regimens SVR12 rate of GT1b

3.3.3.3 Treatment regimens for GT3

Count Row %	SVR12 Negative	Relapse	Total
Epclusa	11 100.00	0 0.00	11
Exviera+Viekirax	2 100.00	0 0.00	2
Harvoni	0 0.00	1 100.00	1
SOF	25 96.15	1 3.85	26
SOF DAC	41 93.18	3 6.82	44
SOF SMP	1 50.00	1 50.00	2
Total	80	6	86

Table 20. Treatment regimens SVR12 rate of GT3

3.3.4. Analysis SVR12 rate of gender

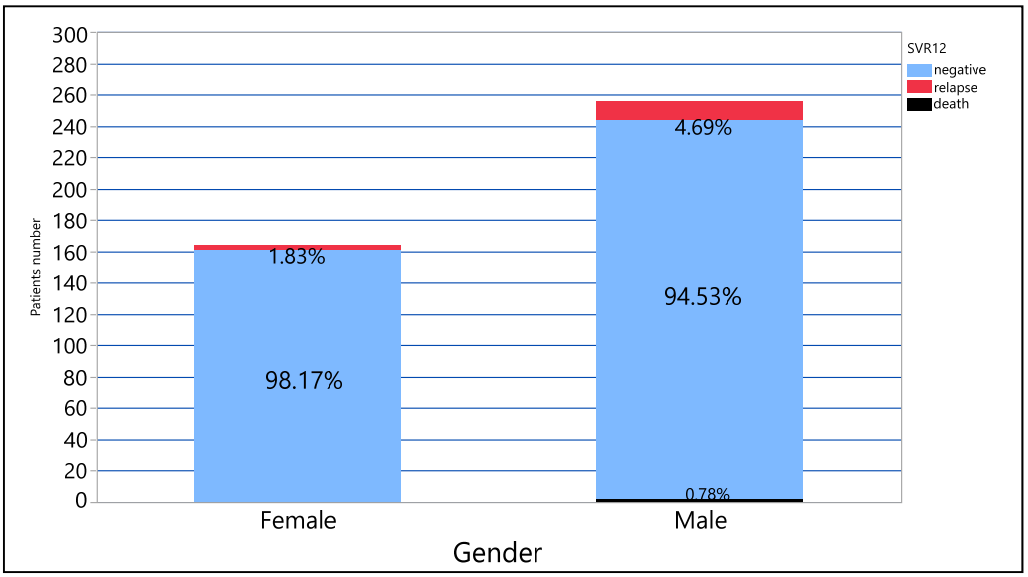

Fig. 12. Analysis SVR12 outcome by gender

Count Row %	Death	Negative	Relapse	Total
Female	0 0.00	161 98.17	3 1.83	164
Male	2 0.78	242 94.53	12 4.69	256
Total	2	403	15	420

Table 21. Analysis SVR12 outcome by gender

3.3.5. Analysis SVR12 rate by with/without liver cirrhosis

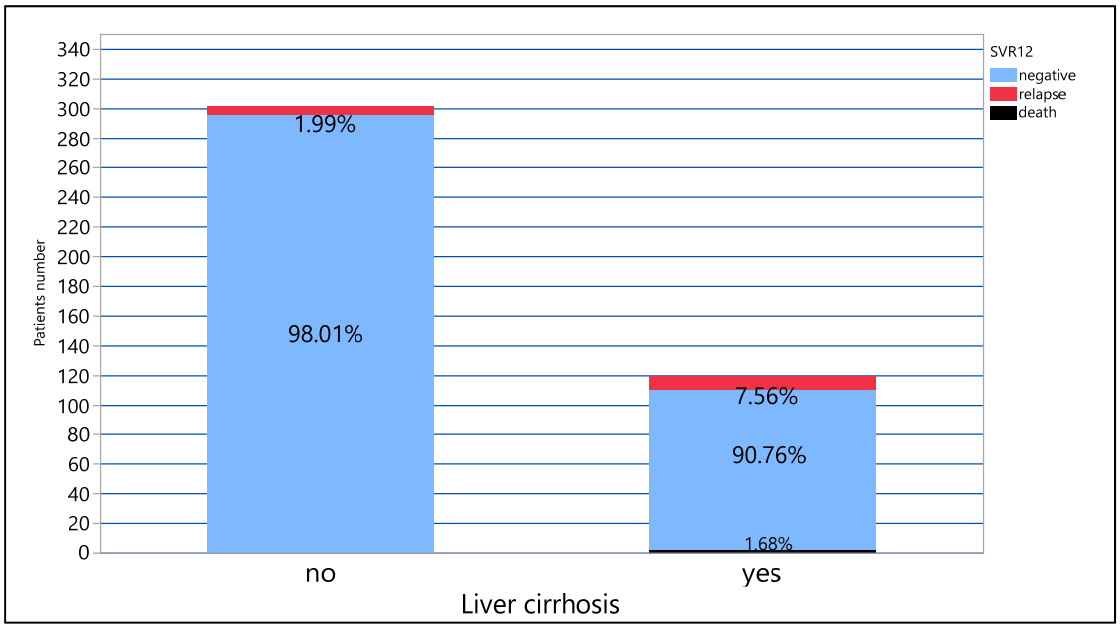

Fig. 13. SVR 12 rate by with/without liver cirrhosis (*P*=0.0016*)

Count Row %	**Negative**	**Relapse**	**Death**	**Total**
Without Liver cirrhosis	295 98.01	6 1.99	0 0.00	301
Liver cirrhosis	108 90.76	9 7.56	2 1.68	119
Total	403	15	2	420

Table 22. SVR 12 rate by with/without liver cirrhosis (*P*=0.0016*)

3.3.6. Analysis of SVR12 rate by with/without liver transplantation

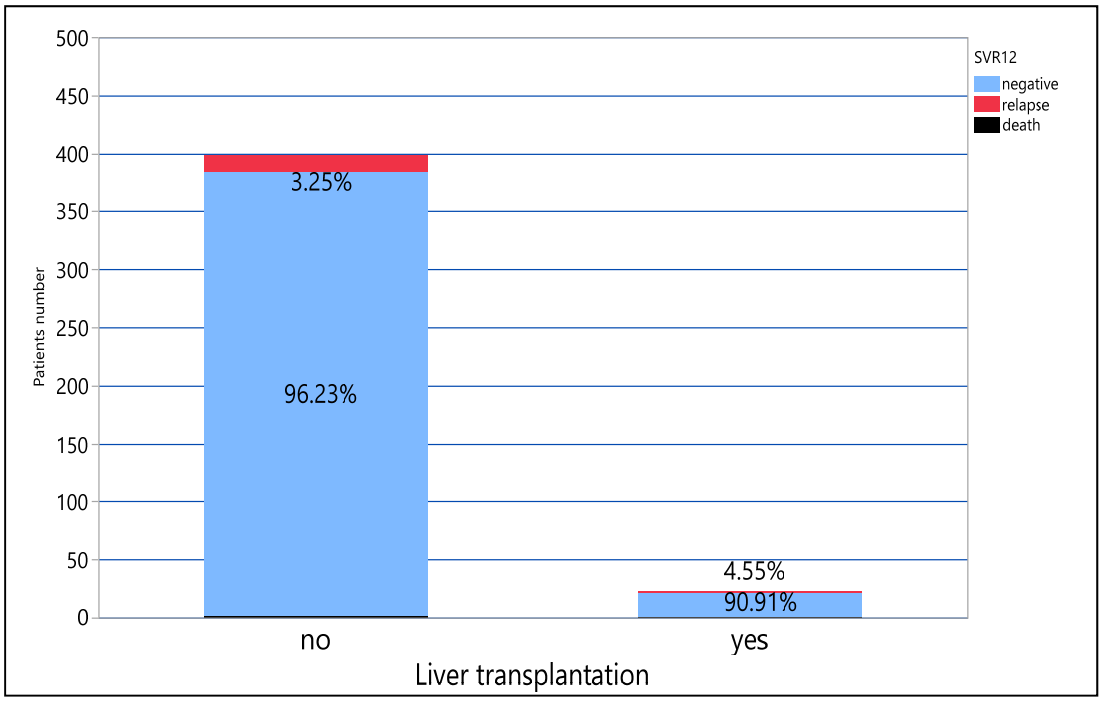

Fig. 14. SVR 12 rate by with/without liver transplantation (*P*=0.0166*)

Count Row %	**Negative**	**Relapse**	**Death**	**Total**
Without Liver transplantation	383 96.23	14 3.52	1 0.25	398
Liver transplantation	20 90.91	1 4.55	1 4.55	22
Total	403	15	2	420

Table 23. SVR 12 rate by with/without liver transplantation (*P*=0.0166*)

3.3.7. Analysis of SVR12 rate by baseline PLT (Platelets)

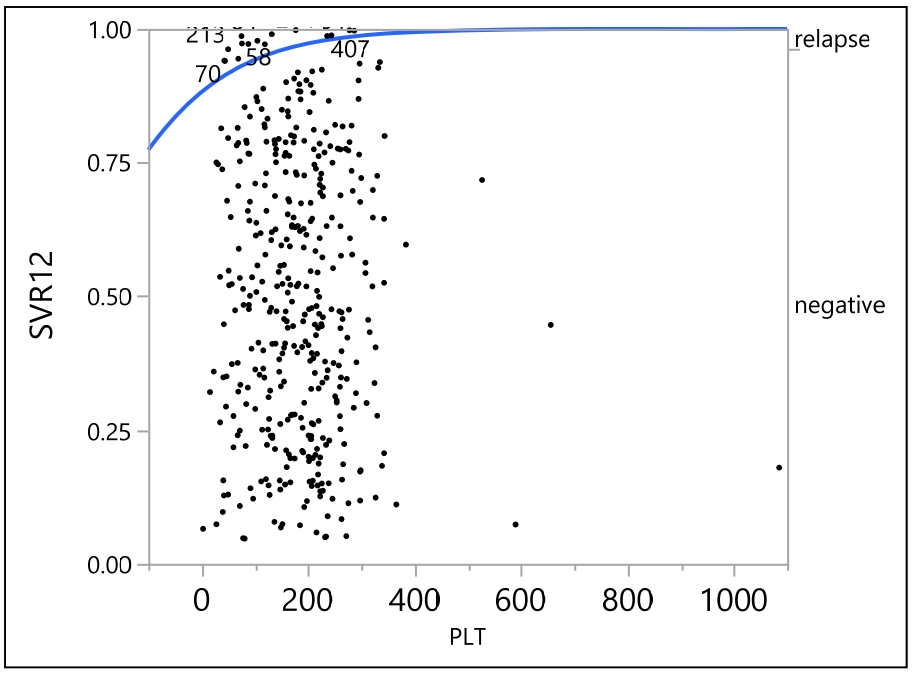

Fig. 15. Logistic fitting of SVR12 rate by baseline PLT (*P*=0.0068*)

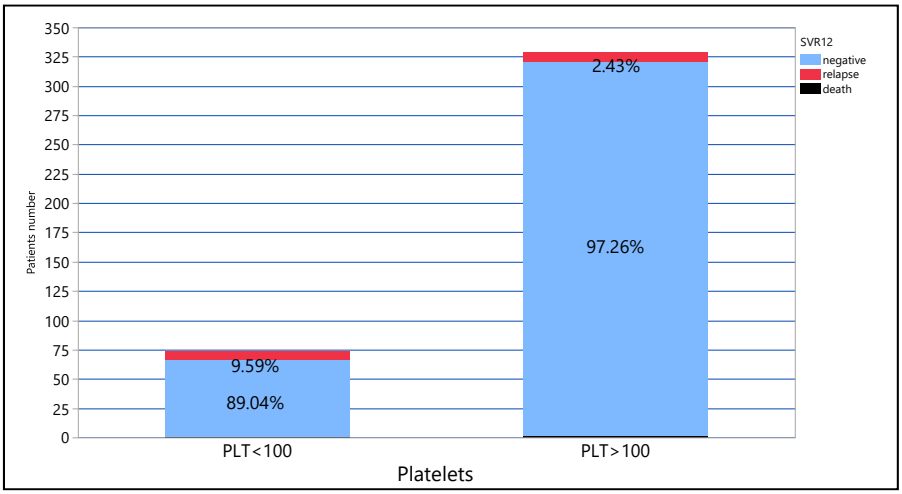

Fig. 16. SVR12 rate by baseline PLT (*P*=0.0068*)

Count Row %	**Negative**	**Relapse**	**Death**	**Total**
PLT<100	65 89.04	7 9.59	1 1.37	73
PLT>100	320 97.26	8 2.43	1 0.30	329
Total	385	15	2	402 (18 patients no data)

Table 24. SVR12 rate by baseline PLT (*P*=0.0068*)

3.3.8. Analysis of SVR12 rate by with/without pre-therapy

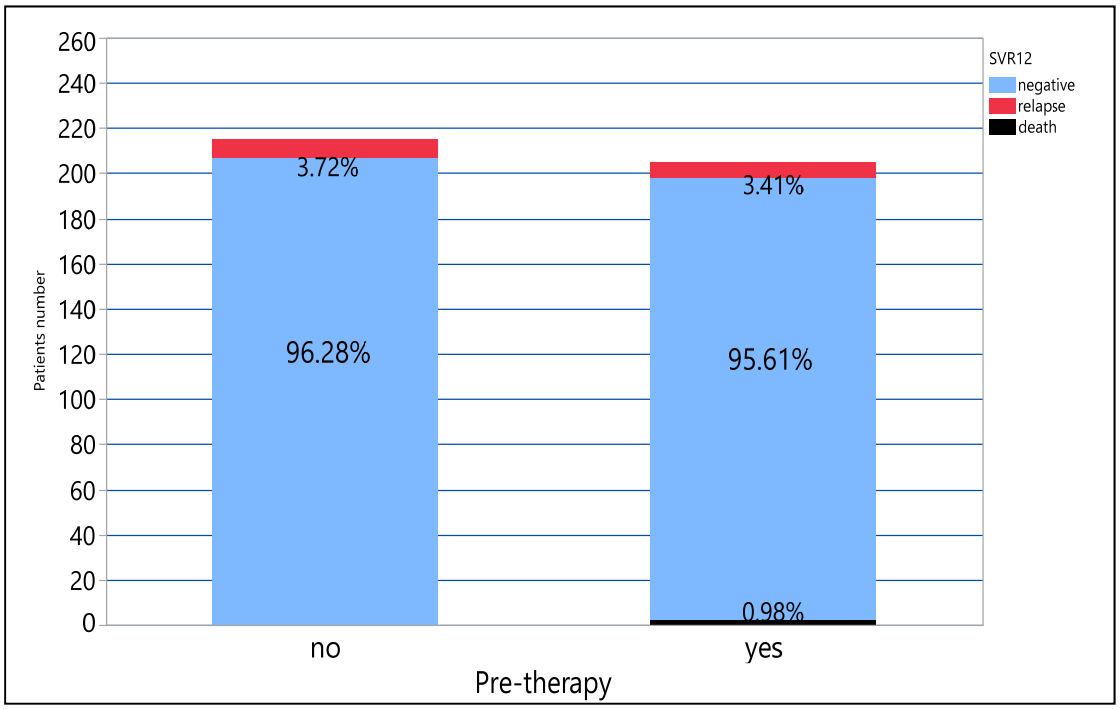

Fig. 17. SVR12 rate by with/without pre-therapy

Count Row %	**Negative**	**Relapse**	**Death**	**Total**
Without Pre-therapy	207 96.28	8 3.72	0 0.00	215
With Pre-therapy	196 95.61	7 3.41	2 0.98	205
Total	403	15	2	420

Table 25. SVR12 rate by with/without pre-therapy

3.3.9. Analysis of SVR12 rate by treatment duration

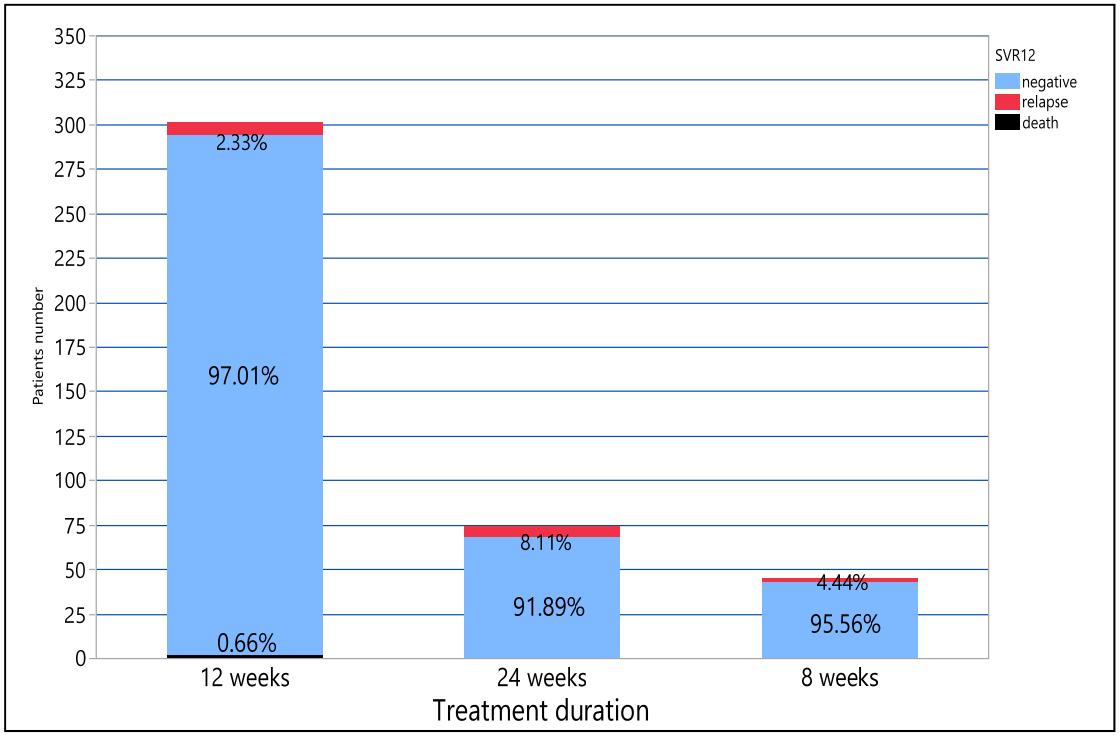

Fig. 18. SVR12 rate of treatment duration

Count Row %	**Negative**	**Relapse**	**Death**	**Total**
8 weeks	43 95.56	2 4.44	0 0.00	45
12 weeks	292 97.01	7 2.33	2 0.66	301
24 weeks	68 91.89	6 8.11	0 0.00	74
Total	403	15	2	420

Table 26. SVR12 rate of treatment duration

3.3.10. Analysis of SVR12 outcome by Age

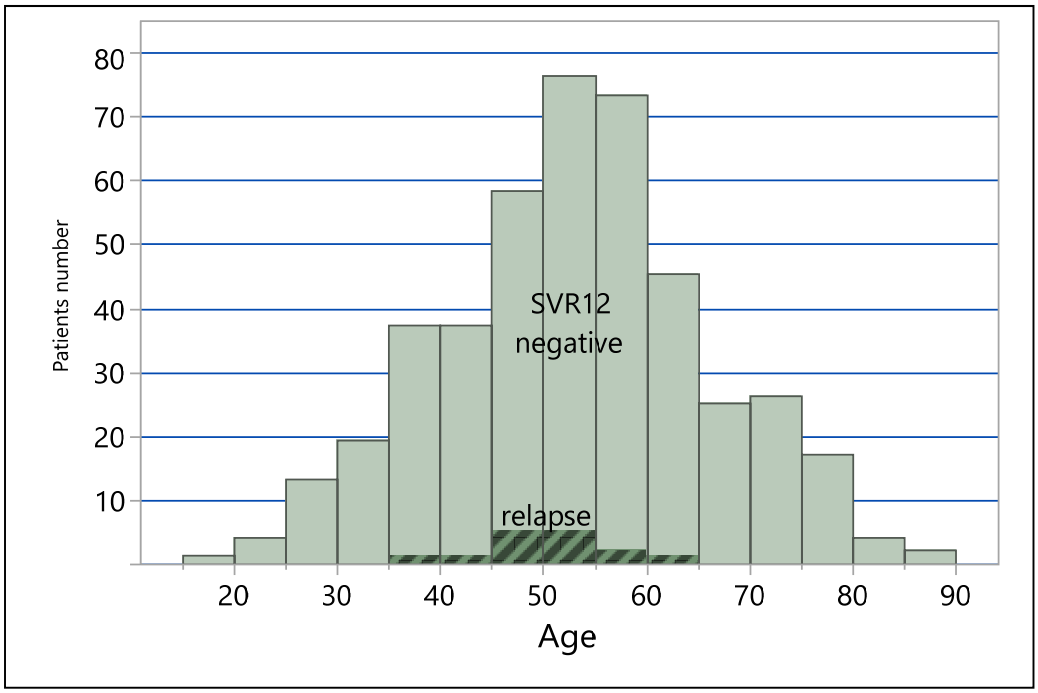

Fig. 19. SVR12 outcome by Age distribution

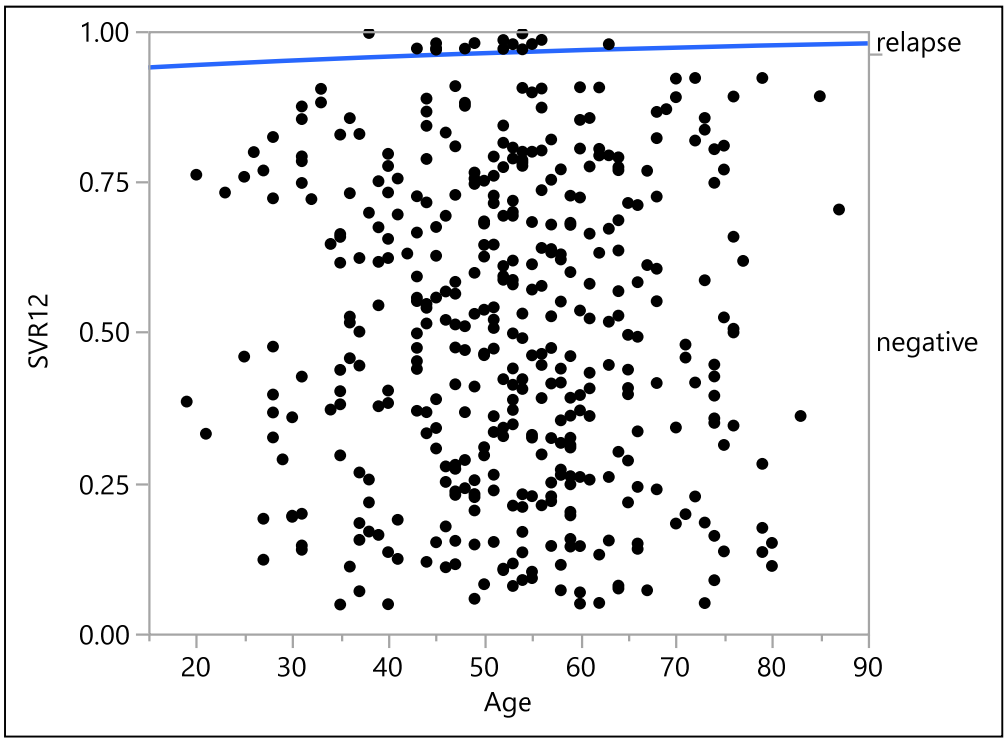

Fig. 20. Logistic fitting of SVR12 rate by age

Analysis of SVR12 outcome by Age>60

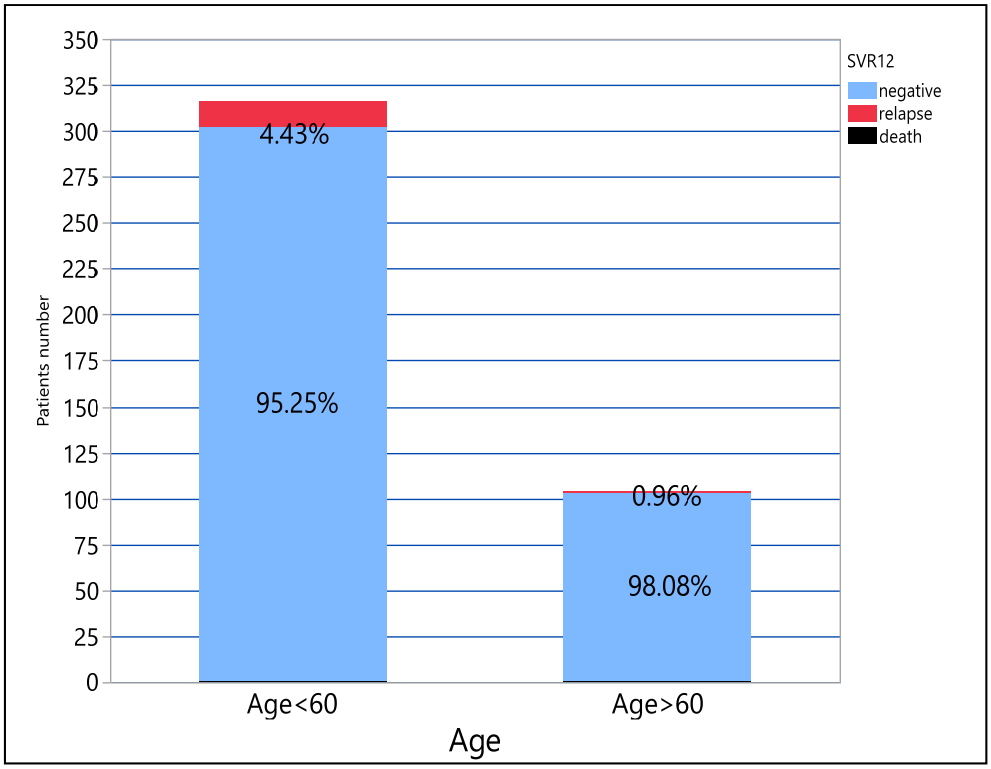

Fig. 21. Analysis SVR12 rate by age>60

Count Row %	**Relapse**	**SVR12 Negative**	**Death**	**Total**
Age<60	14 4.43	301 95.25	1 0.32	329
Age>60	1 0.93	107 99.07	1 0.96	108
Total	15	403	2	420

Table 27. Analysis SVR12 rate by age>60

3.3.11 Analysis of SVR12 outcome by baseline viral load

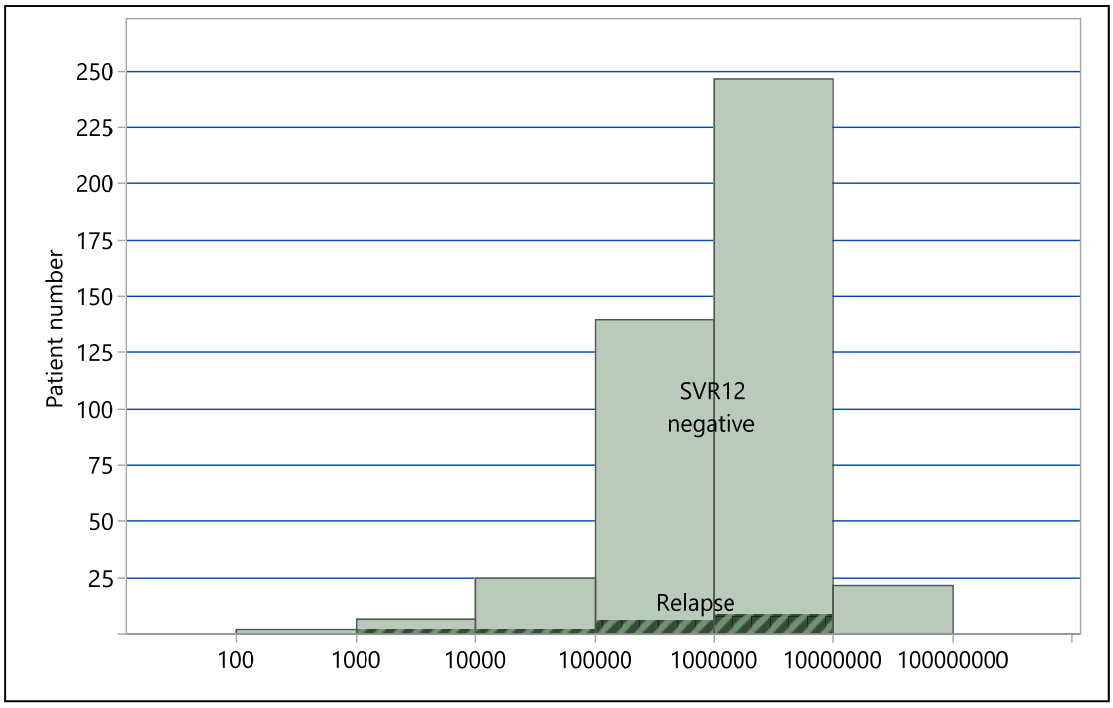

Fig. 22. SVR12 rate by baseline viral load distribution

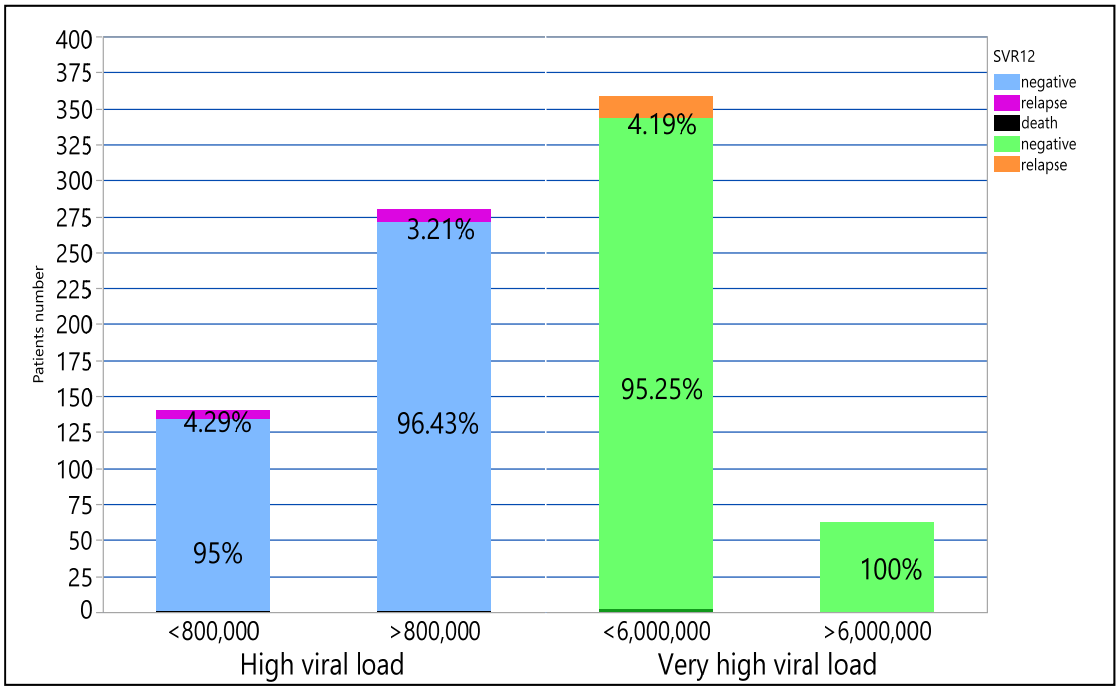

Fig. 23. SVR12 rate by high viral load

Count Row %	**SVR12** **Negative**	**Relapse**	**Death**	**Total**
Baseline viral load >800,000	270 96.43	9 3.21	1 0.36	280
Baseline viral load <800,000	133 95.00	6 4.29	1 0.71	140
Total	403	15	2	420

Table 28. Analysis SVR12 rate by high viral load (>800,000 IU/mL)

Count Row %	**SVR12** **Negative**	**Relapse**	**Death**	**Total**
Baseline viral load >6,000,000	62 100.00	0 0.00	0 0.00	62
Baseline viral load <6,000,000	341 95.25	15 4.19	2 0.56	358
Total	403	15	2	420

Table 29. Analysis SVR12 rate by very high viral load (>6,000,000 IU/mL)

3.4. Analysis of genotype

3.4.1. Analysis of treatment regimens by genotypes

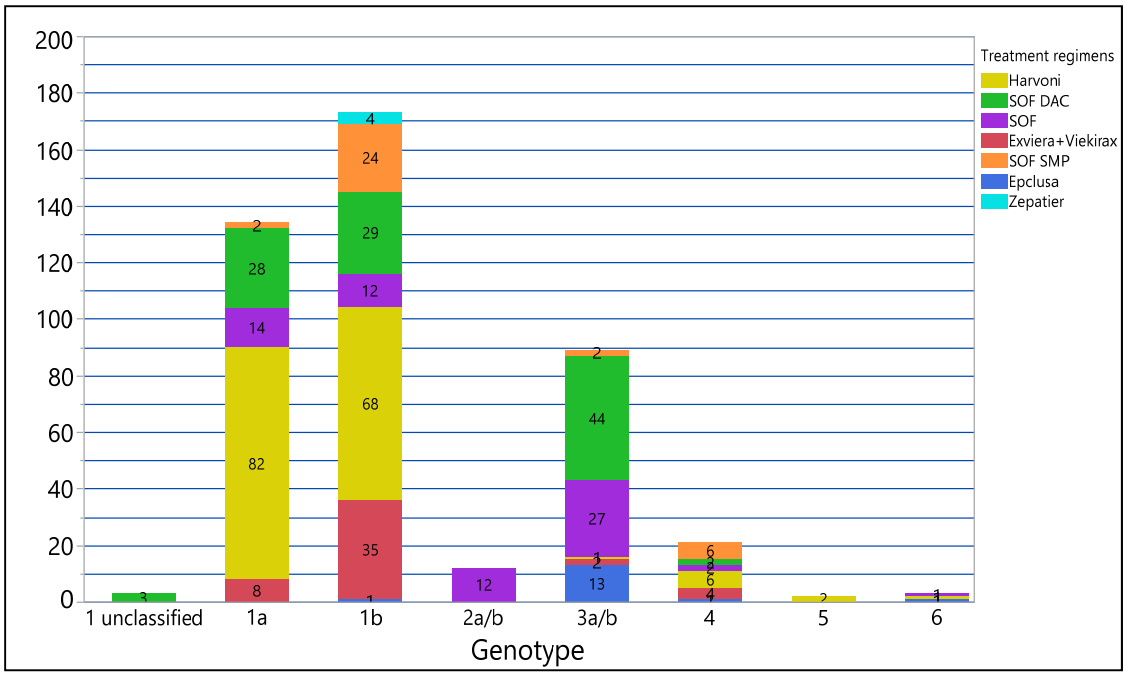

Fig. 24. Analysis genotype by treatment regimens

Count Row %	1 unclassified	1a	1b	2a/b	3a/b	4	5	6	Total
Epclusa	0 0.00	0 0.00	1 6.25	0 0.00	13 81.25	1 6.25	0 0.00	1 6.25	16
Exviera+Viekirax	0 0.00	8 16.33	35 71.43	0 0.00	2 4.08	4 8.16	0 0.00	0 0.00	49
Harvoni	0 0.00	82 51.25	68 42.50	0 0.00	1 0.63	6 3.75	2 1.25	1 0.63	160
SOF	0 0.00	14 20.59	12 17.65	12 17.65	27 39.71	2 2.94	0 0.00	1 1.47	68
SOF DAC	3 2.83	28 26.42	29 27.36	0 0.00	44 41.51	2 1.89	0 0.00	0 0.00	106
SOF SMP	0 0.00	2 5.88	24 70.59	0 0.00	2 5.88	6 17.65	0 0.00	0 0.00	34
Zepatier	0 0.00	0 0.00	4 100.00	0 0.00	0 0.00	0 0.00	0 0.00	0 0.00	4
Total	3	134	173	12	89	21	2	3	437

Table 30. Analysis of genotype by treatment regimens

3.4.2. Analysis of genotype by gender

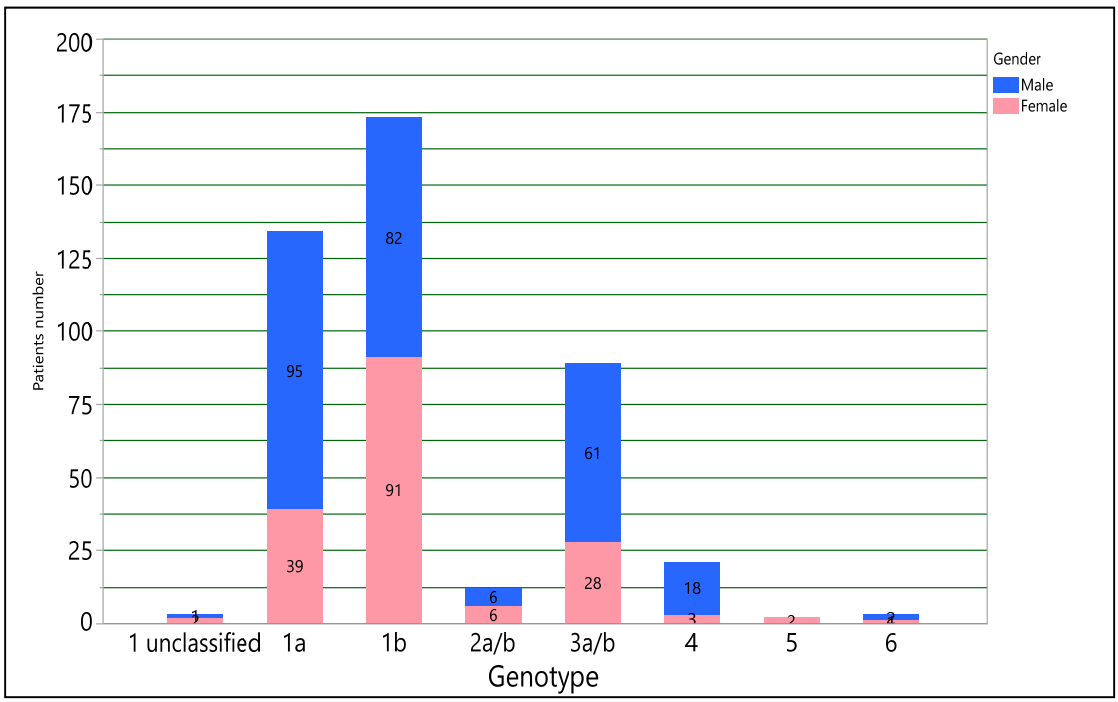

Fig. 25. Analysis of genotype by gender

Count Row %	**1 unclassified**	**1a**	**1b**	**2a/b**	**3a/b**	**4**	**5**	**6**	**Total**
Female	2 1.16	39 22.67	91 52.91	6 3.49	28 16.28	3 1.74	2 1.16	1 0.58	172
Male	1 0.38	95 35.85	82 30.94	6 2.26	61 23.02	18 6.79	0 0.00	2 0.75	265
Total	3	134	173	12	89	21	2	3	437

Table 31. Analysis of genotype by gender

3.4.3. Analysis of relapse by genotype

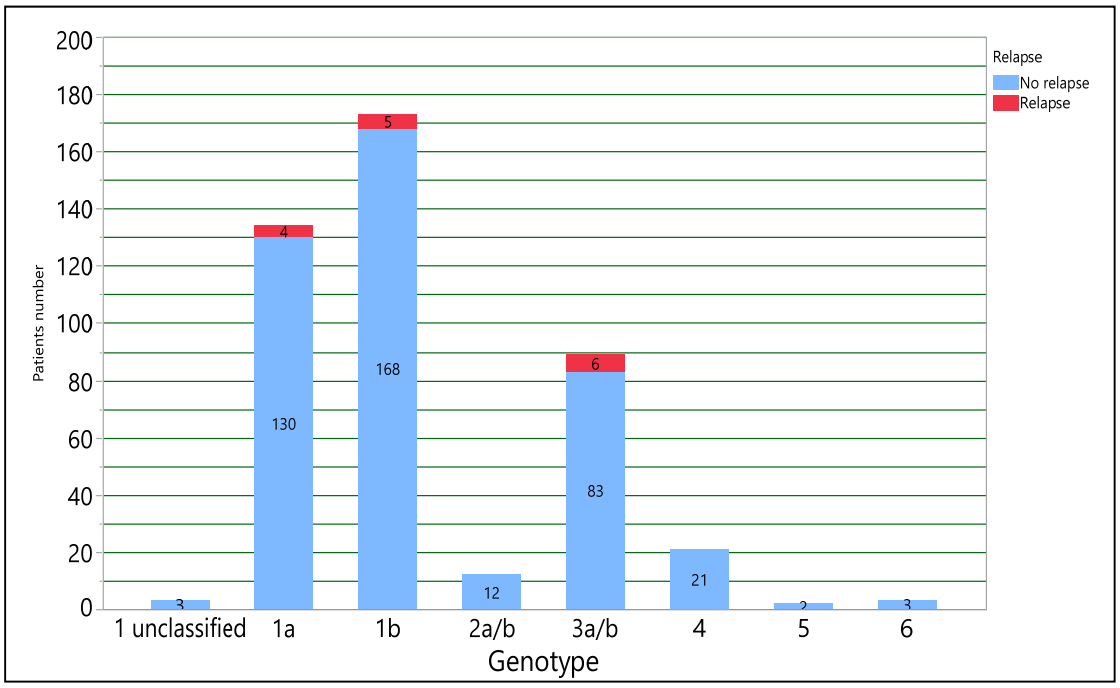

Fig. 26. Analysis of relapse by genotype

Count Row %	1 unclassified	1a	1b	2a/b	3a/b	4	5	6	Total
No relapse	3 0.71	130 30.81	168 39.81	12 2.84	83 19.67	21 4.98	2 0.47	3 0.71	422
Relapse	0 0.00	4 26.67	5 33.33	0 0.00	6 40.00	0 0.00	0 0.00	0 0.00	15
Total	3	134	173	12	89	21	2	3	437

Table 32. Analysis of relapse by genotype

3.4.4. Analysis of liver cirrhosis by genotype

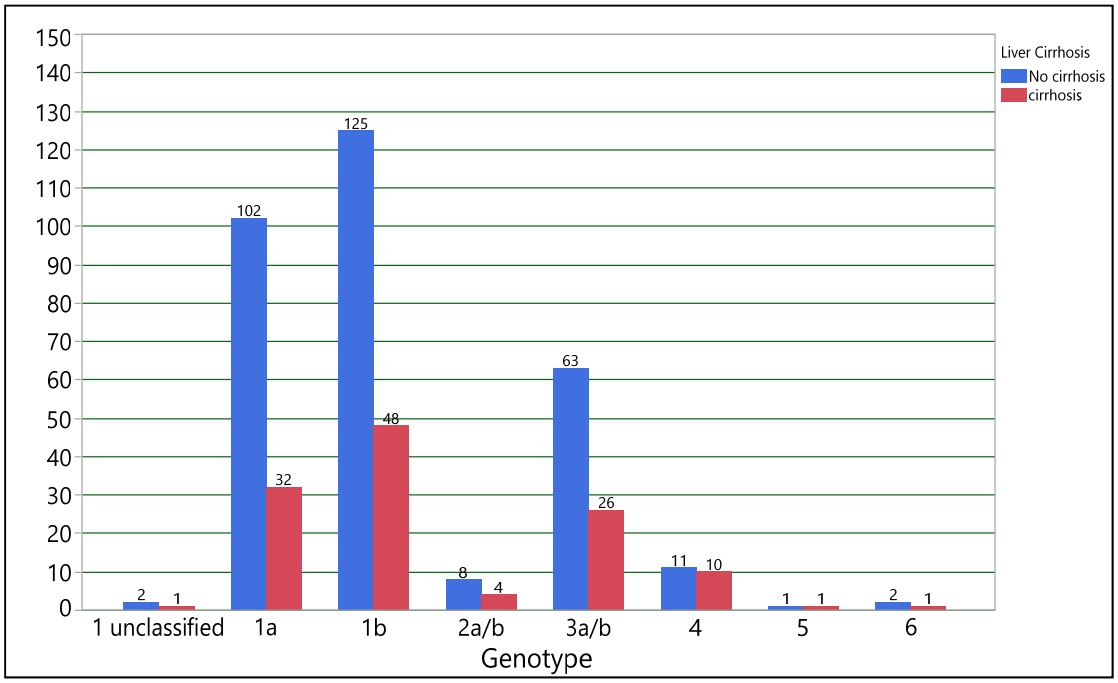

Fig. 27. Analysis of liver cirrhosis by genotype

3.4.5. Analysis of genotype by treatment duration

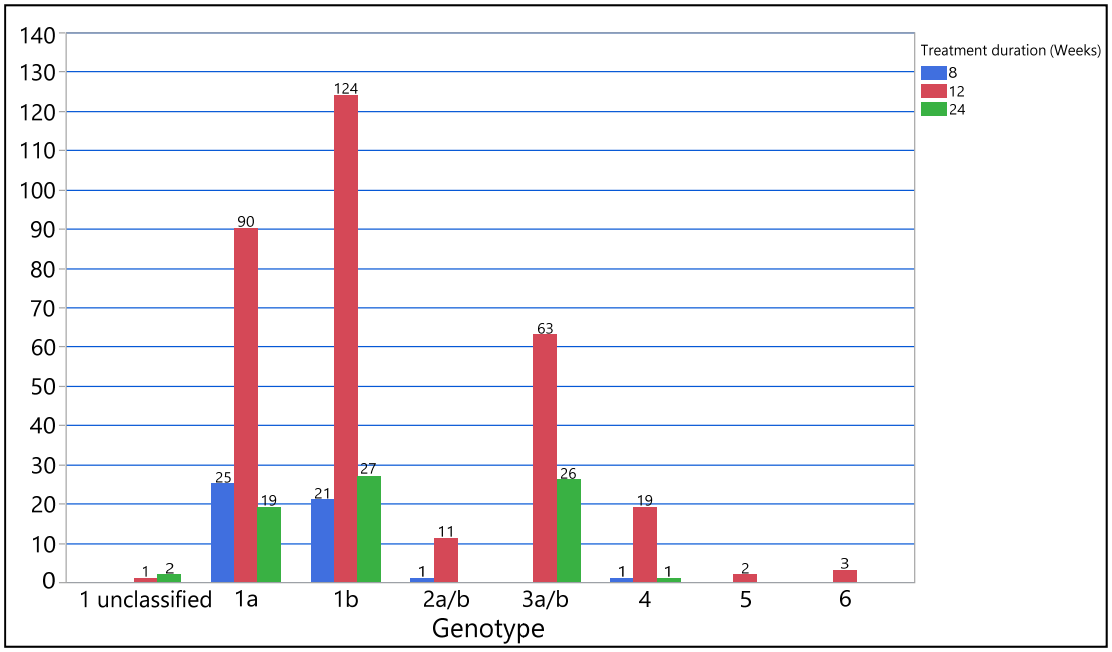

Fig. 28. Analysis of genotype by treatment duration

3.5. Viral load

3.5.1. Baseline viral load

The baseline viral load ranges of 437 patients range from 672 to 70,700,000 IU/mL. The distribution of baseline viral load result shows the majority of patients are in the range from 1,000,000 to 10,000,000 IU/m, followed by the range from 100,000 to 1,000,000 IU/mL. Few patients with viral load <100,000 IU/mL or >10,000,000 IU/mL.

3.5.2. High viral load vs. Low viral load

The baseline viral load is divided into three levels, include low viral load, high viral load and very high rival load. High viral load is defined as PCR ≥800,000 IU/ml or ≥2.000.000 IU/mL, and ≥6.000.000 IU/mL is considered to very high viral load. Low viral load is considered to <800,000 IU/ml.

In this study, There are 145 (33.18%) patients with low viral load (<800.000 IU/mL). Most of the patients 292 (67.73%) with high viral load ≥800.000 IU/mL. A small part of the patient with the very high viral load: 99 (22.65%) ≥6.000.000 IU/mL; 4 (0.92%) ≥25.000.000 IU/mL.

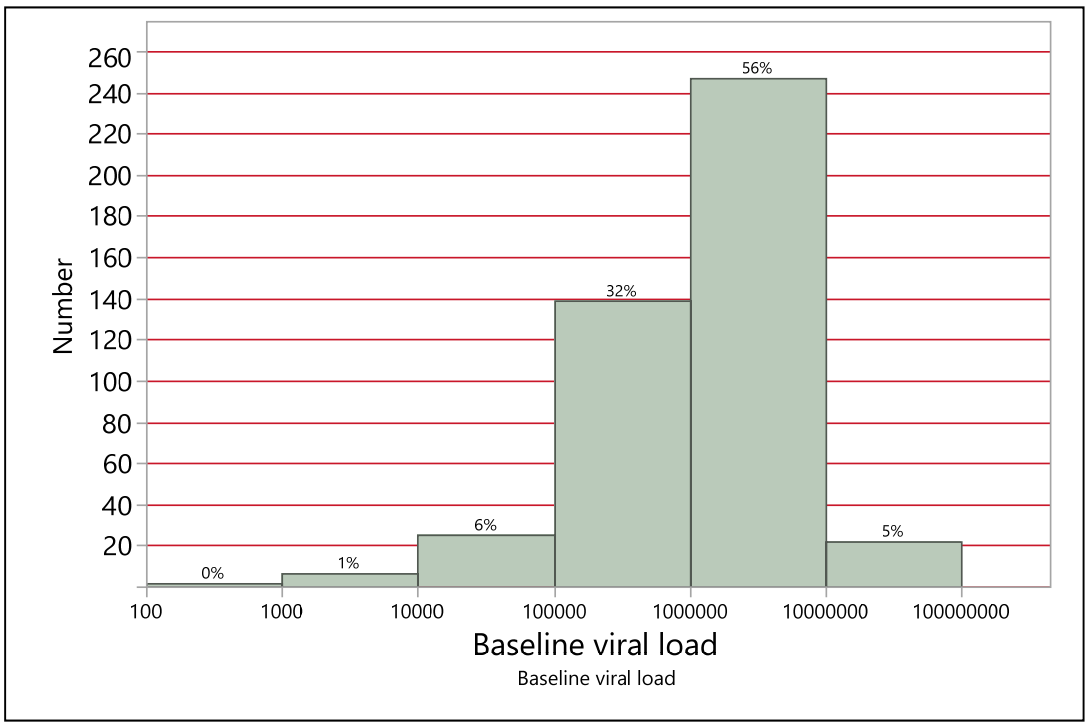

Fig. 29. Frequency distribution of baseline viral load

Baseline Viral load		Number	Proporiton
<800.000 IU/mL	Low viral load	145	(33.18%)
≥800.000 IU/mL	High viral load	292	(67.73%)
≥6.000.000 IU/mL	Very high viral load	99	(22.65%)
≥25.000.000 IU/mL	Very high viral load	4	(0.92%)

Table 33. High viral load vs. Low viral load

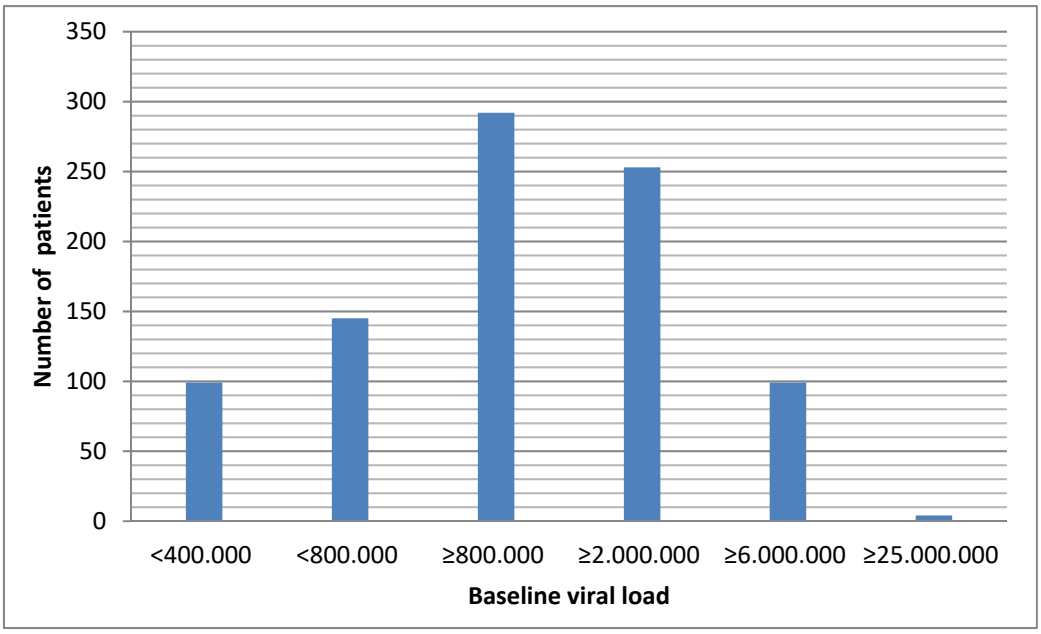

Fig. 30. High viral load vs. Low viral load of hepatitis C patient's proportion

Baseline Viral load	
Mean	3109403.73
Min	672
Max	70.700.000
Std Dev	5470164.4
Upper 95% Mean	3624889.5
Lower 95% Mean	2593918
75.0% Quartile	3870000
50.0% Median	1500000
25.0% Quartile	478000

Table 34. Analysis of Baseline Viral load

3.5.3. Min time of viral load drop to zero during treatment

Analysis of the PCR 0 min time showed that most patients' viral load drop to zero at 4weeks (220, 50.69%), followed by 12 weeks (144, 33.18%), and even some patients in the second week, the viral load decline to zero with rapid speed. Total 434 patients were included in the analysis because there are 3 patients' viral load' data were incomplete during treatment.

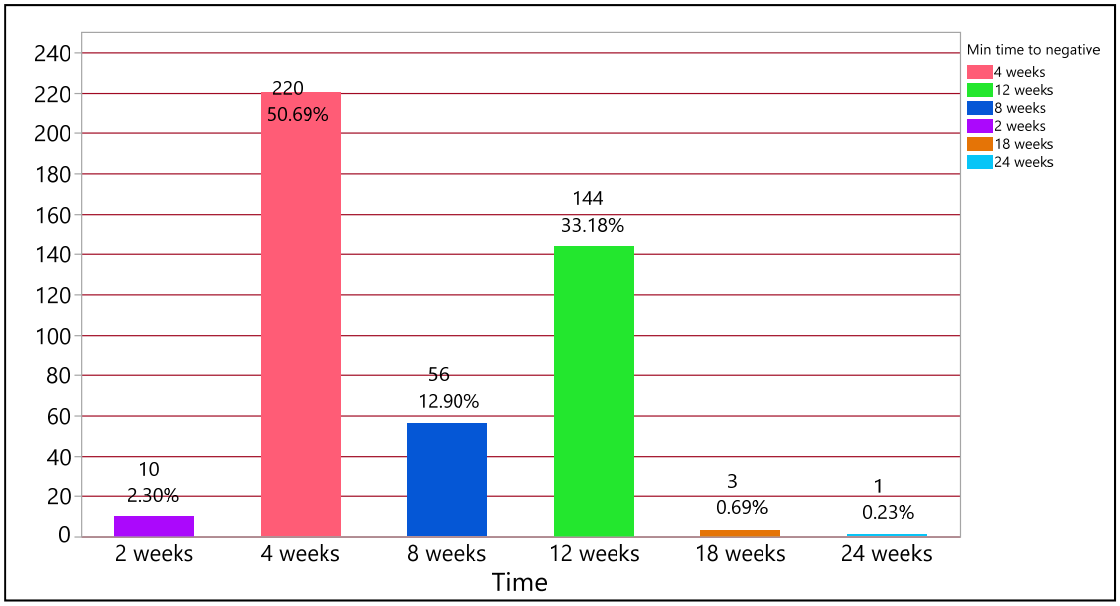

Fig. 31. Min time of viral load drop to 0 during treatment

3.5.4. PCR-negative/positive ratio at different times

For the analysis of the PCR negative / positive ratios at different times, the results showed that the viral load of most patients in the fourth week decreased to zero. The viral load of some patients dropped to zero in the second week. From the fourth week to the eighth week, the majority of patients PCR have turned to negative.

Because the patient did not perform PCR testing every week, most patients were tested in the fourth week from the beginning of the treatment, so most of the data in the second week was unknown.

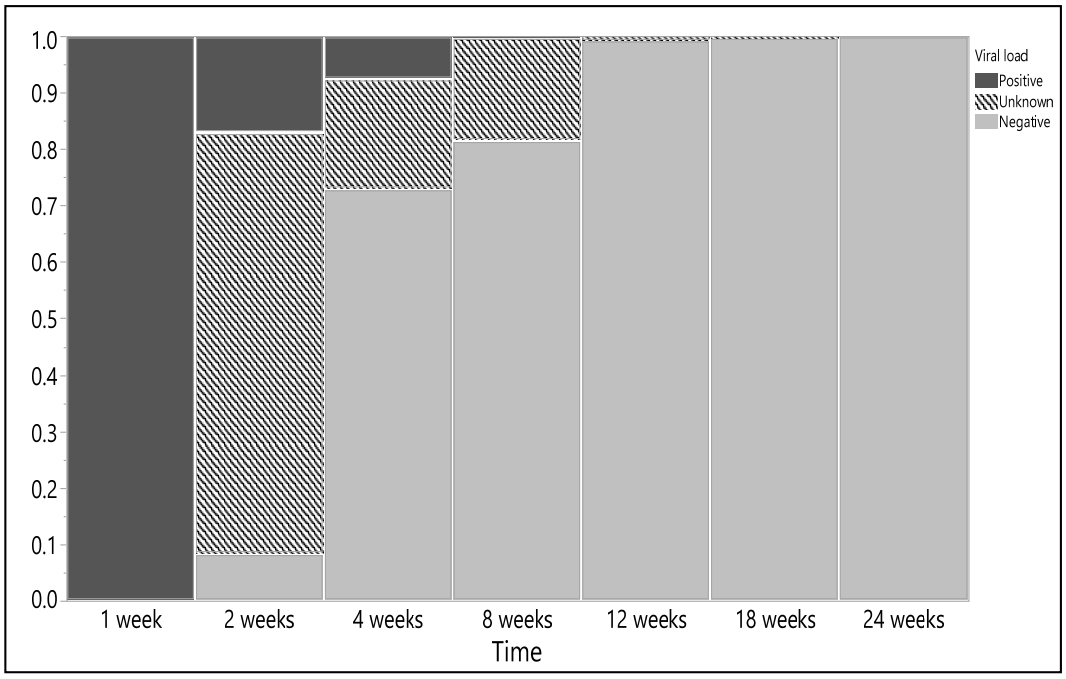

Fig. 32. Viral load negative/positive percentage at different time

3.5.5. Viral load changed with time

From the graph of PCR trend with time, it can be observed that most patients' viral load were reduced to zero in the fourth week.

Patients with a very high viral load of more than 25.000.000 IU/mL have decreased at a faster rate compared to other patients with viral load less than 25.000.000 IU/mL. Patients with very high viral load ≥25.000.000 IU/mL were dropped rapidly to zero within the fourth week. Even the patients with viral loads greater than 50.000.000 IU/mL and more than 70.000.000 IU/mL also decrease rapidly to 0 in the fourth week. On the other hand, Some patients with viral load levels below 25.000.000 IU/mL have slower PCR rates, PCR of viral load were reduced to 0 at week 12 or even 24 weeks.

The changes in viral load changing with time were analyzed by using Bivariate Fit analysis, show in the fitting curve of figure 33.

Viral load changing with time

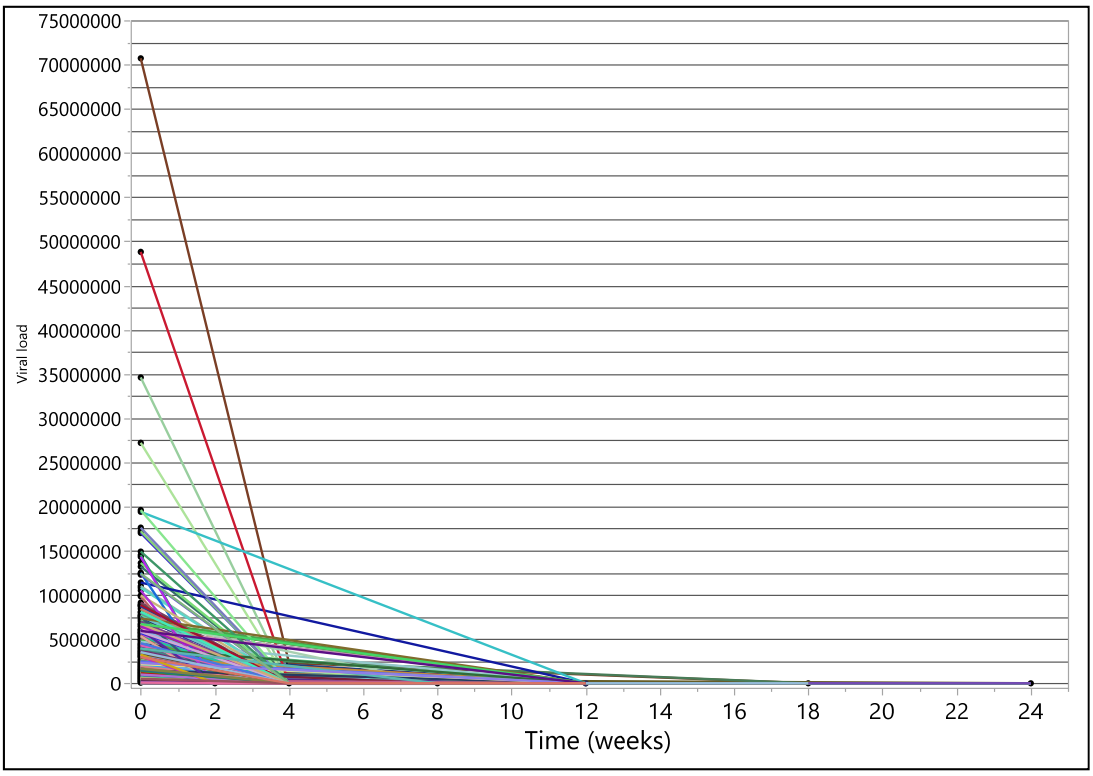

Fig. 33. Viral load changing with Time (437 patients)

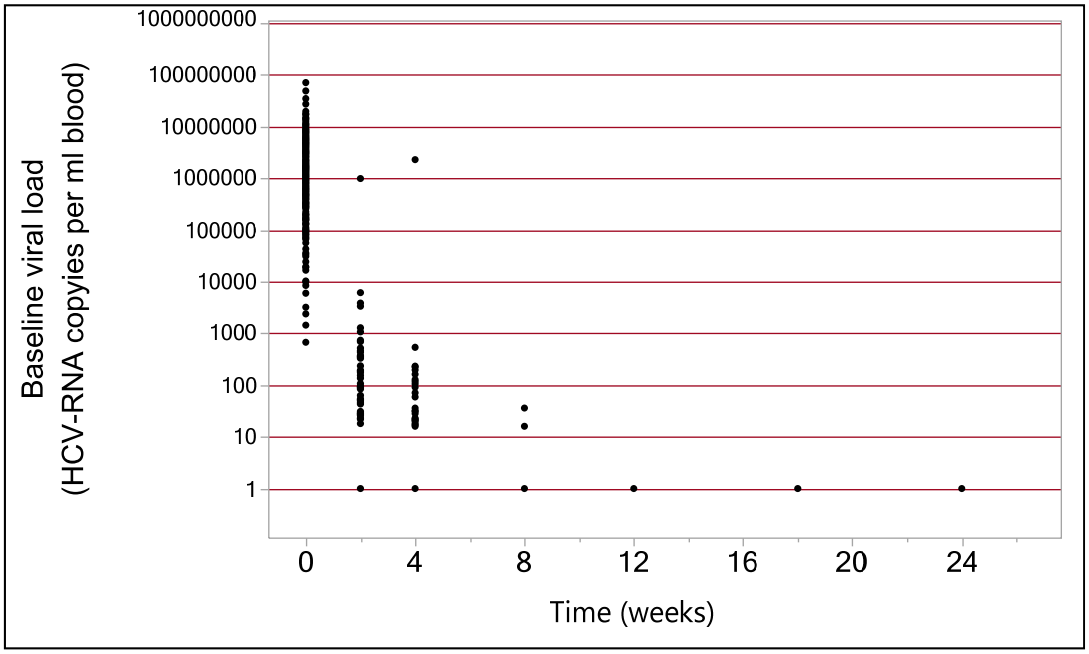

Fig. 34. Viral load changing with Time (437 patients)

3.5.6. Patients with very high viral load

There are four patients with the very high viral load > 25.000.000 IU/mL, The Patient Nr. 042 with the highest viral load PCR>70.000.000 IU/mL shows decreased rapidly, in 4weeks fell to <800.000 IU/mL, and PCR drop to zero in 8 weeks. The other four patients with viral load > 25.000.000 IU/mL (Patient Nr. 042 Baseline Viral load 70.700.000 IU/mL; Patient Nr. 211 Baseline Viral load 48.800.000 IU/mL; Patient Nr. 128 Baseline Viral load 34.600.000 IU/mL; Patient Nr. 173 Baseline Viral load 27.200.000 IU/mL), their viral load dropped very quickly to zero within 4 weeks. The changes in viral load changing with time were analyzed by using Bivariate Fit analysis, shown in figure 35.

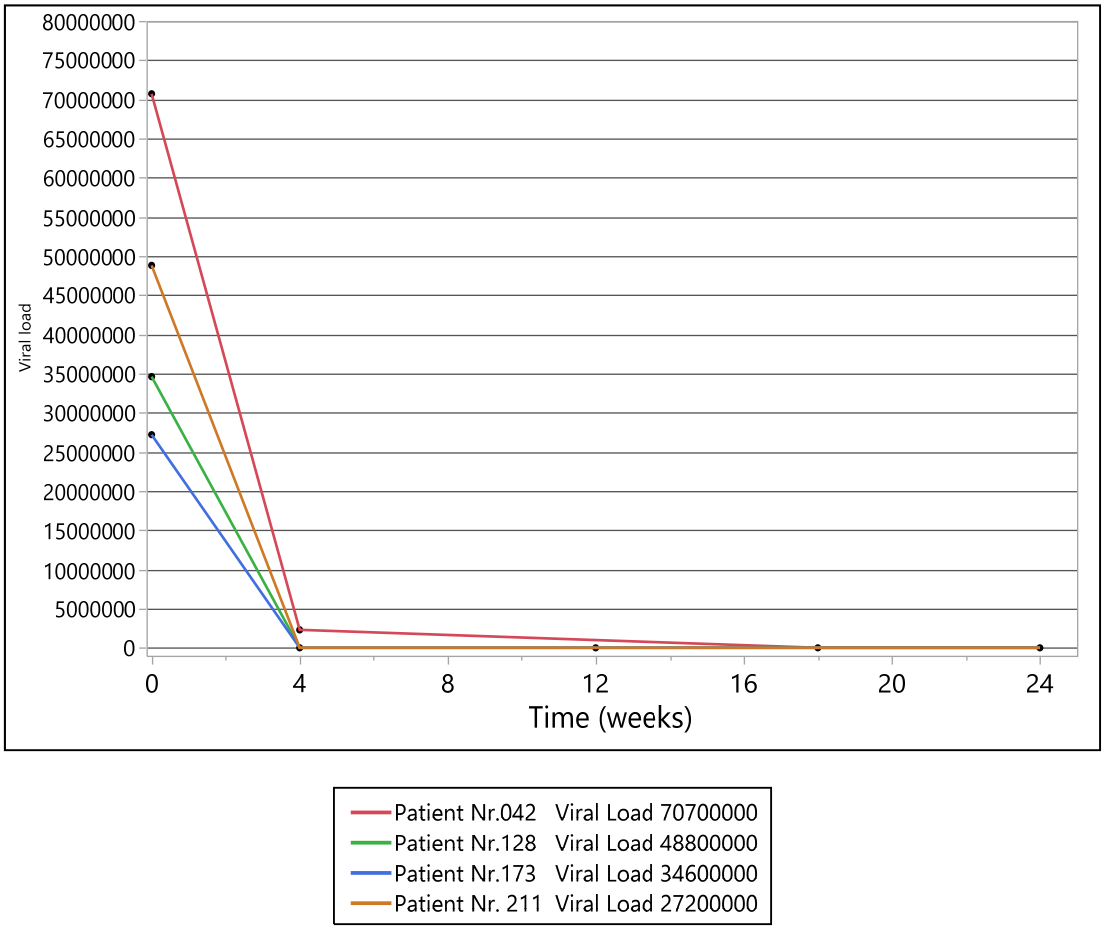

Fig. 35. Very high viral load changing with time (PCR >25,000,000)

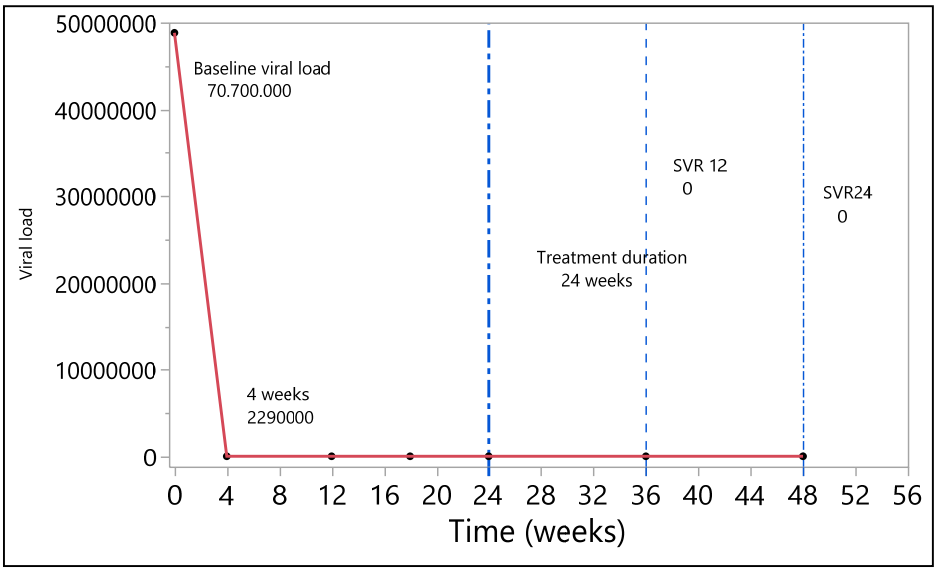

Fig. 36. Patient Nr. 042 Very high viral load 70,700,000 (PCR>60,000,000)

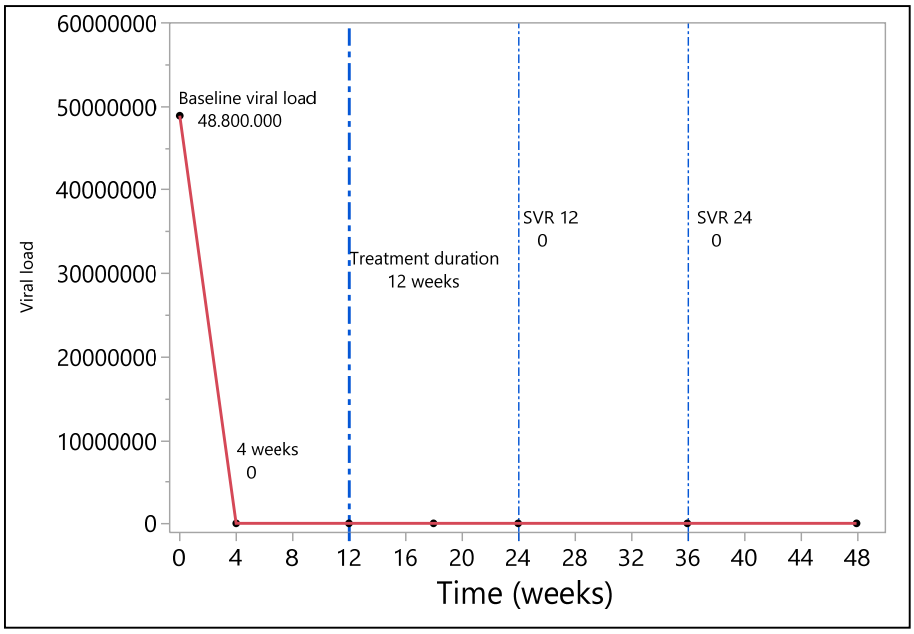

Fig. 37. Patient Nr. 211 Very high viral load 48,800,000 (PCR >25,000,000)

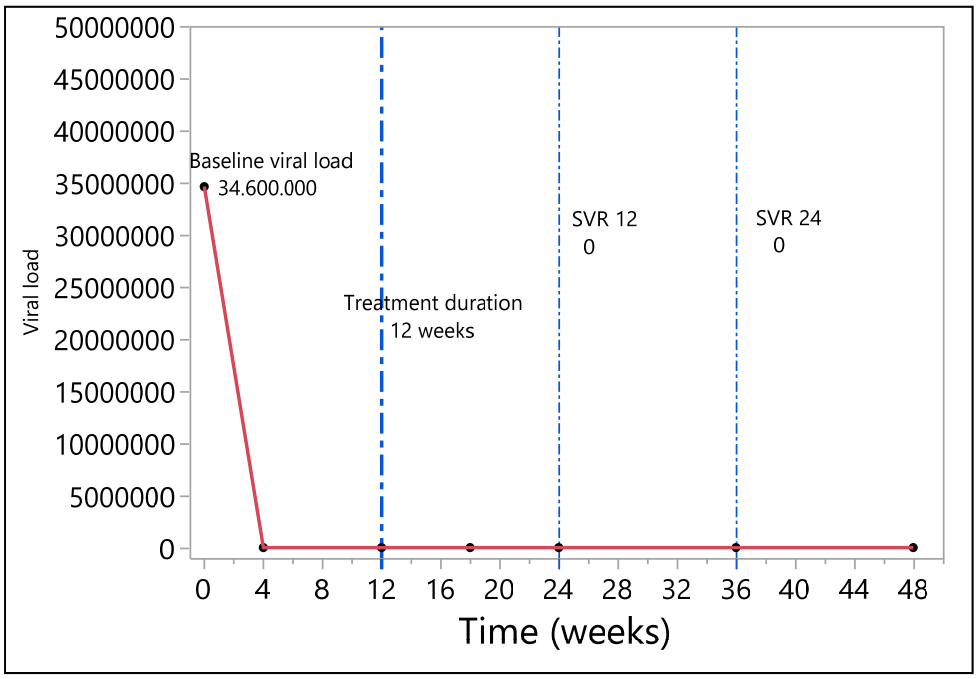

Fig. 38. Patient Nr. 128 Very high viral load 34,600,000 (PCR >25,000,000)

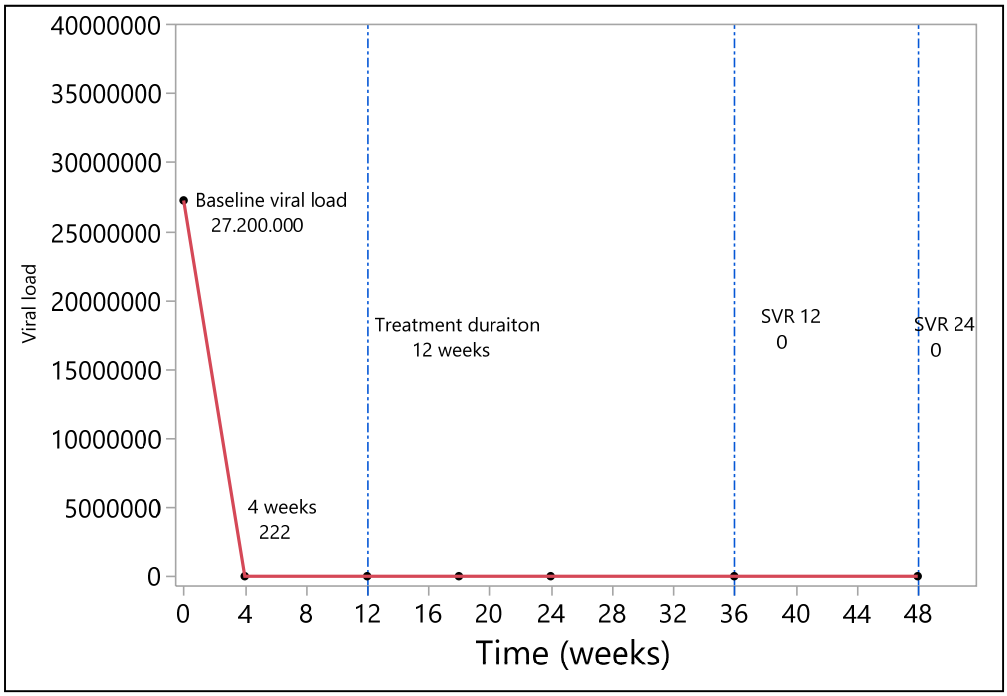

Fig. 39. Patient Nr. 173 Very high viral load 27,200,000 (PCR >25,000,000)

3.6. Relapse in Hepatitis C patients

3.6.1. General characters of the relapse patients

There are 15 relapse patients in the total 437 hepatitis C patients. It can be observed from these relapse patients that: the gender ratio shows male patients (12/15, 80%) obviously more than female patients (3/15, 20%), with significant statistical differences (P<0.01).

The age of all relapse patients range from 38 (Min) to 63 (Max), the average age was 50.13±6.28 (MV±SD).

The treatment duration includes 24 weeks, 12 weeks and 8 weeks. There are six relapsed patients with 24 weeks treatment duration (6/15, 40%), seven patients with 12 weeks treatment duration (7/15, 46.67%), and two patients with 8 weeks duration (2/15, 13.33%).

The relapsed patients had three kinds of genotypes: 1a, 1b, 3a/b. There are 3 patients with genotype 1a (4/15, 26.67%), 5 patients with 1b (5/15, 33.33%), and 6 patients with 3a/b (6/15, 40%).

The treatment regimens included SOF (4/15, 26.67%), SOF DAC (5/15, 31.25%), Harvoni (5/15, 33.33%), SOF SMP (1/15, 6.67%).

The number of special patients with liver cirrhosis is 9 (60%), with significant statistical differences (P<0.01*). There are no patients with liver transplantation and nephrectomy in the relapse patients.

After treatment, there are 8 (53.33%) relapse patients' SVR12 with high viral load (> 6 Mio IU/mL) vs. 7 (46.67%) patients with low viral load (< 6 Mio IU/mL); 2 (13.33%) patients' SVR24 with high viral load (> 6 Mio IU/mL) vs 13 (86.67%) patients with low viral load (< 6 Mio IU/mL); and 5 (33.33%) patients' SVR48 with high viral load (> 6 Mio IU/mL) vs. 10 (66.67%) patients with low viral load (< 6 Mio IU/mL).

Relapse patients	**n, % N=15**
Gender	
Male	12 (80%)
Female	3 (20%)
Age	
Mean±SD (Min - Max)	50.13±6.28 (38 - 63)
>60 yr	1 (6.67%)
Treatment duration	
24 weeks	6 (40%),
12 weeks	7 (46.67%),
8 weeks	2 (13.33%).
Genotype	
1a	4 （26.67%）
1b	5 （33.33%）
3a/b	6 （40%）
Treatment regimens	
SOF	4 (26.67%)
SOF DAC	5 (31.25%)
Harvoni	5 (33.33%)
SOF SMP	1 (6.67%)
Special patients	
Liver cirrhosis	9 (60%) *P*<0.01*
Liver transplantation	0
Nephrectomy	0
Coinfection	
HBV	1 (6.67%)
HIV	0

Pre-therapy	**n, %** **N=15**
With	6 (40%)
without	9 (60%)
Baseline platelet count	
Low platelets (≤100/nL)	7 (46.67%)
Baseline viral load	
<800.000 IU/mL Low viral load	6 (40%)
≥800.000 IU/mL High viral load	9 (60%)
≥6.000.000 IU/mL Very high viral load	0
SVR 12	
≥800.000 IU/mL High viral load	8
≥6.000.000 IU/mL Very high viral load	2
SVR 24	
≥800.000 IU/mL High viral load	10
≥6.000.000 IU/mL Very high viral load	2
SVR 48	
≥800.000 IU/mL High viral load	11
≥6.000.000 IU/mL Very high viral load	0

Baseline clinical chemistry		Mean±SD (Min - Max)	
ALT	(IU/1)	74.21±44.96	(21-191)
Platelets	(1000/μL)	34.87±87.09	(43-287)
WBC	(/μL)	5010±1817.43	(1840-8090)
Hemoglobin	(g/dL)	13.53±2.96	(8.7-18)
Creatinine	(mg/dL)	13.53±2.96	(8.7-18)
Bilirubin	(mg/dL)	1.09±0.64	(0.4-2.3)
INR	(INR)	1.14±0.12	(1-1.3)

Table 35. Characteristics of relapse patients

3.6.2. Viral load of relapse patients

From the table of viral load changing during treatment and after treatment of relapse patients, 15 patients had relapsed of the 437 patients, and the viral load of these patients had dropped to zero with PCR DNA negative during treatment (within 24 weeks), but during follow-up the viral load was found increased again after treatment.

The results show most of the 15 relapsed patients' viral load dropped to zero within 12 weeks, and most of them (11/15, 73.33%) the viral load changed rapidly to negative (zero or less than 15) within 4 weeks. From the 8 weeks to 24 weeks, these patients kept negative, but after treatment, the sustained virological response (SVR12, SVR24 and SVR48) increased a lot, some have even increased several times compared to the baseline viral load. The patient P213 with very low value at baseline viral load (8430) increased 70 times during SVR12 (590000) and 150 times during SVR24 (1280000).

Patient Nr.	Baseline Viral load	2 weeks	4 weeks	8 weeks	12 weeks	18 weeks	24 weeks	SVR 12	SVR 24	SVR 48
P030	4890000	6130	195	0	0	0	0	13900000	782000	69100
P070	105000		0		0		0	163000	839000	
P084	1380000	175	<15	0	0	0		1200000	640000	1050000
P213	8430		<15		0	0	0	590000	1280000	170000
P214	1740000		0		0	0	0	0	110000	
P289	3160000	135	0	0	0	0	0	2530000	1810000	2760
P058	10300	<15	0					2190		152000
P091	3390000		<15		0			425000	1120000	1490000
P094	321000	<15	0		0			2670000	955000	2570000
P121	4910000		16		0			17900000	16900000	
P207	408000	<15	0	0				768000	0	
P375	862000		0		0			97600	197000	
P421	3030000	<15	0		0			4490000	8960000	5960000
P237	469000			0	0			1690000	1110000	1870000
P407	2180000		0		0			1270000		

Table 36. Viral load and SVR of 15 relapse patients

3.6.3. Viral load changing during treatment and after treatment of relapse patients

The baseline viral load of most relapse patients were less than 800,000. These initial values were lower than most patients without relapse group. Only one patient (P207) relapse with SVR12 76800 declined to negative zero during SVR24.

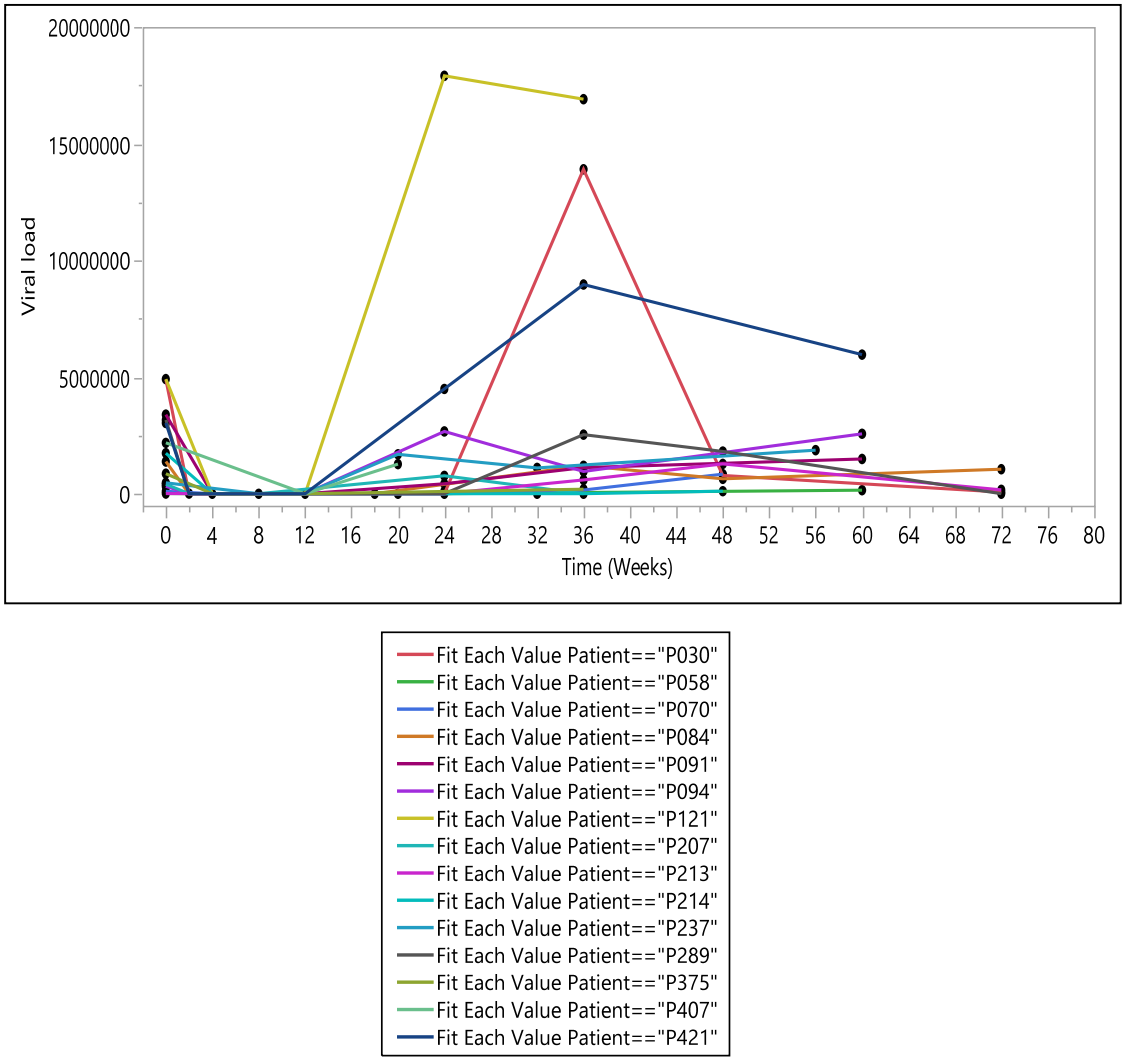

Fig. 40. Viral load changing during treatment and after treatment of 15 relapse patients

3.6.4. Genotype and treatment regimens of the relapse patients

Patient	Gender	Treatment Duration	Age	Genotype	Treatment regimens
P030	Male	24	48	3a/b	SOF DAC
P070	Male	24	53	1b	Harvoni
P084	Female	24	56	3a/b	SOF DAC
P213	Male	24	49	3a/b	Harvoni
P214	Male	24	45	3a/b	SOF
P289	Male	24	52	3a/b	SOF DAC
P058	Male	12	54	1a	SOF
P091	Male	12	38	1b	SOF DAC
P094	Male	12	63	1b	SOF
P121	Male	12	45	3a/b	SOF SMP
P207	Female	12	43	1a	SOF
P375	Male	12	52	1b	Harvoni
P421	Female	12	55	1a	SOF DAC
P237	Male	8	45	1b	Harvoni
P407	Male	8	54	1a	Harvoni

Table 37. Genotype and treatment regimens of the relapse patients

3.6.5. Comorbidity and coinfection of relapse patients

Among 15 relapsed patients, nine patients had liver cirrhosis, with significant statistical relevance. Only one relapse patient had liver transplantation, without statistical relevance. There is no relapse patient with nephrectomy during treatment. All the relapse patients without coinfection HIV. One relapse patient with HBV.

Patient	Liver cirrhosis	Liver transplantation	Nephrectomy	Coinfection HIV	Coinfection HBV
P030	yes	yes	no	no	no
P070	yes	no	no	no	no
P084	yes	no	no	no	no
P213	yes	no	no	no	no
P214	yes	no	no	no	no
P289	yes	no	no	no	no
P058	yes	no	no	no	no
P091	no	no	no	no	no
P094	no	no	no	no	no
P121	no	no	no	no	no
P207	yes	no	no	no	no
P375	yes	no	no	no	no
P421	no	no	no	no	no
P237	no	no	no	no	no
P407	no	no	no	no	yes

Table 38. Comorbidity and Coinfection of relapse patients

3.6.6. Pre-therapy of relapse patients

Among 15 relapse patients, there are half patients (7/15) with pre-therapy and half patients (8/15) without pre-therapy. 9 patients had taken medicine with IFN, 7 patients had taken medicine with RBV, and most relapse patients with pre-therapy had taken both medicines combined with IFN and RBV, and some patients had taken more several drugs such as PEG, IFN, RBV, TVR, NR.

Patient	Pre-therapy	Pre-therapy with	IFN	RBV
P030	yes	RBV	Without	With
P070	yes	IFN, RBV	With	With
P084	yes	IFN, RBV	With	With
P213	no		Without	Without
P214	no		Without	Without
P289	no		Without	Without
P058	yes	PEG, IFN, RBV	With	With
P091	no		Without	Without
P094	no		Without	Without
P121	yes	PEG, IFN, RBV	With	With
P207	no		Without	Without
P375	yes	RBV, IFN	With	With
P421	yes	PEG, IFN, RBV, TVR, NR	With	With
P237	no		Without	Without
P407	no		Without	Without

Table 39. Pre-therapy of relapse patients

3.6.7. Baseline clinical chemistry of the relapse patients

Patient	Hemoglobin	WBC	Platelets	INR	Creatinine	Bilirubin	ALT
P030	10	2690	69	1.2	1		
P070	8.9	1840	44	1.3	0.7	1.4	51
P084	12	3260	75		0.6	1	49
P213	13.9	3350	50	1.1	0.6	1	60
P214	11.6	4710	132	1.3	0.5	2.3	105
P289	12.7	5240	88	1.1	1	1.7	37
P058	17.1	8090	76			0.8	57
P091	18	5240	244	1	0.9	0.6	88
P094	17.6	7760	177	1.2	0.8	0.6	121
P121	14.5	6370	287	1	0.8	0.5	191
P207	13.5	4690	119	1.2	0.7	2.3	21
P375	15.1	3860	105	1.3	0.7	1.4	106
P421	13.5	6670	278			0.6	56
P237	8.7	5060	43	1	1	0.4	29
P407	15.9	6320	236	1	0.7	0.6	68

Table 40. Baseline clinical chemistry of the relapse patients

3.6.8. Analysis viral load changing with time in relapse patients

Patient Nr. 030

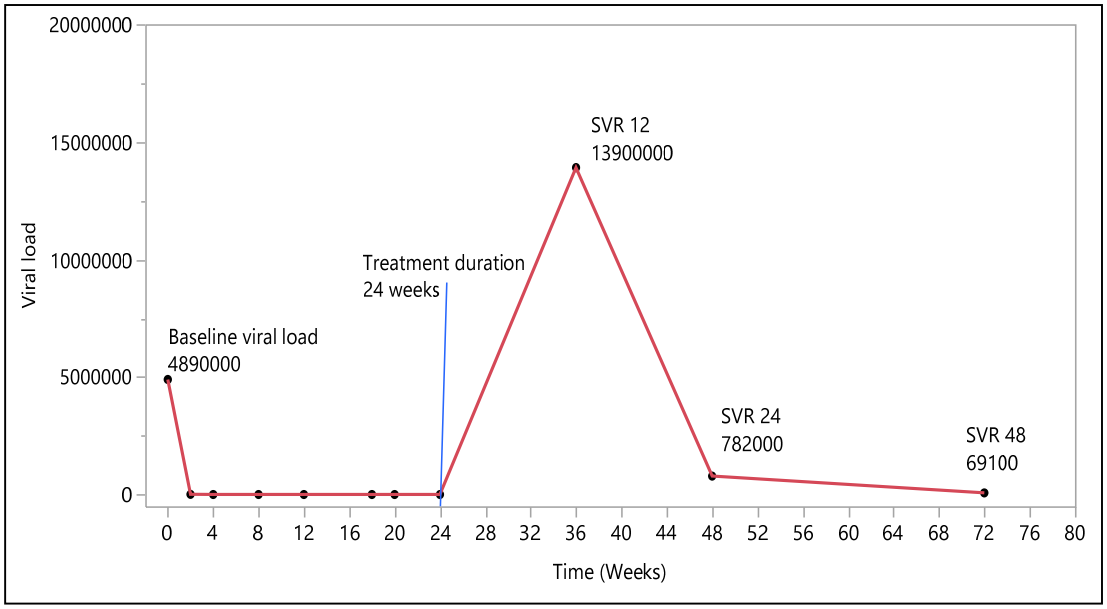

Fig. 41. Viral load changing with time in Hepatitis C relapse patient (P030)

Patient	P030
Gender	Male
Age	48
Genotype	3a/b
Treatment duration	24
Treatment regimens	SOF DAC
Liver cirrhosis	yes
Liver transplantation	yes
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	yes
Pre-therapy with	RBV
Hemoglobin	10
WBC	2690
PLT	69
INR	1.2
Creatinine	1
Bilirubin	no data
ALT	no data
Baseline viral load	4890000

Table 41. Characteristics of Hepatitis C relapse patient (P030)

Patient Nr. 070

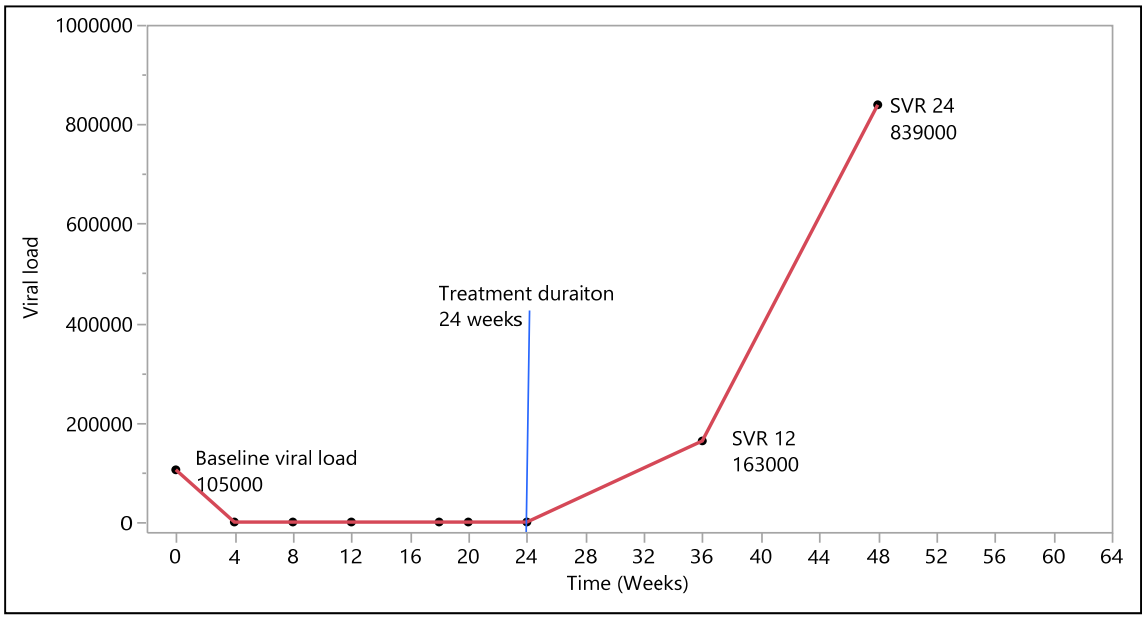

Fig. 42. Viral load changing with time in Hepatitis C relapse patient (P070)

Patient	P070
Gender	Male
Age	53
Genotype	1b
Treatment duration	24
Treatment regimens	Harvoni
Liver cirrhosis	yes
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	yes
Pre-therapy with	IFN, RBV
Hemoglobin	8.9
WBC	1840
PLT	44
INR	1.3
Creatinine	0.7
Bilirubin	1.4
ALT	51
Baseline viral load	105000

Table 42. Characteristics of Hepatitis C relapse patient (P070)

Patient Nr. 084

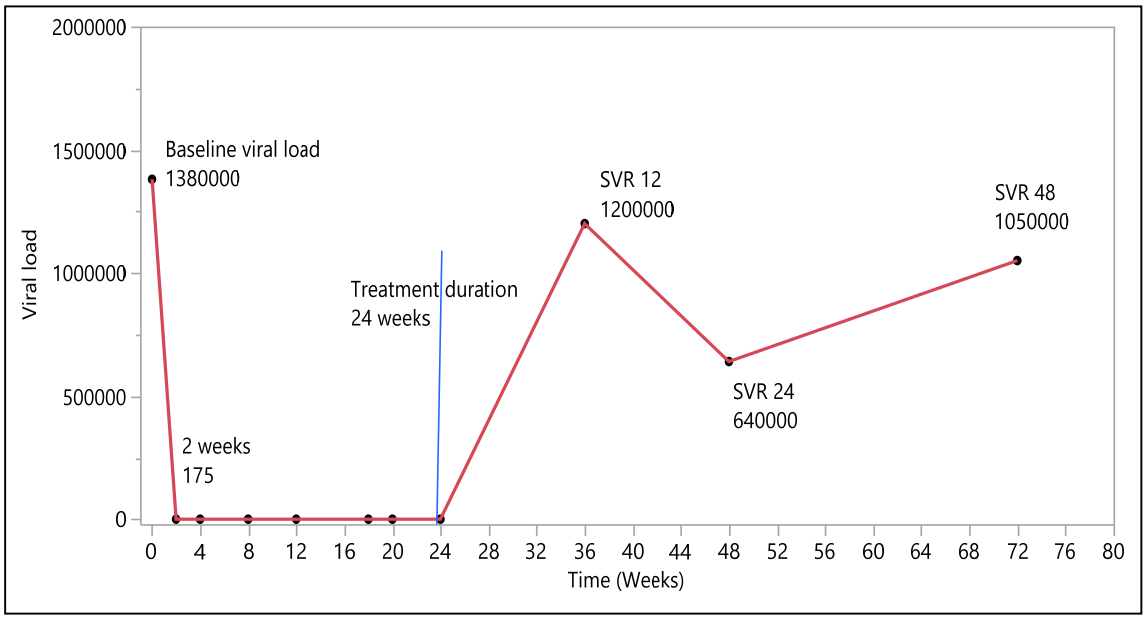

Fig. 43. Viral load changing with time in Hepatitis C relapse patient (P084)

Patient	P084
Gender	Female
Age	56
Genotype	3a/b
Treatment duration	24
Treatment regimens	SOF DAC
Liver cirrhosis	yes
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	yes
Pre-therapy with	IFN,RBV
Hemoglobin	12
WBC	3260
PLT	75
INR	no data
Creatinine	0.6
Bilirubin	1
ALT	49
Baseline viral load	1380000

Table 43. Characteristics of Hepatitis C relapse patient (P084)

Patient Nr. 213

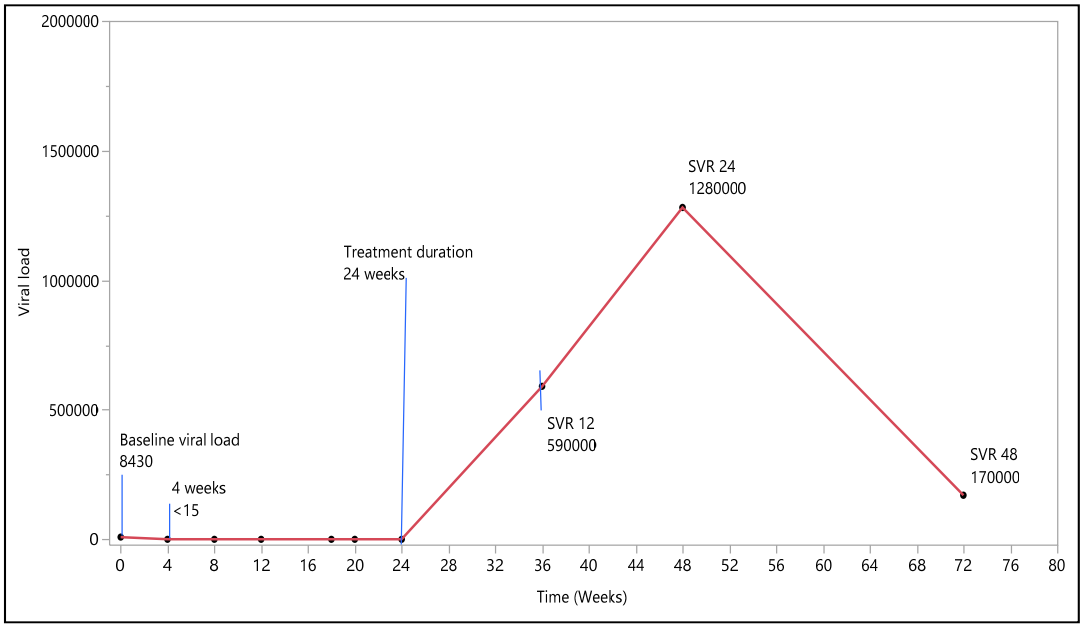

Fig. 44. Viral load changing with time in Hepatitis C relapse patient (P213)

Patient	P213
Gender	Male
Age	49
Genotype	3a/b
Treatment duration	24
Treatment regimens	Harvoni
Liver cirrhosis	yes
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	no
Hemoglobin	13.9
WBC	3350
PLT	50
INR	1.1
Creatinine	0.6
Bilirubin	1
ALT	60
Baseline viral load	8430
SVR 12	590000
SVR 24	1280000
SVR 48	170000

Table 44. Characteristics of Hepatitis C relapse patient (P213)

Patient Nr. 214

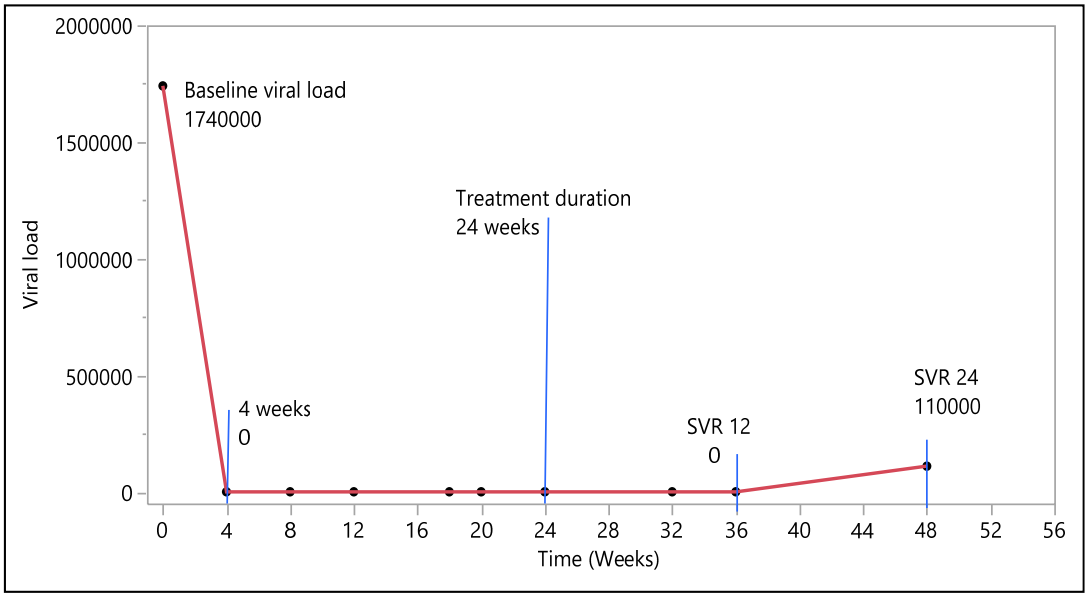

Fig. 45. Viral load changing with time in Hepatitis C relapse patient (P214)

Patient	P214
Gender	Male
Age	45
Genotype	3a/b
Treatment duration	24
Treatment regimens	SOF
Liver cirrhosis	yes
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	no
Hemoglobin	11.6
WBC	4710
PLT	132
INR	1.3
Creatinine	0.5
Bilirubin	2.3
ALT	105
Baseline viral load	1740000

Table 45. Characteristics of Hepatitis C relapse patient (P214)

Patient Nr. 289

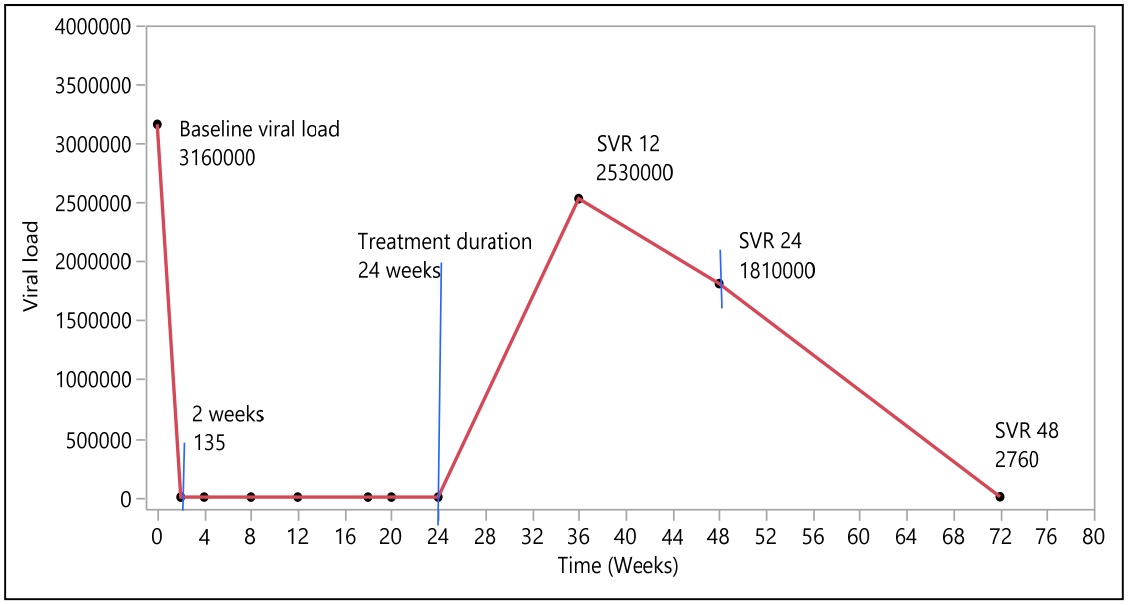

Fig. 46. Viral load changing with time in Hepatitis C relapse patient (P289)

Patient	P289
Gender	Male
Age	52
Genotype	3a/b
Treatment duration	24
Treatment regimens	SOF DAC
Liver cirrhosis	yes
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	no
Hemoglobin	12.7
WBC	5240
PLT	88
INR	1.1
Creatinine	1
Bilirubin	1.7
ALT	37
Baseline viral load	3160000

Table 46. Characteristics of Hepatitis C relapse patient (P289)

Patient Nr. 058

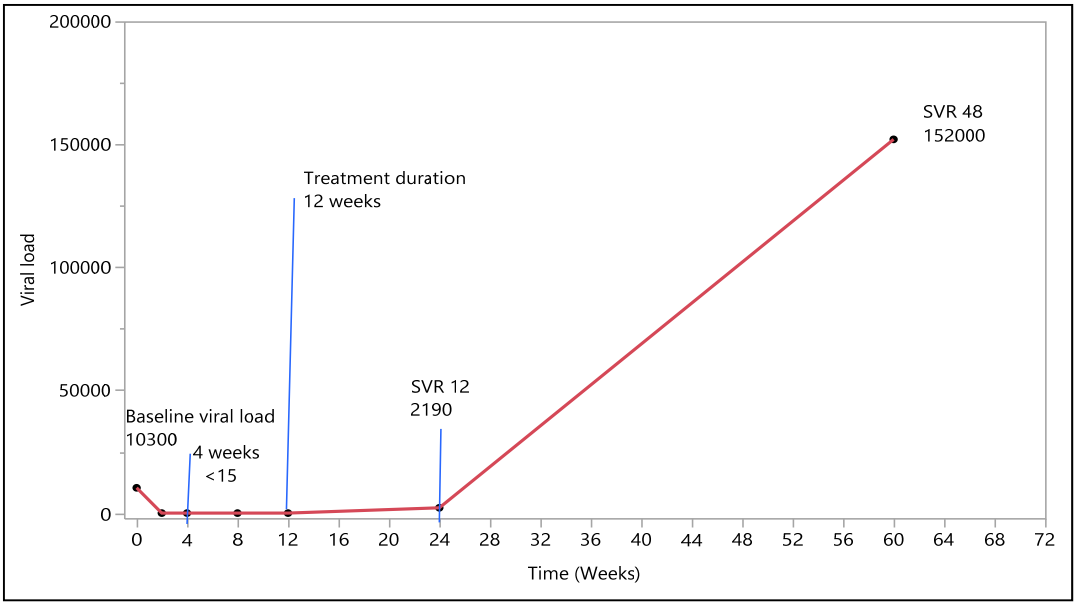

Fig. 47. Viral load changing with time in Hepatitis C relapse patient (P058)

Patient	P058
Gender	Male
Age	54
Genotype	1a
Treatment duration	12
Treatment regimens	SOF
Liver cirrhosis	yes
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	yes
Pre-therapy with	PEG, IFN, RBV
Hemoglobin	17.1
WBC	8090
PLT	76
INR	no data
Creatinine	no data
Bilirubin	0.8
ALT	57
Baseline viral load	10300

Table 47. Characteristics of Hepatitis C relapse patient (P058)

Patient Nr. 091

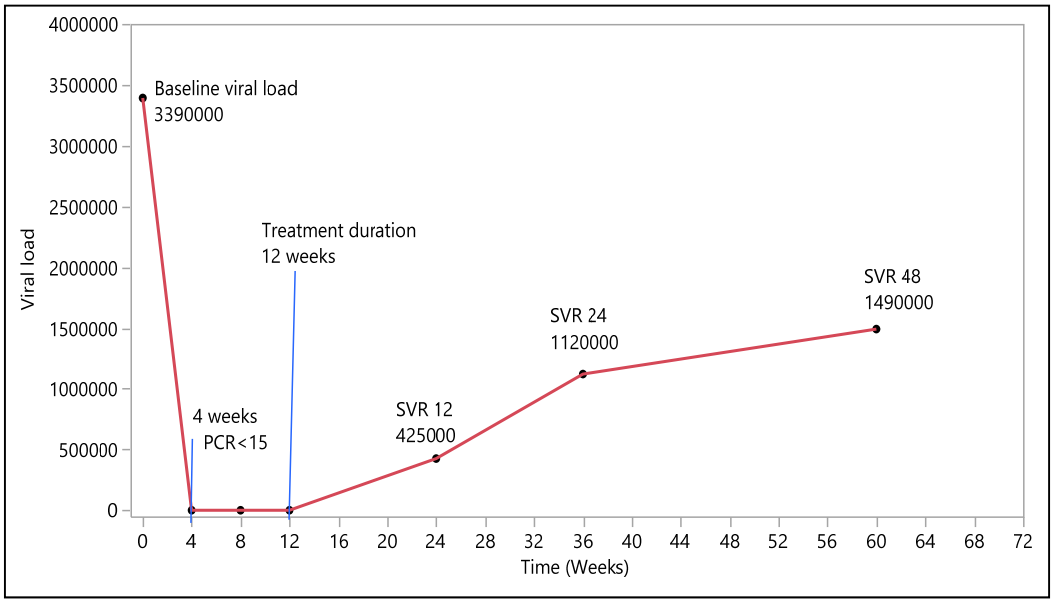

Fig. 48. Viral load changing with time in Hepatitis C relapse patient (P091)

Patient	P091
Gender	Male
Age	38
Genotype	1b
Treatment duration	12
Treatment regimens	SOF DAC
Liver cirrhosis	no
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	no
Hemoglobin	18
WBC	5240
PLT	244
INR	1
Creatinine	0.9
Bilirubin	0.6
ALT	88
Baseline viral load	3390000

Table 48. Characteristics of Hepatitis C relapse patient (P091)

Patient Nr. 094

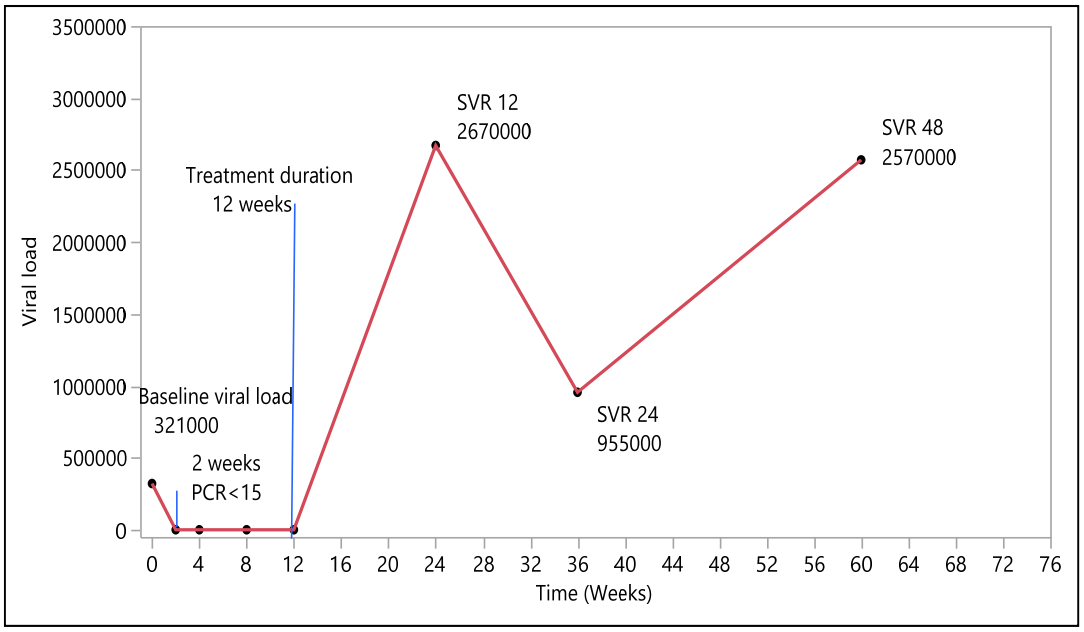

Fig. 49. Viral load changing with time in Hepatitis C relapse patient (P094)

Patient	P094
Gender	Male
Age	63
Genotype	1b
Treatment duration	12
Treatment regimens	SOF
Liver cirrhosis	no
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	no
Hemoglobin	17.6
WBC	7760
PLT	177
INR	1.2
Creatinine	0.8
Bilirubin	0.6
ALT	121
Baseline viral load	321000

Table 49. Characteristics of Hepatitis C relapse patient (P094)

Patient Nr. 121

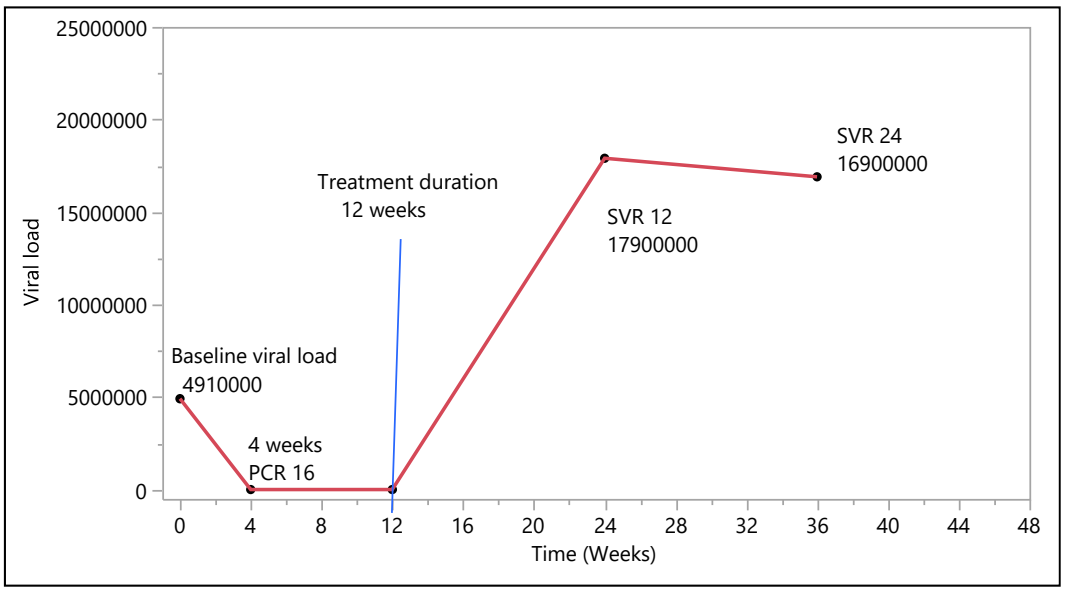

Fig. 50. Viral load changing with time in Hepatitis C relapse patient (P121)

Patient	P121
Gender	Male
Age	45
Genotype	3a/b
Treatment duration	12
Treatment regimens	SOF SMP
Liver cirrhosis	no
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	yes
Pre-therapy with	PEG, IFN, RBV
Hemoglobin	14.5
WBC	6370
PLT	287
INR	1
Creatinine	0.8
Bilirubin	0.5
ALT	191
Baseline viral load	4910000

Table 50. Characteristics of Hepatitis C relapse patient (P094)

Patient Nr. 207

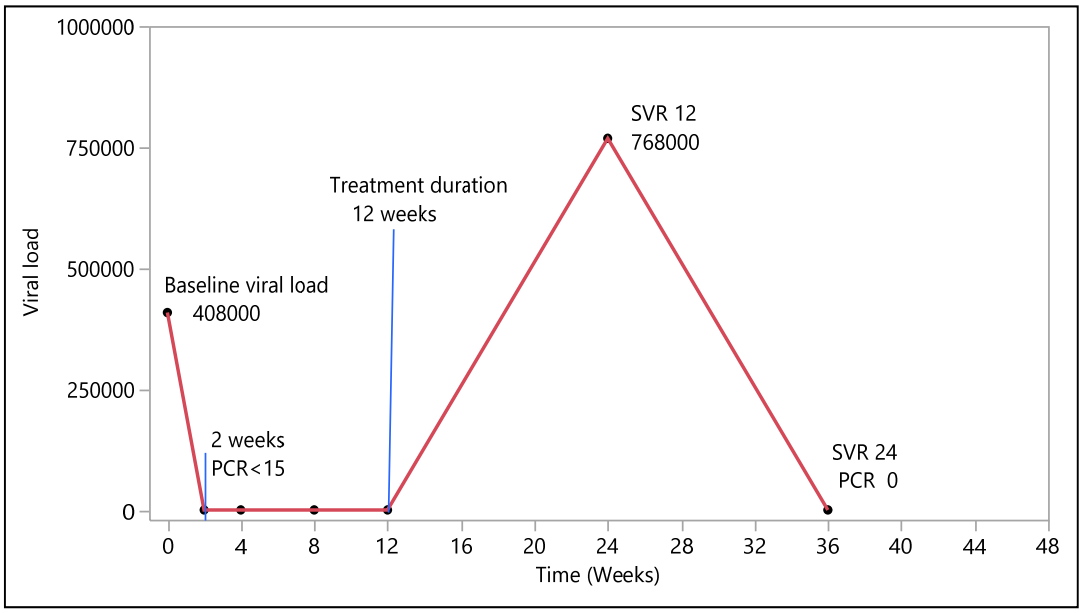

Fig. 51. Viral load changing with time in Hepatitis C relapse patient (P207)

Patient	P207
Gender	Female
Age	43
Genotype	1a
Treatment duration	12
Treatment regimens	SOF
Liver cirrhosis	yes
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	no
Hemoglobin	13.5
WBC	4690
PLT	119
INR	1.2
Creatinine	0.7
Bilirubin	2.3
ALT	21
Baseline viral load	408000

Table 51. Characteristics of Hepatitis C relapse patient (P207)

Patient Nr. 375

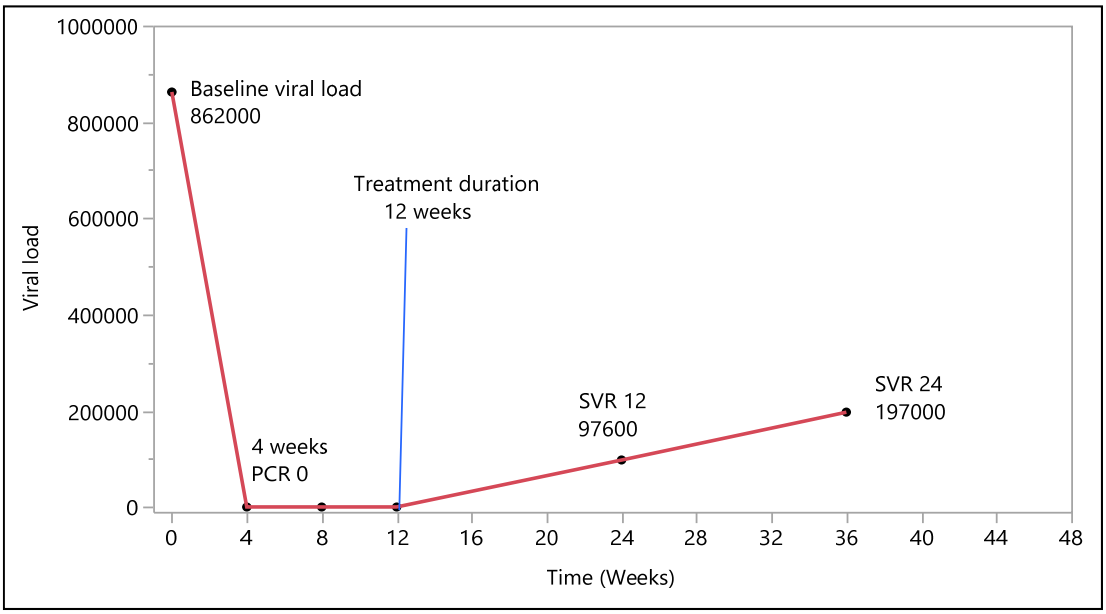

Fig. 52. Viral load changing with time in Hepatitis C relapse patient (P375)

Patient	P375
Gender	Male
Age	52
Genotype	1b
Treatment duration	12
Treatment regimens	Harvoni
Liver cirrhosis	yes
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	yes
Pre-therapy with	RBV, IFN
Hemoglobin	15.1
WBC	3860
PLT	105
INR	1.3
Creatinine	0.7
Bilirubin	1.4
ALT	106
Baseline viral load	862000

Table 52. Characteristics of Hepatitis C relapse patient (P375)

Patient Nr. 421

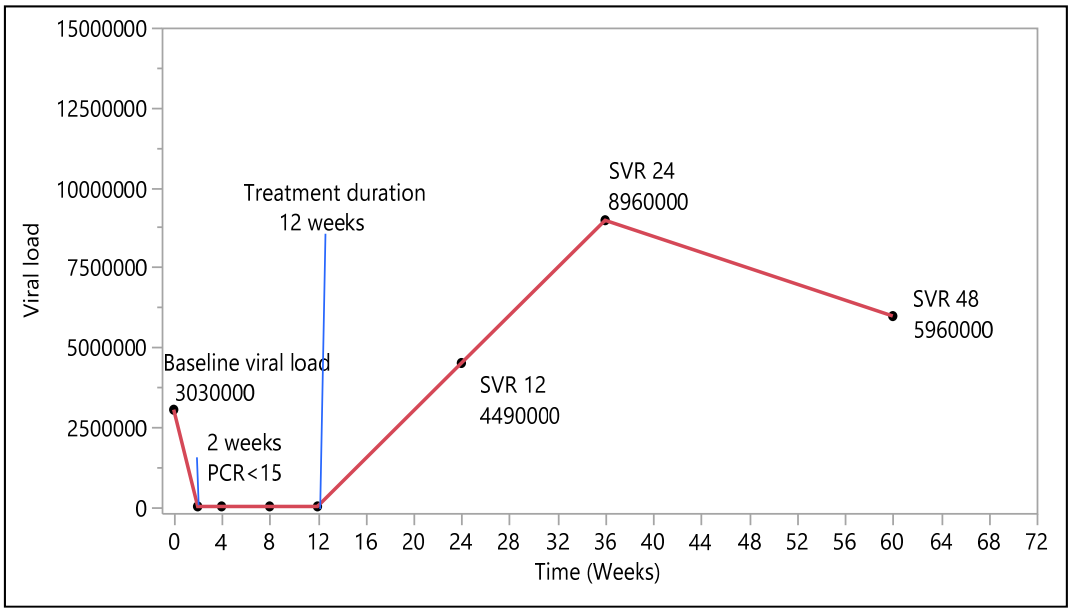

Fig. 53. Viral load changing with time in Hepatitis C relapse patient (P421)

Patient	P421
Gender	Female
Age	55
Genotype	1a
Treatment duration	12
Treatment regimens	SOF DAC
Liver cirrhosis	no
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	yes
Pre-therapy with	PEG, IFN, RBV, TVR, NR
IFN	Without
RBV	Without
Hemoglobin	13.5
WBC	6670
PLT	278
INR	no data
Creatinine	no data
Bilirubin	0.6
ALT	56
Baseline viral load	3030000

Table 53. Characteristics of Hepatitis C relapse patient (P421)

Patient Nr. 237

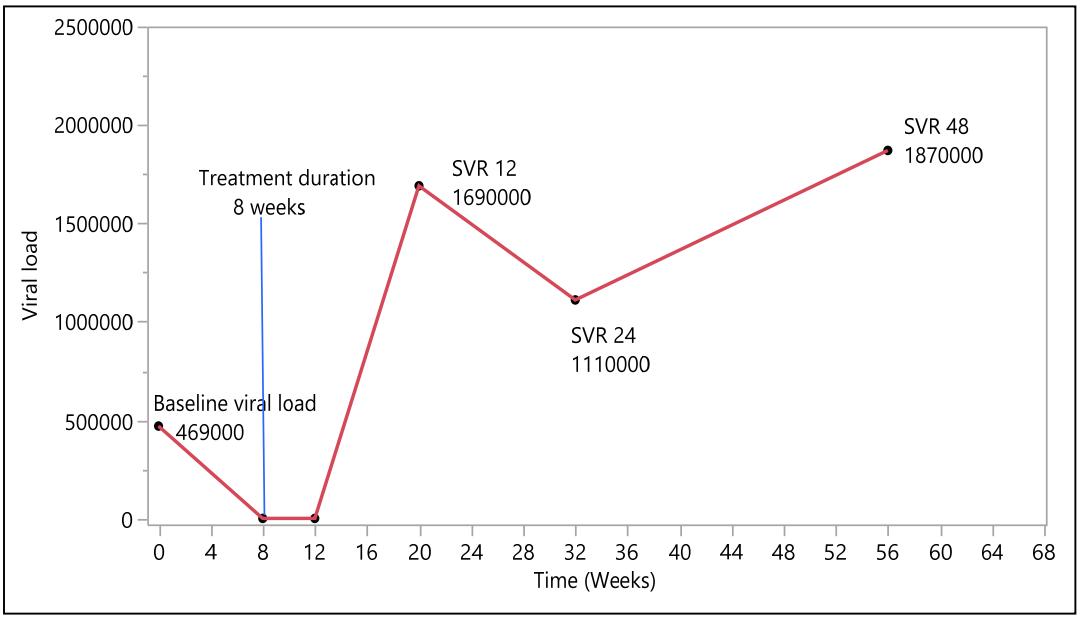

Fig. 54. Viral load changing with time in Hepatitis C relapse patient (P237)

Patient	P237
Gender	Male
Age	45
Genotype	1b
Treatment duration	8
Treatment regimens	Harvoni
Liver cirrhosis	no
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	no
Pre-therapy	no
Hemoglobin	8.7
WBC	5060
PLT	43
INR	1
Creatinine	1
Bilirubin	0.4
ALT	29
Baseline viral load	469000

Table 54. Characteristics of Hepatitis C relapse patient (P237)

Patient Nr. 407

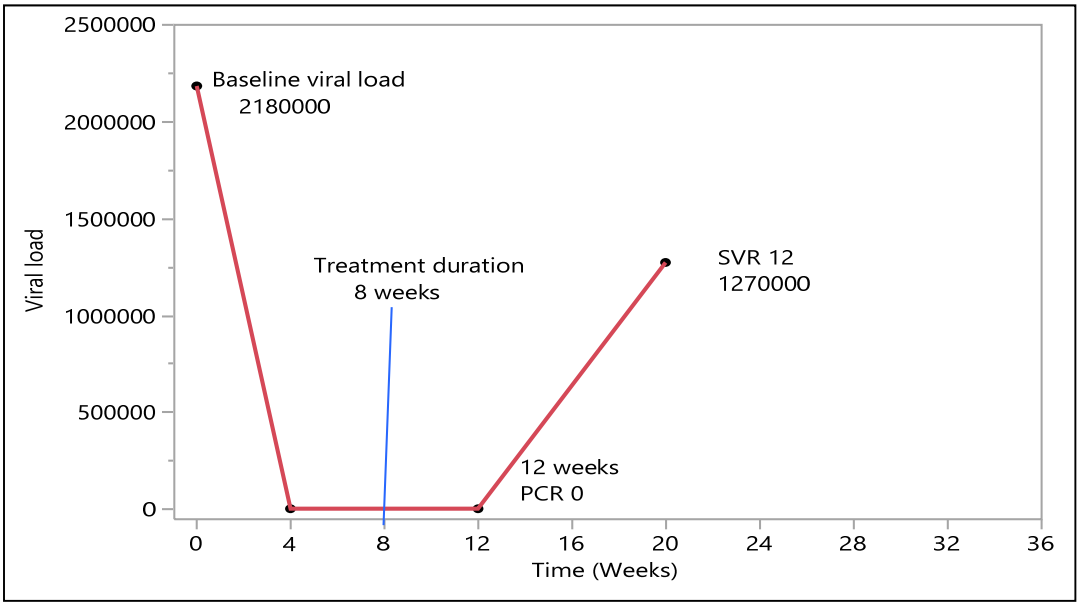

Fig. 55. Viral load changing with time in Hepatitis C relapse patient (P407)

Patient	P407
Gender	Male
Age	54
Genotype	1a
Treatment duration	8
Treatment regimens	Harvoni
Liver cirrhosis	no
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	yes
Pre-therapy	no
Hemoglobin	15.9
WBC	6320
PLT	236
INR	1
Creatinine	0.7
Bilirubin	0.6
ALT	68
Baseline viral load	2180000

Table 55. Characteristics of Hepatitis C relapse patient (P407)

3.6.8. Analysis correlation of relapse by Multiple factors

All factors that may affect the relapse were analyzed by statistical correlation method, including gender, age, genotype, initial viral load, treatment regimens, coinfection HIV, coinfection HBV, liver cirrhosis, liver transplantation, nephrectomy, pre-therapy, etc. Treatment duration, liver cirrhosis, baseline PLT has a significant statistical correlation to the relapse.

3.6.9. Analysis correlation of relapse by treatment duration

Correlation analysis for relapse and treatment duration, there was a statistically significant correlation between the relapse and the treatment duration, P=0.0174* (P<0.05). Relapse patients with 24 weeks treatment duration had the highest proportion.

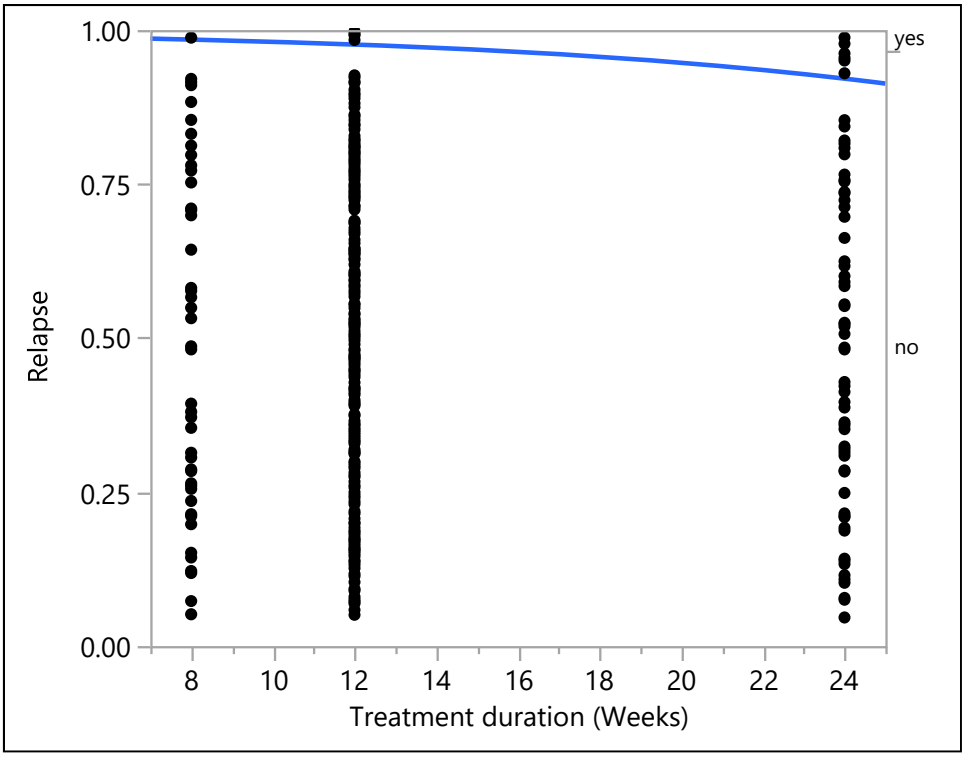

Fig. 56. Logistic curve fitting of relapse by treatment duration (P<0.001**)

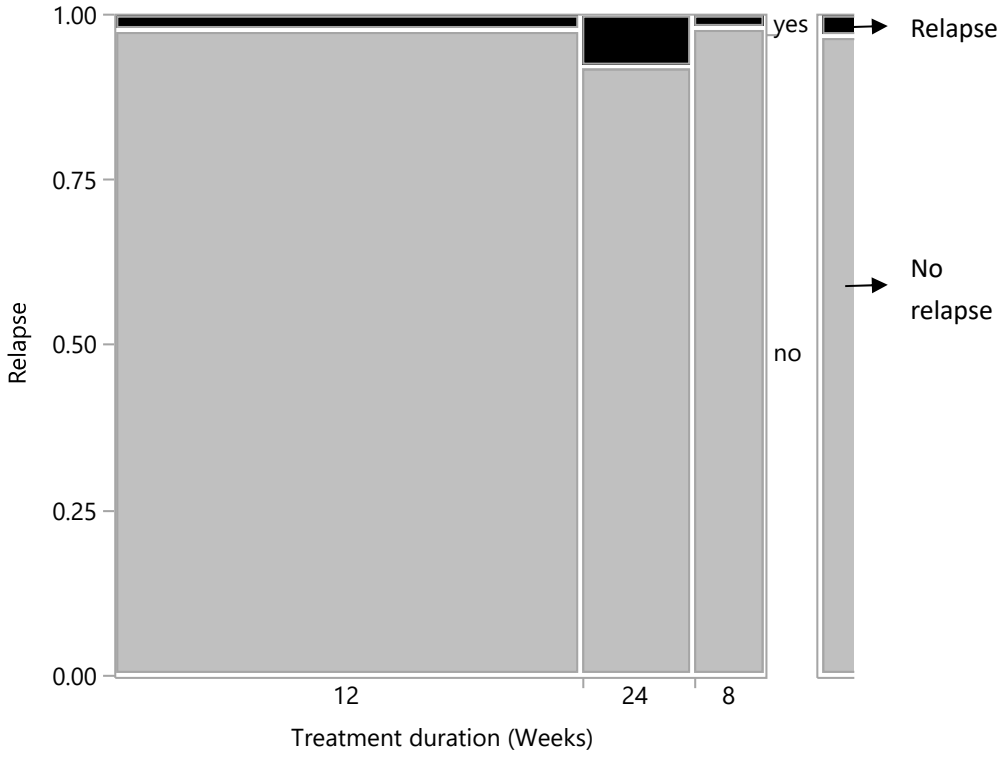

Fig. 57. Logistic fitting of relapse by treatment duration

Count Row %	Without relapse	Relapse	Total
12 weeks	294 97.67	7 2.33	301
24 weeks	68 91.89	6 8.11	74
8 weeks	43 95.56	2 4.44	45
Total	405	15	420

Table 56. Contingency of treatment duration by Relapse

3.6.10. Analysis correlation of relapse by Liver cirrhosis

Correlation analysis for relapse and liver cirrhosis *P*=0.0056*（*P*<0.05）There was a statistically significant correlation between the relapse and the cirrhosis.

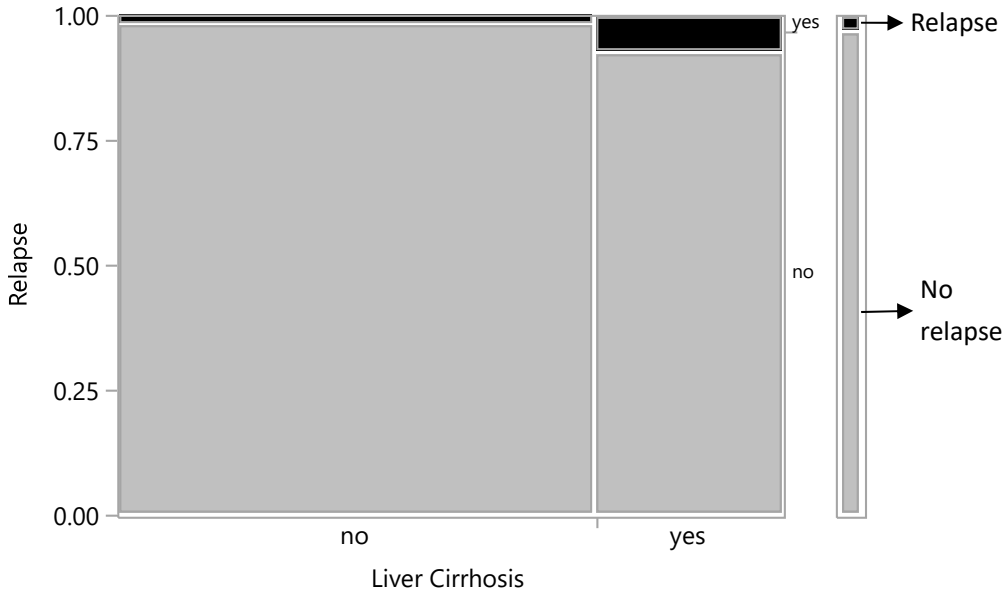

Fig. 58. Correlation between relapse and Liver cirrhosis (*P*=0.0056*)

Count Row %	Without relapse	Relapse	Total
Non-cirrhotic patients	295 98.01	6 1.99	301
Liver cirrhosis	110 92.44	9 7.32	119
Total	405	15	420

Table 57. Contingency of liver cirrhosis by relapse (*P*=0.0056*)

3.6.11. Analysis relapse correlation by Liver cirrhosis

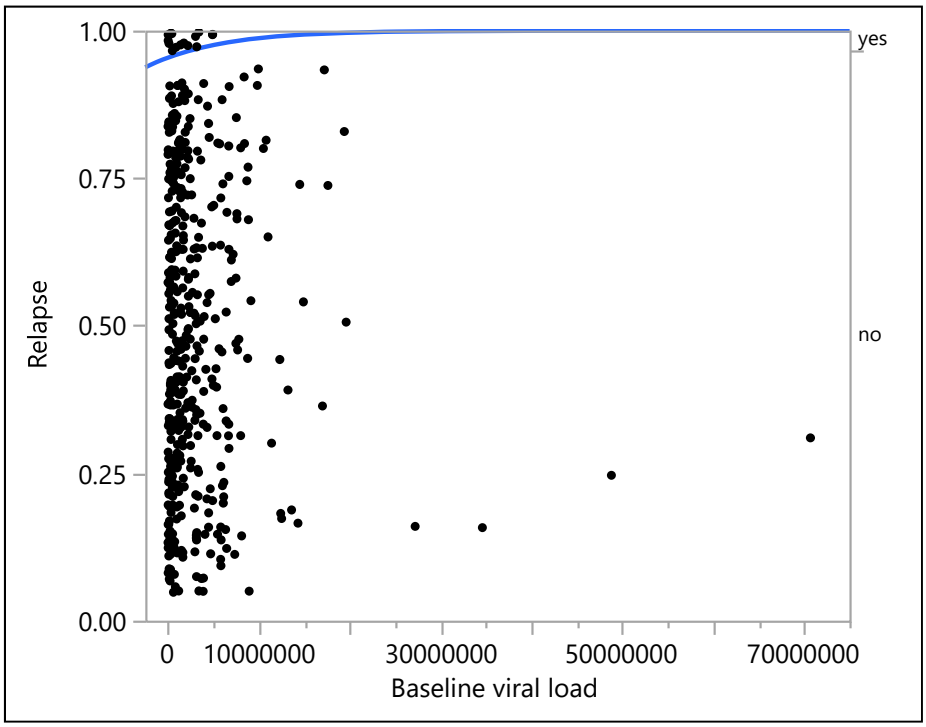

Fig. 59. Logistic fitting of relapse by baseline viral load (P<0.001*)

3.6.12. Analysis correlation of relapse by baseline viral load

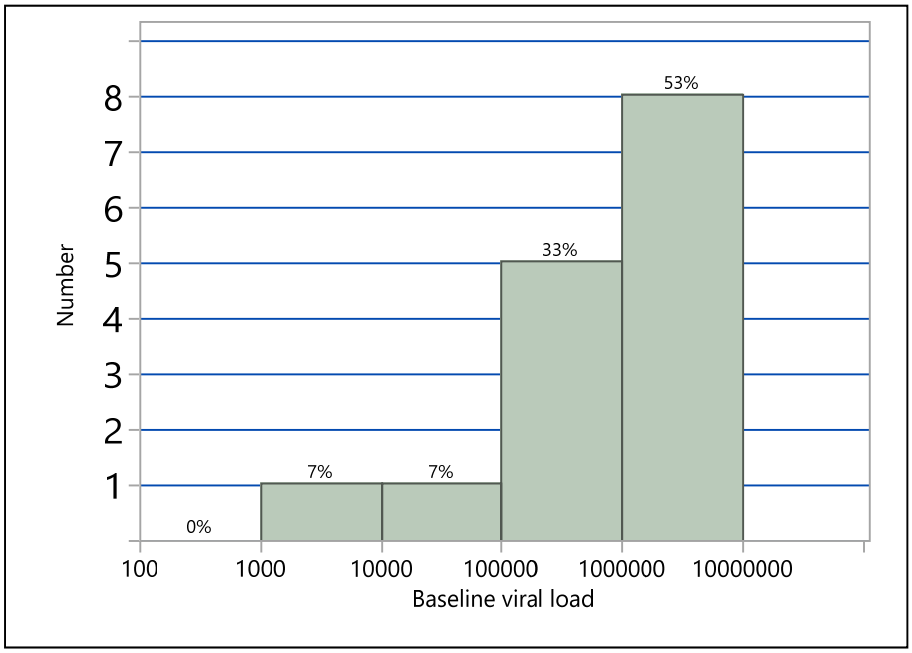

Fig. 60. Baseline viral load of relapse patients

3.6.13. Analysis correlation of relapse by age

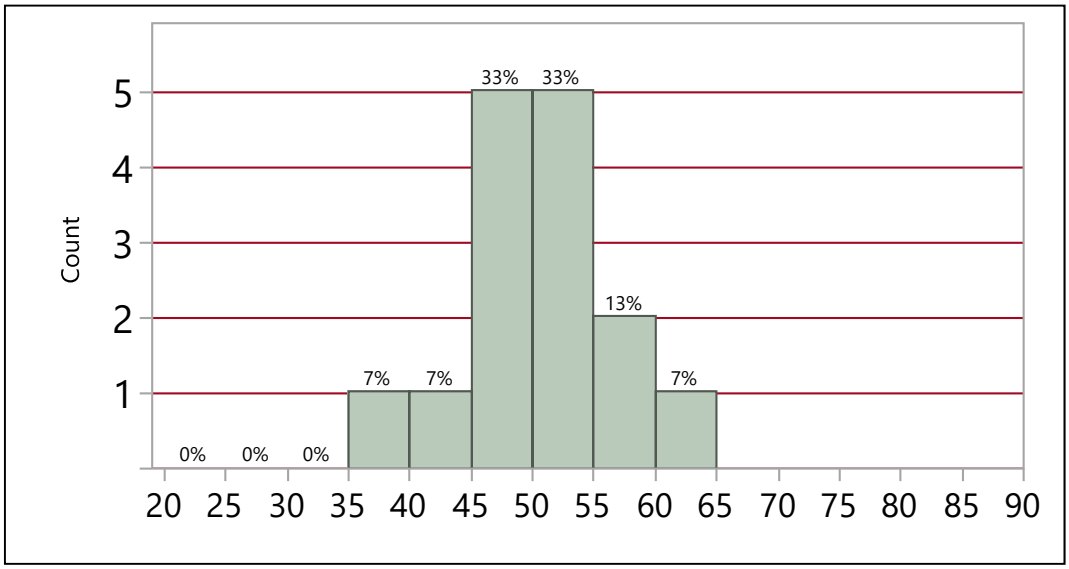

Fig. 61. Relapse correlation with age

Count Row %	**Relapse**	**SVR12 negative**	**Total**
Age<60	14 4.26	315 95.74	329
Age>60	1 0.93	107 99.07	108
Total	15	422	437

Table 58. Analysis relapse by age

3.6.14. Analysis correlation of relapse by genotype

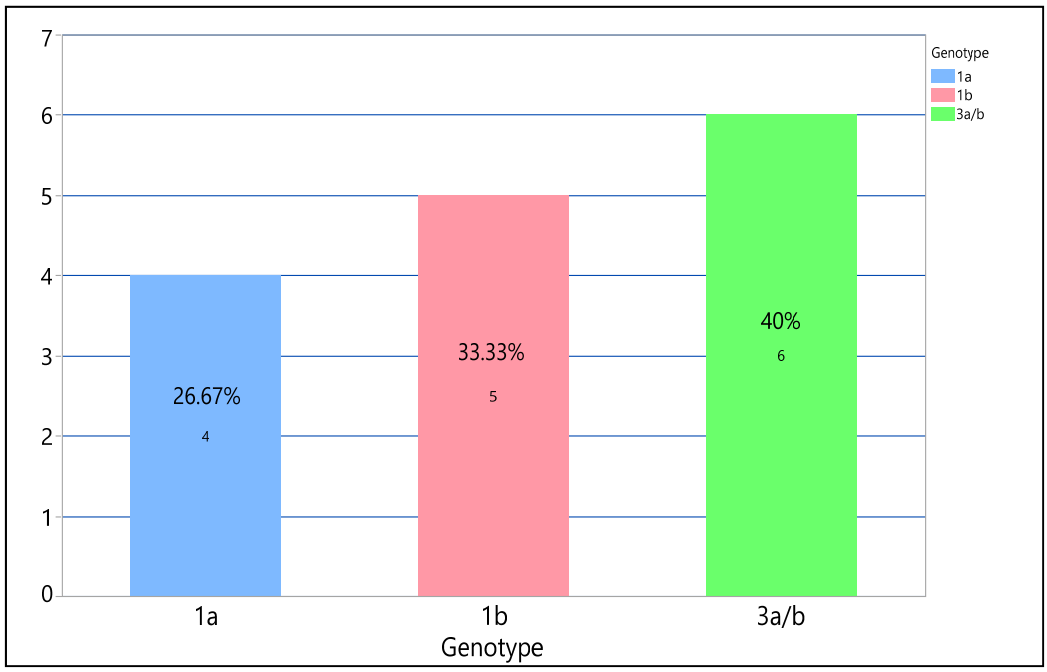

Fig. 62. Relapse correlation with genotype

3.6.15. Analysis correlation of relapse by gender

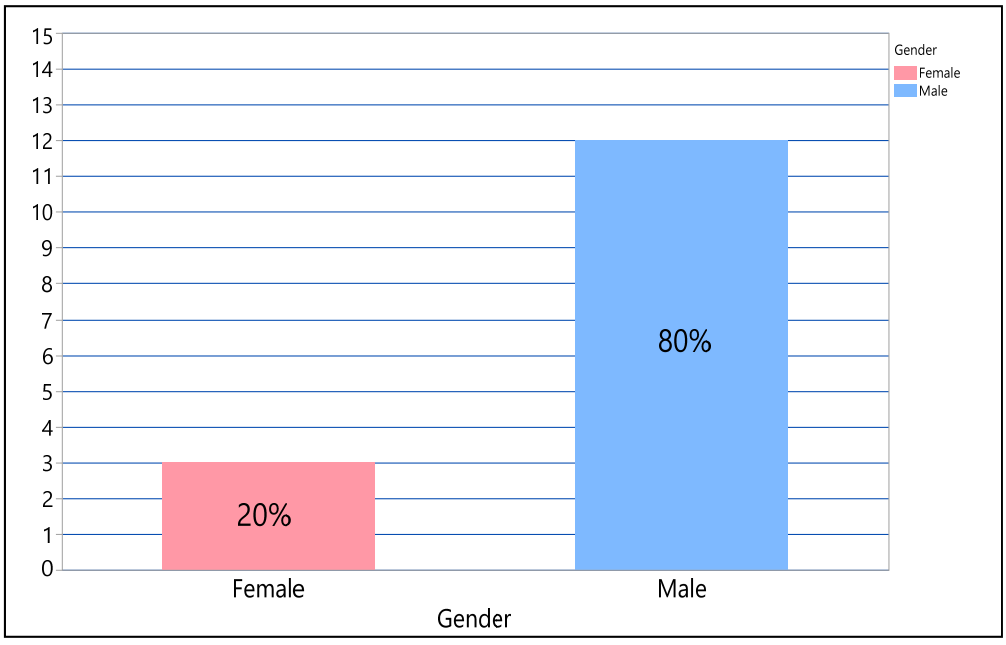

Fig. 63. Relapse correlation with gender

3.6.16. Analysisc correlation of relapse by Liver cirrhosis

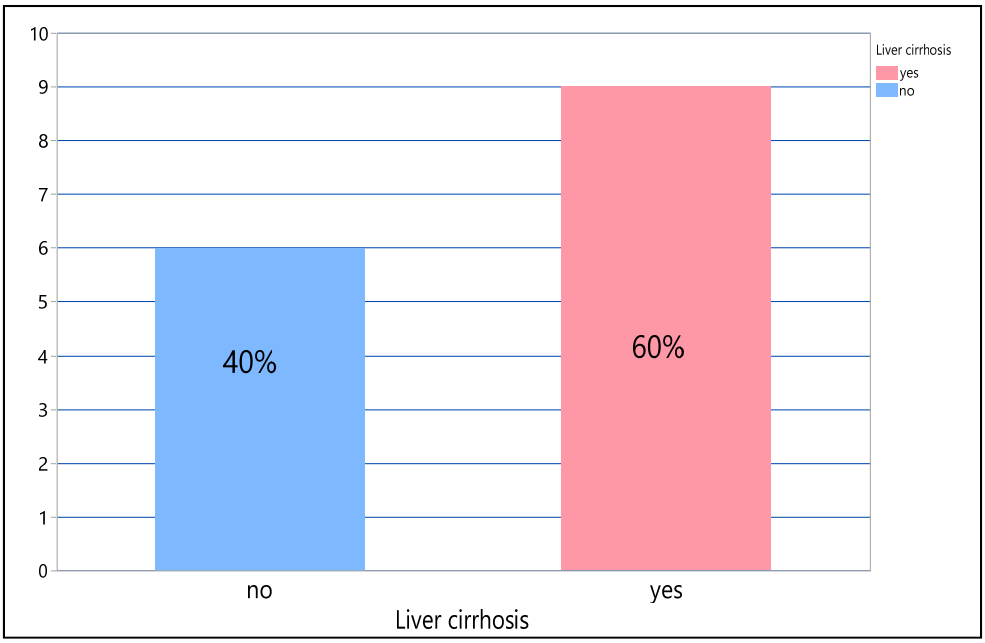

Fig. 64. Relapse correlation with Liver cirrhosis

3.6.17. Analysisc correlation of relapse by Liver transplantation

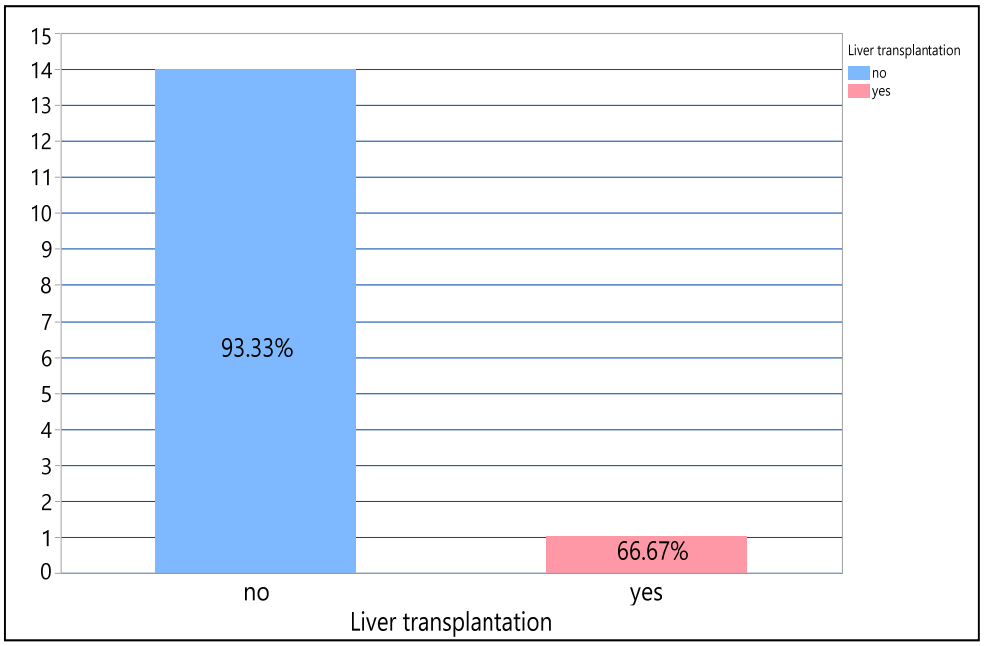

Fig. 65. Relapse correlation with Liver transplantation

3.7. Hepatitis C patients coinfection with HIV or HBV

3.7.1. General characters of hepatitis C patients coinfection with HIV or HBV

There are 8 coinfection patients in hepatitis C patients: 6 patients coinfection with HIV, 2 patients coinfection with HBV. Only one patient is female (P029, coinfection HIV), the gender ratio shows male patients (7/8, 87.5%) obviously more than female patients (1/8, 12.5%), with significant statistical differences (P<0.01).

The treatment duration included 24 weeks, 12 weeks and 8 weeks. There are four coinfection patients had 24 weeks treatment duration (4/8, 50%), three patients had 12 weeks treatment duration (3/8, 37.5%), and only one patient had 8 weeks duration (1/8, 12.5%).

The age of all coinfection HIV or HBV patients range from 43 (Min) to 72 (Max), the average age was 54.75±8.69 (MV±SD).

All the patients coinfection with HIV or HBV included only three kinds of genotypes: 1a (5/8, 62.5%); 3a/b (1/8, 12.5%); 4 (2/8, 25%). All the patient's coinfection with HBV had only one genotypes 1a.

The treatment regiments includes SOF (1/8, 12.5%); SOF DAC (3/8, 50%); Harvoni (2/8,37.5%).

Among eight coinfection patients, there are six patients without pre-therapy and two patients with pre-therapy with IFN and RBV

3.7.2. Special patients and relapse hepatitis C patients coinfection with HIV or HBV

Among eight coinfection patients, The number of special patients combined with liver cirrhosis is 3 (37.5%) all from HIV; is two patients with liver transplantation (1 HIV, 1 HBV). There are no patients combined with nephrectomy in the coinfection HIV or HBV patients.

After treatment, there are only one relapse patients (P407, HBV) SVR12 with high viral load (1.270.000 IU/mL >800.000 IU/mL);

Coinfection with HIV or HBV patients	n % N=8
Coinfection	
HIV	6 (6/437, 1.37%)
HBV	2 (2/437, 0.46%)
Gender	
Male	7 (7/8, 87.5%) *P*<0.01%*
Female	1 (1/8, 12.5%)
Age	
Mean±SD (Min - Max)	54.75±8.69 (43 - 72)
>60 yr	1 (1/8, 12.5%)
Genotype	
1a	5 （62.5%） (3 HIV, 2 HBV)
3a/b	2 （25%） (2 HIV)
4	1 （12.5%） (1 HIV)
Relapse	
HIV	0
HBV	1
Treatment duration	
24 weeks	4 (40%),
12 weeks	3 (37.5%)
8 weeks	1 (12.5%)
Treatment regimens	
SOF	1 (12.5%)
SOF DAC	4 (40%),
Harvoni	3 (37.5%)

Special patients	
Liver cirrhosis	3 (37.5%) (3 HIV)
Liver transplantation	2 （25%） (1 HIV, 1 HBV)
Nephrectomy	0
Pre-therapy	
With	2 (25%)
without	6 (75%)
Baseline platelet count	
Low platelets (≤100/nL)	2 (25%)
Baseline viral load	
<800.000 IU/mL Low viral load	3 (37.5%)
≥800.000 IU/mL High viral load	5 (62.5%)
≥6.000.000 IU/mL Very high viral load	2 (25%)
≥25.000.000 IU/mL Very high viral load	0
SVR 12 rate	
HIV	6/6 (100%)
HBV	1/2 (50%)
Baseline clinical chemistry	**Mean ± SD (Min - Max)**
ALT (IU/1)	1856.63±30.78 (18-99)
Platelets (thousand/µL)	173.75±103.52 (42-344)
WBC (/uL)	7010.00±2024.97 (4710-11170)
Hemoglobin (g/dL)	13.53±1.89 (11.2-16.6)
Creatinine (mg/dL)	0.76±0.21 (0.6-1.1)
Total Bilirubin (mg/dL)	11.53±27.81 (0.3-80.2)
INR	1.06±0.15 (0.9-1.3)

Table 59. Characteristics of hepatitis C patients coinfection with HIV or HBV

3.7.3. Proportion of hepatitis C patients coinfection with HIV or HBV

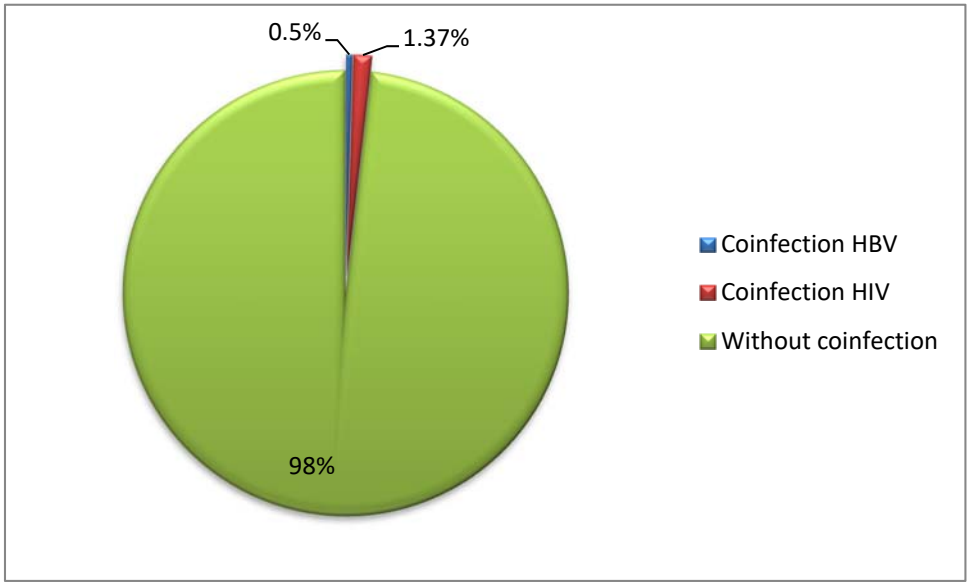

Fig. 66. Proportion of hepatitis C patients coinfection with HIV or HBV

3.7.4. Characteristics of hepatitis C patients coinfection with HIV

Patient	PCR 0	PCR 2	PCR4	PCR 8	PCR12	PCR18	PCR24	SVR 4	SVR12	SVR 24	SVR48
P012	1260000				0		0		0	0	
P029	4830000		0		<15	0	0		0	0	0
P041	3210			0	0				0		
P097	254000	<15	0	0	0	0	0	0	0		0
P242	684000	49	0		0				0	0	
P328	6970000				0				0		

Table 60. Viral load of hepatitis C patients coinfection with HIV

Patient	Gender	Age	Genotype	Treatment regiment	Treatment duration	Treatment completed	Pre-therapy
P012	Male	72	3a/b	SOF	24	completed	no
P029	Female	56	1a	SOF DAC	24	completed	no
P041	Male	53	1a	Harvoni	12	completed	no
P097	Male	43	4	SOF DAC	24	completed	no
P242	Male	58	1a	SOF DAC	12	completed	no
P328	Male	56	4	Harvoni	12	completed	yes

Table 61. General characteristics of hepatitis C patients coinfection with HIV

Patient	Relapse	HIV	HBV	Liver Cirrhosis	Liver transplantation	Nephrectomy
P012	no	yes	no	no	no	no
P029	no	yes	no	yes	no	no
P041	no	yes	no	yes	no	no
P097	no	yes	no	yes	yes	no
P242	no	yes	no	no	no	no
P328	no	yes	no	no	no	no

Table 62. Complication of coinfection HIV patients

Patient	Hemoglobin	WBC	PLT	Quick	INR	Creatinine	Bilirubin	ALT
P012	13.9	6830	123			1.1	1.3	39
P029	13.4	5730	116	78	1.1		1.3	34
P041	12	4710	156	57	1.3	0.8	2.1	33
P097	13	6180	42				6	99
P242	16.6	11170	282	>120	0.9	0.6	0.4	63
P328	12.2	8720	344	106	1	0.6	0.3	18

Table 63. Baseline clinical chemistry of coinfection HIV patients

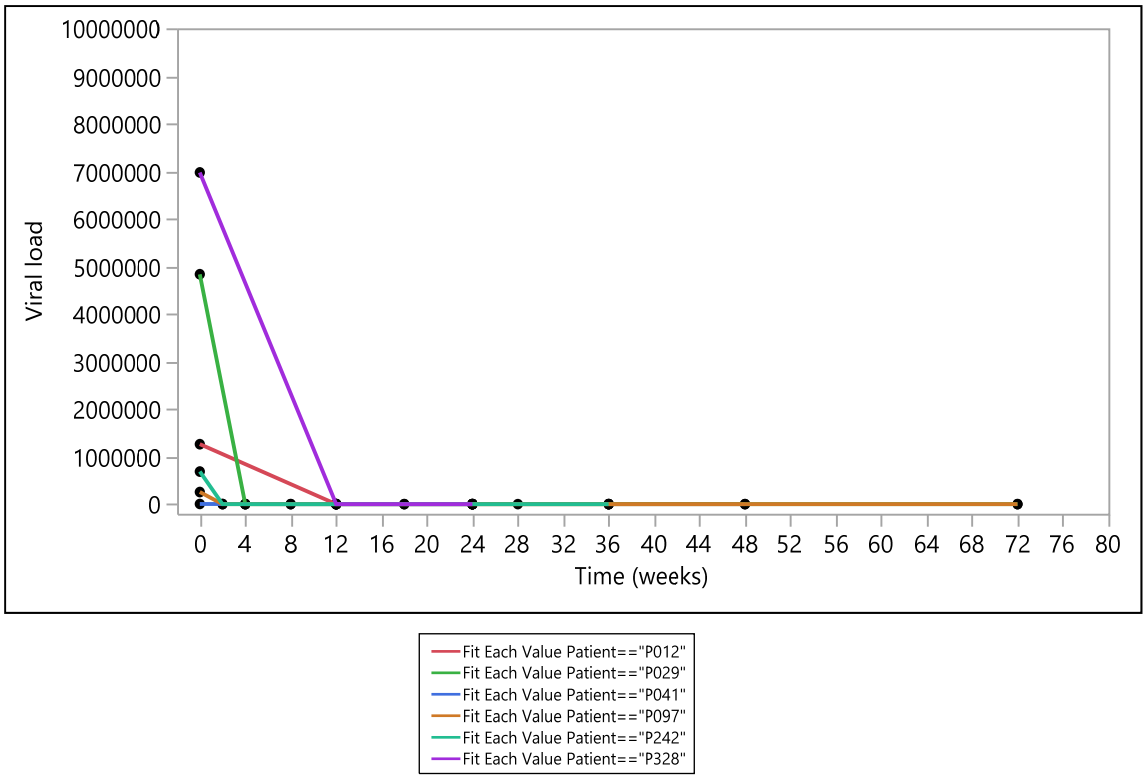

Fig. 67. Viral load changing with time of HCV coinfection HIV

3.7.5. Characteristics of hepatitis C patients coinfection with HBV

Patient	PCR 0	PCR 2	PCR4	PCR 8	PCR12	PCR18	PCR24	SVR 4	SVR12	SVR 24	SVR48
P024	13200000		0		0		0		0		0
P407	2180000				0				1270000		

Table 64. Viral load of hepatitis C patients coinfection with HBV

Patient	Gender	Age	Genotype	Treatment regiment	Treatment duration	Treatment completed	Pre-therapy
P024	Male	46	1a	SOF DAC	24	completed	yes
P407	Male	54	1a	Harvoni	8	relapse	no

Table 65. General characteristics of hepatitis C patients coinfection with HBV

Patient	Relapse	HIV	HBV	Liver Cirrhosis	Liver transplantation	Nephrectomy
P024	no	no	yes	no	yes	no
P407	yes	no	yes	no	no	no

Table 66. Complication of hepatitis C patients coinfection with HBV

Patient	Hemoglobin	WBC	PLT	Quick	INR	Creatinine	Bilirubin	ALT
P024	11.2	6420	91				80.2	99
P407	15.9	6320	236	96	1	0.7	0.6	68

Table 67. Baseline clinical chemistry of hepatitis C patients coinfection with HBV

3.7.6. Viral load changing with time in hepatitis C patients coinfection HIV

Patient Nr. 012

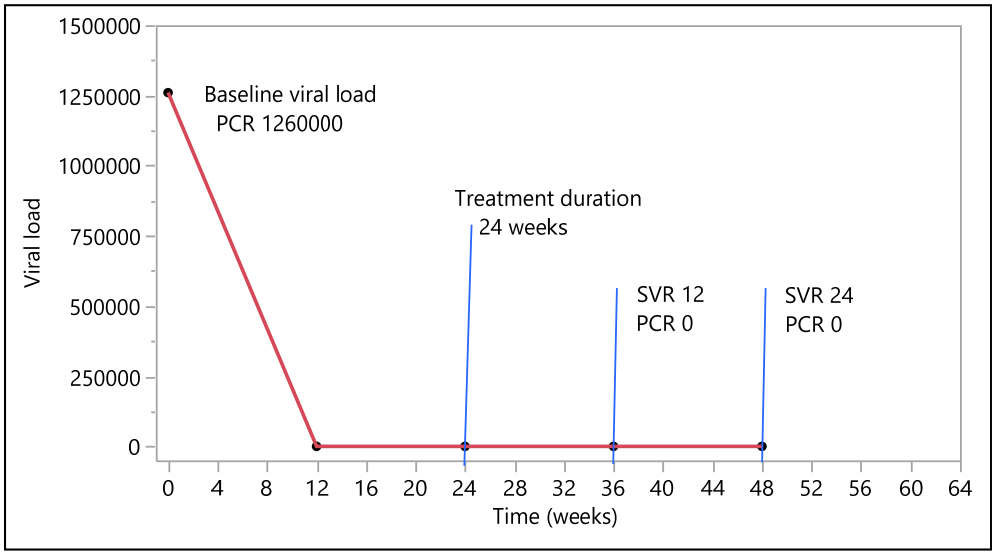

Fig. 68. Viral load changing with time in hepatitis C patients coinfection with HIV (P012)

Patient	**P012**
Gender	Male
Age	72
Genotype	3a/b
Treatment regiment	SOF
Treatment duration (Weeks)	24
Treatment completed	completed
Relapse	no
HIV	yes
HBV	no
Liver Cirrhosis	no
Liver transplantation	no
Nephrectomy	no
Pre-therapy	no
Hemoglobin	13.9
WBC	6830
PLT	123
INR	
Creatinine	1.1
Bilirubin	1.3
ALT	39
Baseline viral load	1260000

Table 68. Characteristics of in hepatitis C patients coinfection with HIV (P012)

Patient Nr. 029

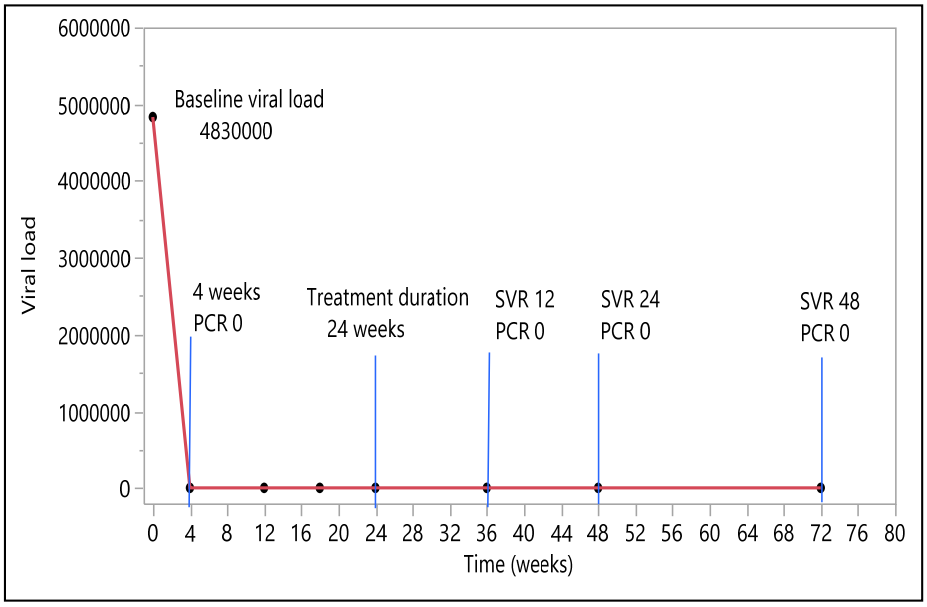

Fig. 69. Viral load changing with time in hepatitis C patients coinfection with HIV (P029)

Patient	**P029**
Gender	Female
Age	56
Genotype	1a
Treatment regiment	SOF DAC
Treatment duration (Weeks)	24
Treatment completed	completed
Relapse	no
HIV	yes
HBV	no
Liver Cirrhosis	yes
Liver transplantation	no
Nephrectomy	no
Pre-therapy	no
Hemoglobin	13.4
WBC	5730
PLT	116
INR	1.1
Creatinine	
Bilirubin	1.3
ALT	34
Baseline viral load	4830000

Table 69. Characteristics of in hepatitis C patients coinfection with HIV (P029)

Patient Nr. 041

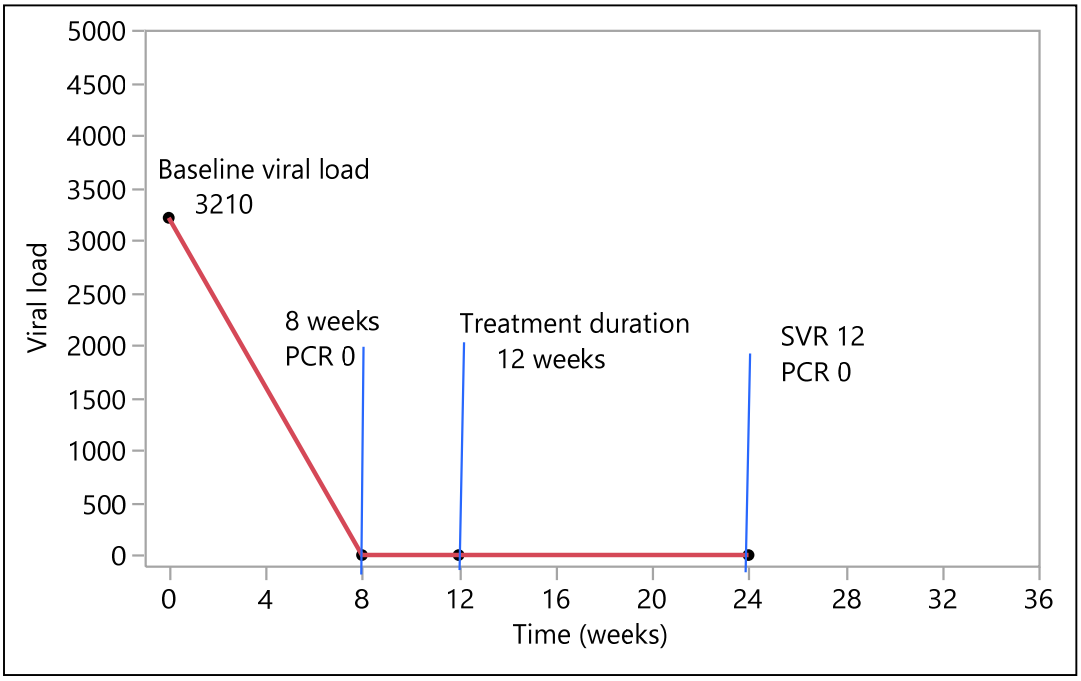

Fig. 70. Viral load changing with time in hepatitis C patients coinfection with HIV (P041)

Patient	**P041**
Gender	Male
Age	53
Genotype	1a
Treatment regiment	Harvoni
Treatment duration (Weeks)	12
Treatment completed	completed
Relapse	no
HIV	yes
HBV	no
Liver Cirrhosis	yes
Liver transplantation	no
Nephrectomy	no
Pre-therapy	no
Hemoglobin	12
WBC	4710
PLT	156
INR	1.3
Creatinine	0.8
Bilirubin	2.1
ALT	33
Baseline viral load	3210

Table 70. Characteristics of in hepatitis C patients coinfection with HIV (P041)

Patient Nr. 097

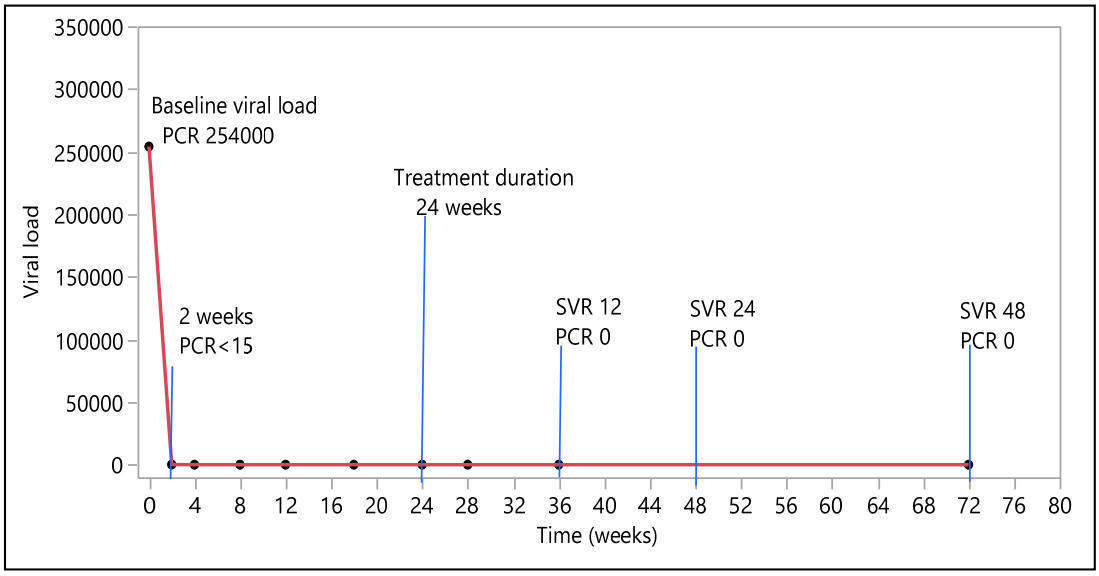

Fig. 71. Viral load changing with time in hepatitis C patients coinfection with HIV (P097)

Patient	**P097**
Gender	Male
Age	43
Genotype	4
Treatment regiment	SOF DAC
Treatment duration (Weeks)	24
Treatment completed	completed
Relapse	no
HIV	yes
HBV	no
Liver Cirrhosis	yes
Liver transplantation	yes
Nephrectomy	no
Pre-therapy	no
Hemoglobin	13
WBC	6180
PLT	42
INR	
Creatinine	
Bilirubin	6
ALT	99
Baseline viral load	254000

Table 71. Characteristics of in hepatitis C patients coinfection with HIV (P097)

Patient Nr. 242

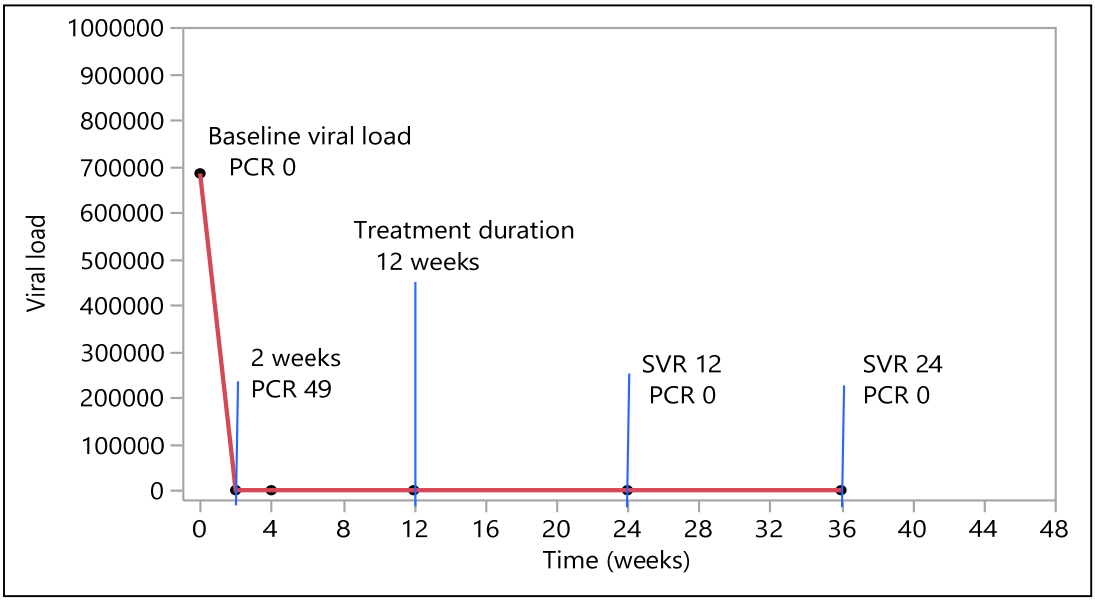

Fig. 72. Viral load changing with time in hepatitis C patients coinfection with HIV (P242)

Patient	**P242**
Gender	Male
Age	58
Genotype	1a
Treatment regiment	SOF DAC
Treatment duration (Weeks)	12
Treatment completed	completed
Relapse	no
HIV	yes
HBV	no
Liver Cirrhosis	no
Liver transplantation	no
Nephrectomy	no
Pre-therapy	no
Hemoglobin	16.6
WBC	11170
PLT	282
INR	0.9
Creatinine	0.6
Bilirubin	0.4
ALT	63
Baseline viral load	684000

Table 72. Characteristics of in hepatitis C patients coinfection with HIV (P242)

Patient Nr. 328

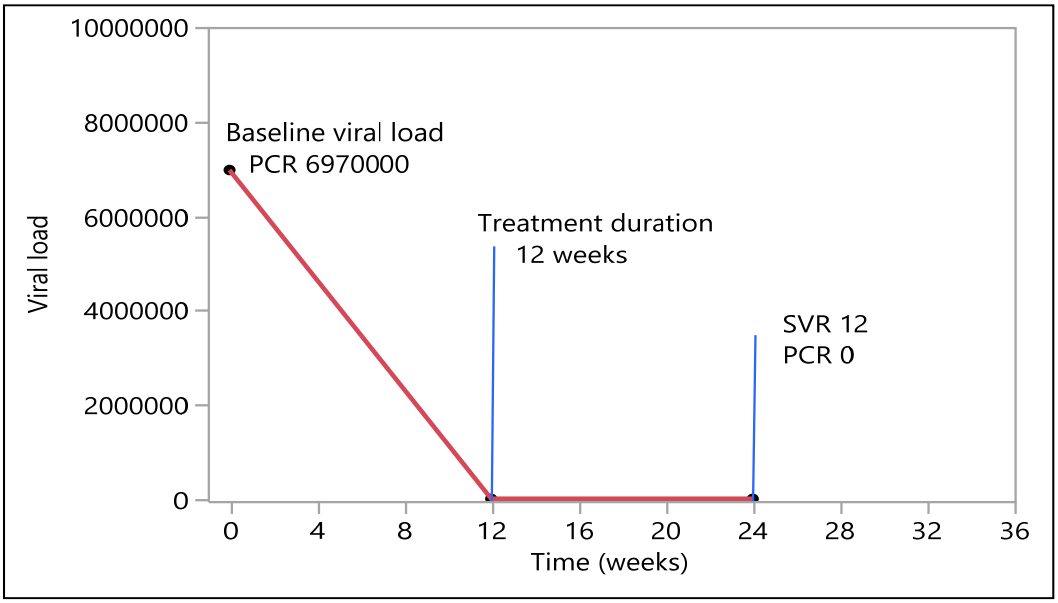

Fig. 73. Viral load changing with time in hepatitis C patients coinfection with HIV (P328)

Patient	**P328**
Gender	Male
Age	56
Genotype	4
Treatment regiment	Harvoni
Treatment duration (Weeks)	12
Treatment completed	completed
Relapse	no
HIV	yes
HBV	no
Liver Cirrhosis	no
Liver transplantation	no
Nephrectomy	no
Pre-therapy	yes
Hemoglobin	12.2
WBC	8720
PLT	344
INR	1
Creatinine	0.6
Bilirubin	0.3
ALT	18
Baseline viral load	6970000

Table 73. Characteristics of in hepatitis C patients coinfection with HIV (P328)

3.7.7 Viral load changing with time in Hepatitis C patients coinfection HBV

Patient Nr. 024

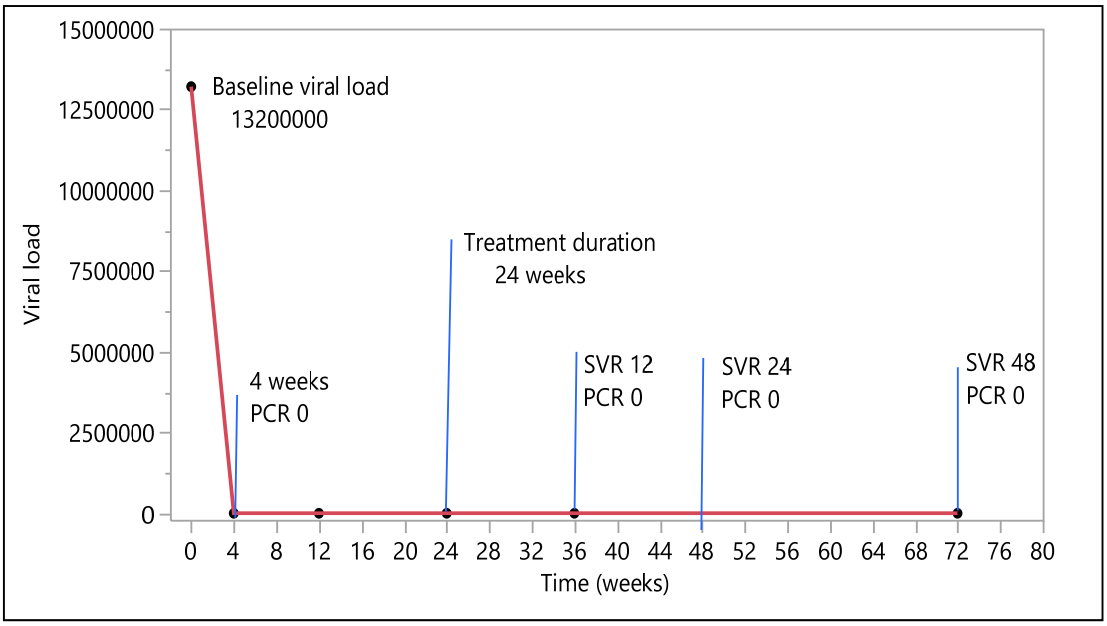

Fig. 74. Viral load changing with time in hepatitis C patients coinfection with HIV (P024)

Patient	**P024**
Gender	Male
Age	46
Genotype	1a
Treatment regiment	SOF DAC
Treatment duration (Weeks)	24
Treatment completed	completed
Relapse	no
HIV	no
HBV	yes
Liver Cirrhosis	no
Liver transplantation	yes
Nephrectomy	no
Pre-therapy	yes
Hemoglobin	11.2
WBC	6420
PLT	91
INR	no data
Creatinine	no data
Bilirubin	80.2
ALT	99
Baseline viral load	13200000

Table 74. Characteristics of in hepatitis C patients coinfection with HBV (P024)

Patient Nr. 407

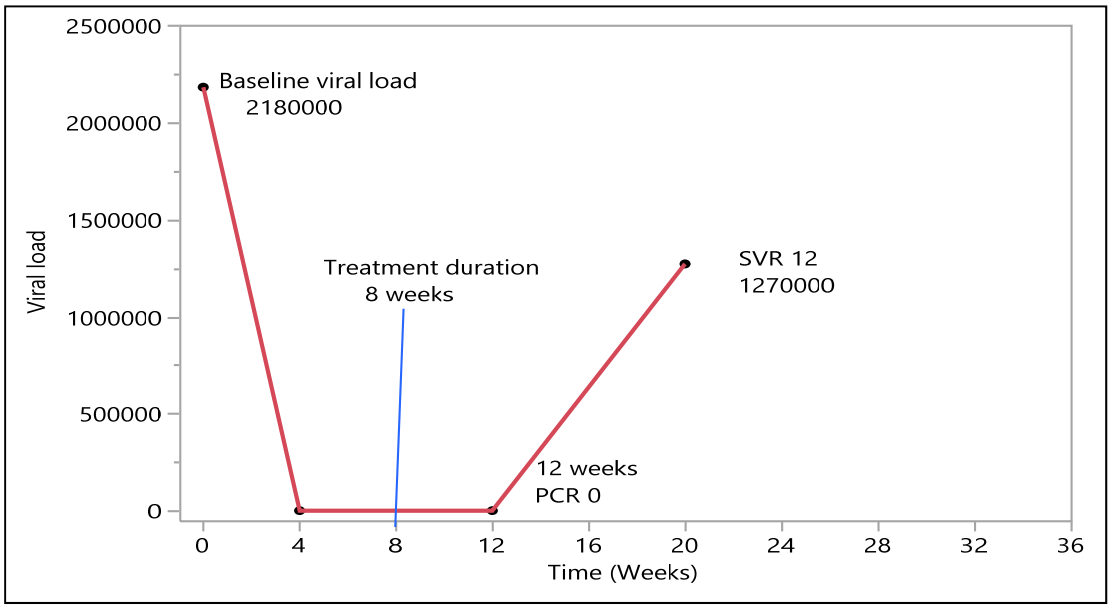

Fig. 75. Viral load changing with time in hepatitis C patients coinfection with HBV (P407)

Patient	P407
Gender	Male
Age	54
Genotype	1a
Treatment duration	8
Treatment regiments	Harvoni
Liver cirrhosis	no
Liver transplantation	no
Nephrectomy	no
Coinfection HIV	no
Coinfection HBV	yes
Pre-therapy	no
Hemoglobin	15.9
WBC	6320
PLT	236
INR	1
Creatinine	0.7
Bilirubin	0.6
ALT	68
Baseline viral load	2180000

Table 75. Characteristics of in hepatitis C patients coinfection with HBV (P407)

3.8 HCV patients with liver cirrhosis

3.8.1 Special patients population

Comorbidity	N %
Liver cirrhosis	123 (28.15%)
Liver transplantation	22 (5.03%)
Nephrectomy	6 (1.37%)

Table 76. Special patients population

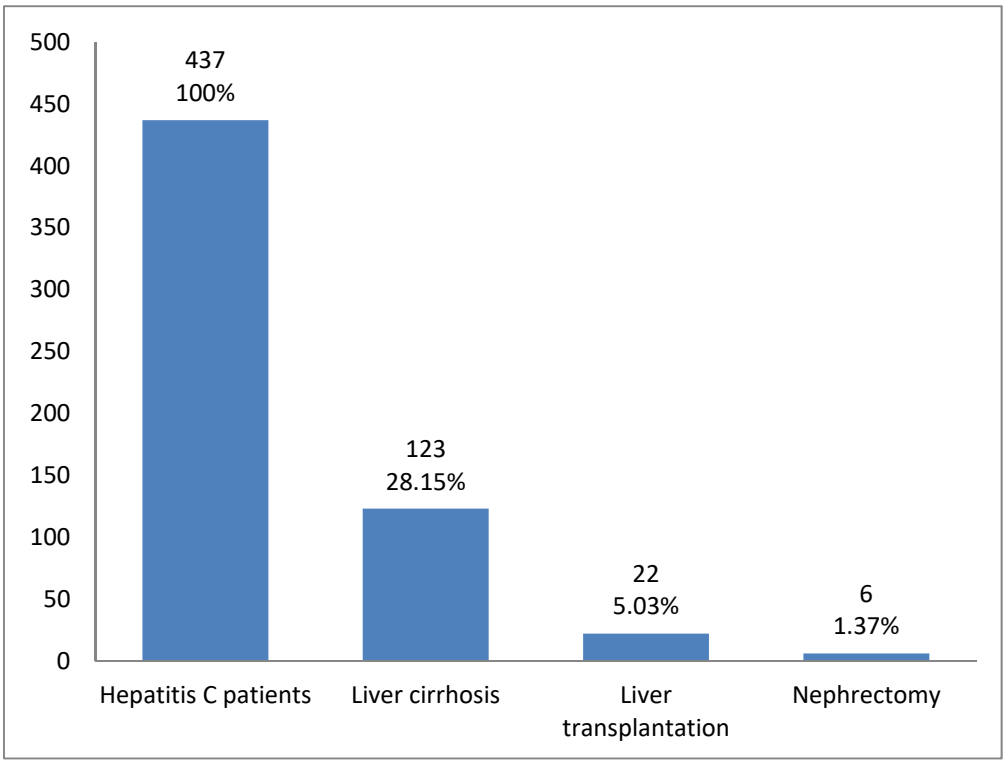

Fig. 76. The proportion of patients with comorbidity

3.8.2 The proportion of hepatitis C patients with liver cirrhosis

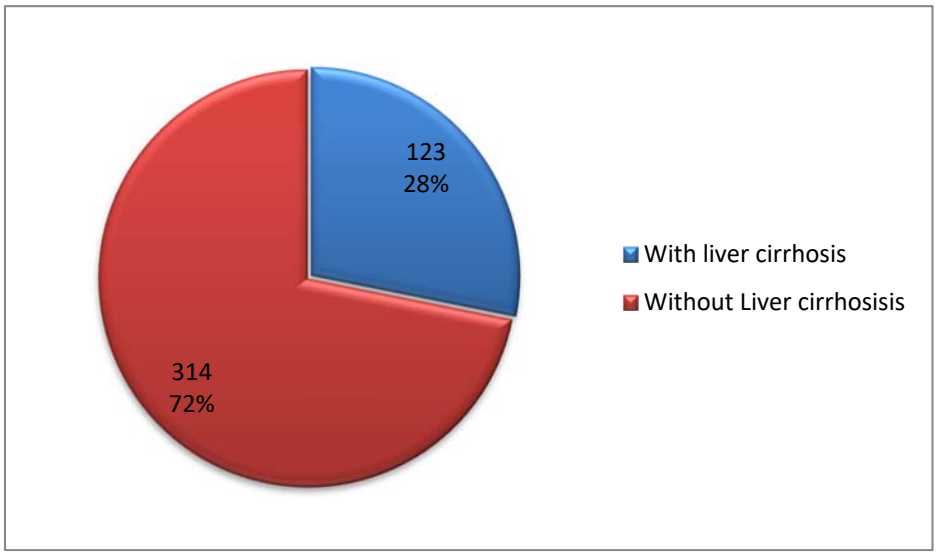

Fig. 77. The proportion of hepatitis C patients with liver cirrhosis

3.8.3 Treatment regimens of liver cirrhosis patients

Treatment regimens	Number of patients	Proportion
Epclusa	6	4.88%
Exviera+Viekirax	7	5.69%
Harvoni	36	29.27%
SOF	12	9.76%
SOF DAC	45	36.59%
SOF SMP	16	13.01%
Zepatier	1	0.81%
Total	123	100%

Table 77. Treatment regimens of liver cirrhosis patients

3.8.4 Characteristics of hepatitis C patients with liver cirrhosis

Liver cirrhosis patients	n, % N=123
Gender	
Male	81 (65.85%)
Female	42 (34.15%)
Age	
Mean±SD (Min-Max)	55.82±10.97 (28 - 84)
≥60 yr	28 (22.76%)
≥80 yr	3 (1.62%)
Treatment duration	
12 weeks	69 (56.56%)
24 weeks	53 (43.44%),
Termination (Death)	1 (6.67%).
Genotype	
1a	32 (26.01%)
1b	48 (39.02%)
1 unclassified	1 (0.81%)
2a/b	4 (3.25%)
3a/b	26 (21.14%)
4	10 (8.13%)
5	1 (0.81%)
6	1 (0.81%)

Treatment regimens	
Epclusa	6 （4.88%）
Exviera+Viekirax	7 （5.69%）
Harvoni	36 （29.27%）
SOF	12 （9.76%）
SOF DAC	45 （36.59%）
SOF SMP	16 （13.01%）
Zepatier	1 （0.81%）
Special patients	
Liver transplantation	7 (5.69%)
Nephrectomy	2 (1.64%)
Death	1 (0.81%)
Coinfection	
HBV	0
HIV	3 (2.44%)
Pre-thrapy	
With	76 (61.79%)
without	47 (38.21%)
Baseline platelet count	
Low platelets (≤100/nL)	57 (46.34%)
Baseline viral load	
<800.000 IU/mL Low viral load	51 (41.46%)
≥800.000 IU/mL High viral load	72 (58.53%)
≥6.000.000 IU/mL Very high viral load	4 (3.25%)

Relapse	
With	9 (7.32%)
Without	114 (92.69)
SVR 12	
Negative	9 (7.32%)
Positive	114 (92.69)
Baseline clinical chemistry	**Mean±SD (Min - Max)**
Hemoglobin (g/dL)	13.45±1.99 (8.4-17.5)
WBC (/μL)	5265.92±2165.00（1520-12710）
PLT (1000/μL)	113.28± 62.74 （23-366）
Quick	75.88±20.07 （28-121）
INR (INR)	1.24±0.42 （0.9-4.4）
Creatinine (mg/dL)	0.74±0.25 （0-2.2）
Bilirubin (mg/dL)	1.58±2.39 （0.3-23.6）
ALT (IU/1)	81.90±55.04 （20-295）

Table 78. Characteristics of liver cirrhosis patients

3.8.5 Age analysis of liver cirrhosis patients

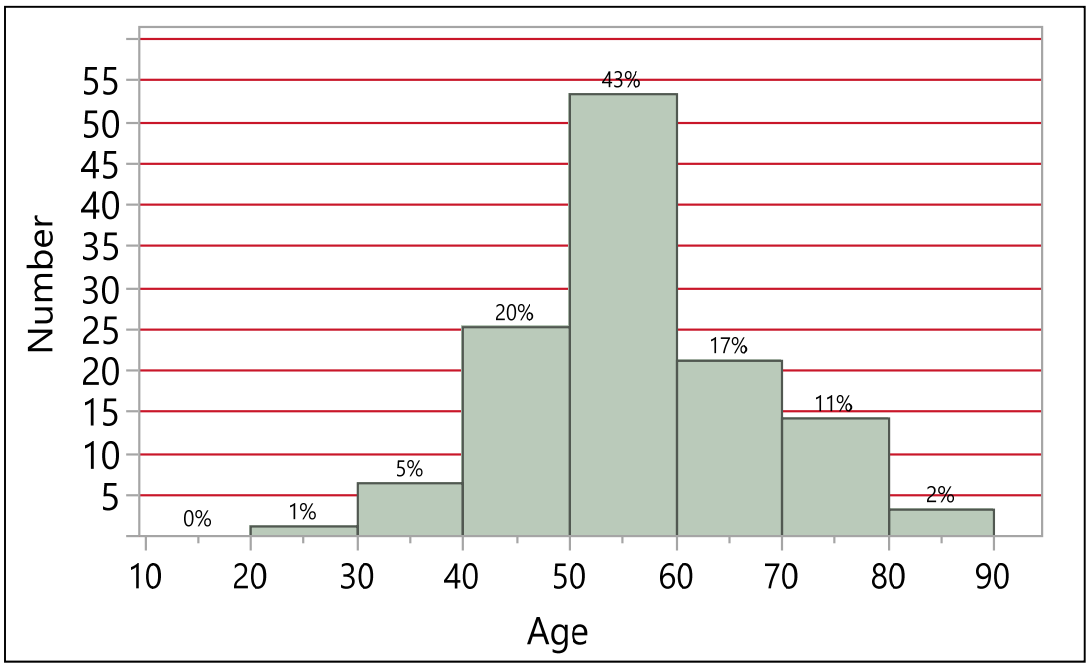

Fig. 78. Age distribution of liver cirrhosis patients

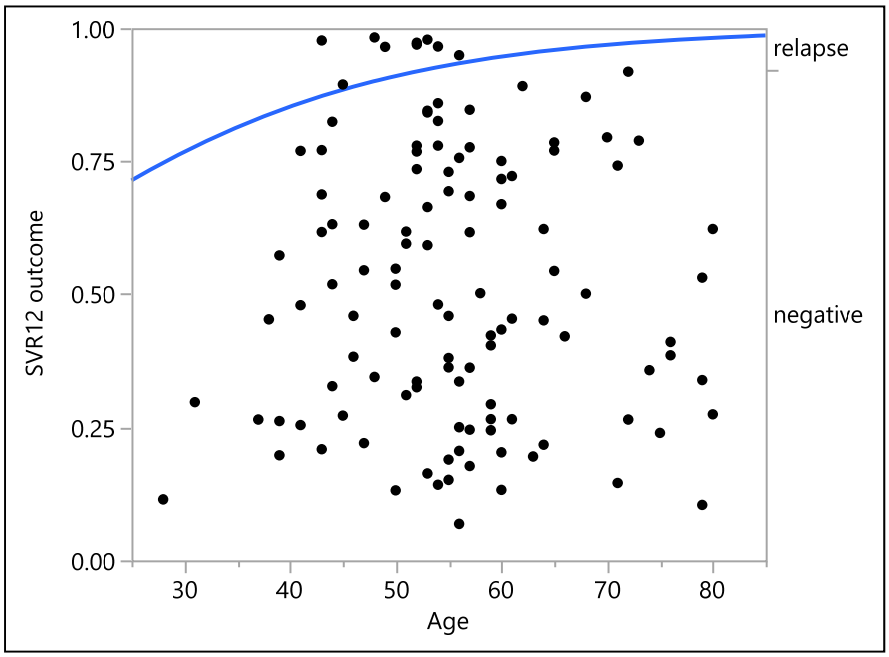

Fig. 79. Logistic fitting of age by SVR12 outcome in liver cirrhosis patients

3.8.6 Gender analysis of hepatitis C patients with/without liver cirrhosis

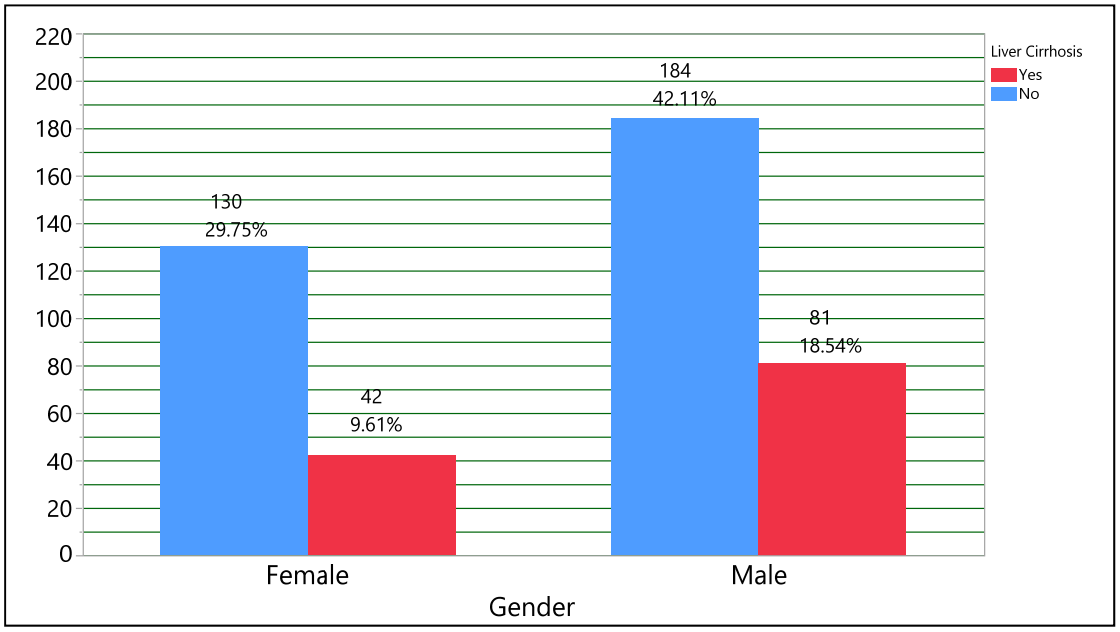

Fig. 80. Liver cirrhosis proportion by gender in hepatitis C patients

3.8.7 Time duration of hepatitis C patients with/without liver cirrhosis

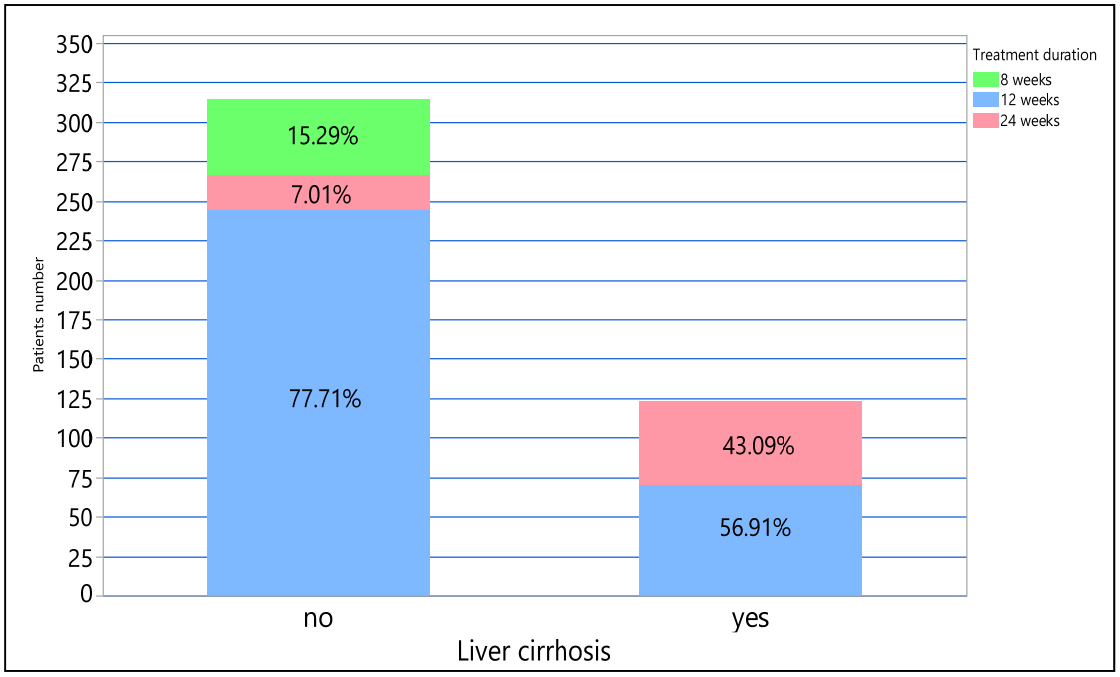

Fig. 81. Time duration of hepatitis C patients with/without liver cirrhosis

3.8.8 Genotype analysis of liver cirrhosis patients

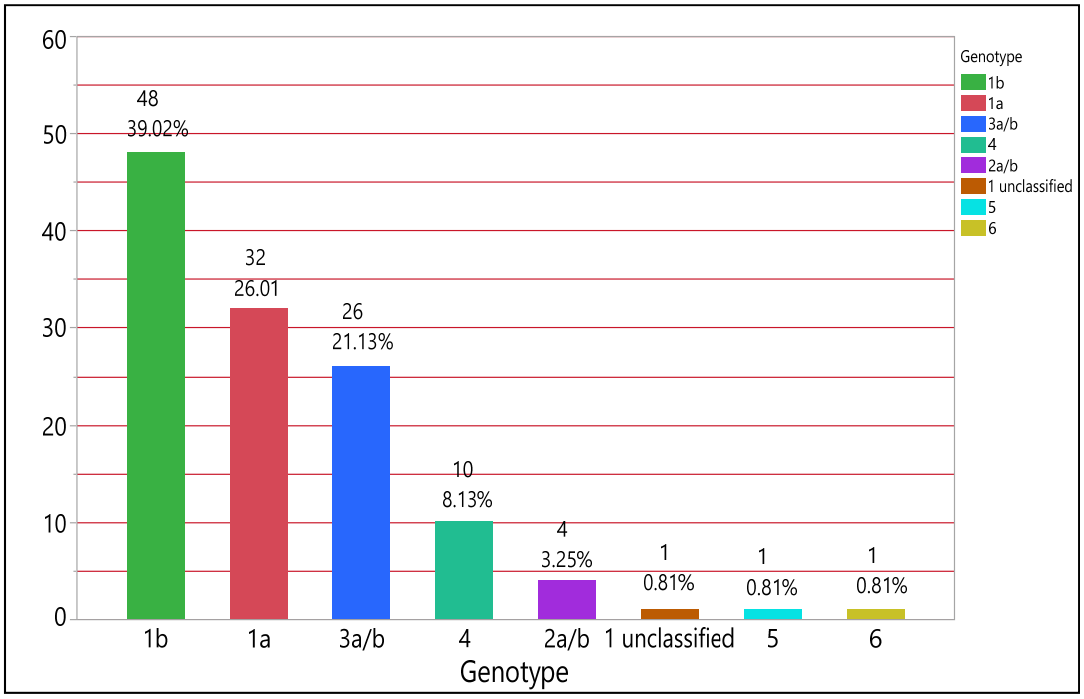

Fig. 82. Genotype proportion of liver cirrhosis patients

3.8.9 Baseline viral load analysis of liver cirrhosis patients

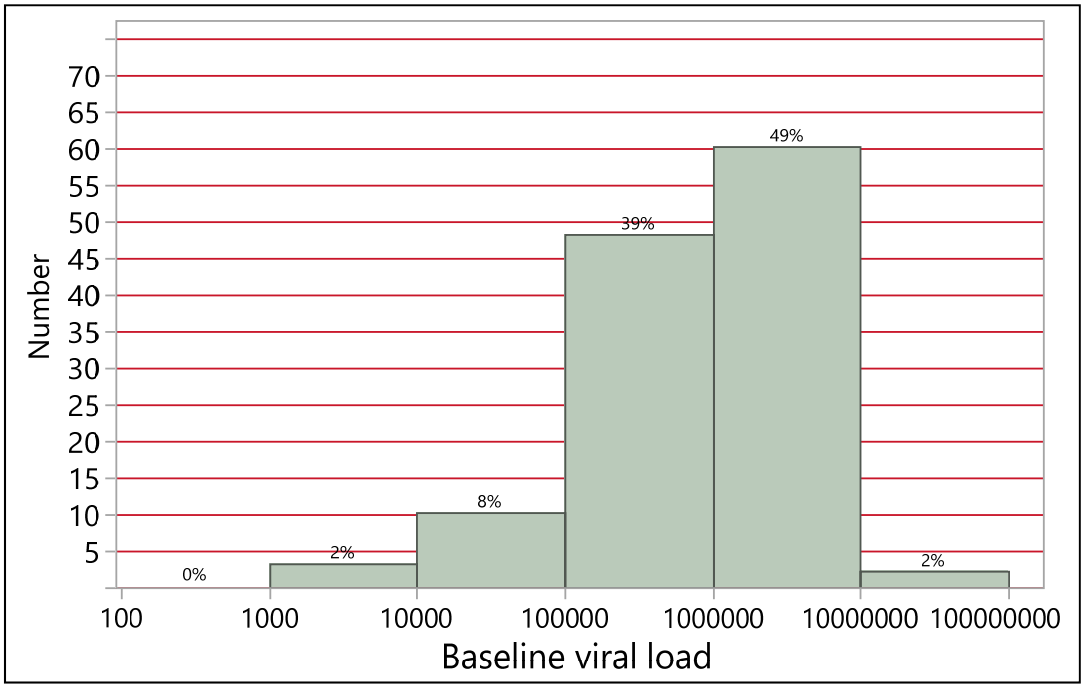

Fig. 83. Distribution of age in liver cirrhosis patients

3.8.10 Analysis liver cirrhosis patients by baseline viral load

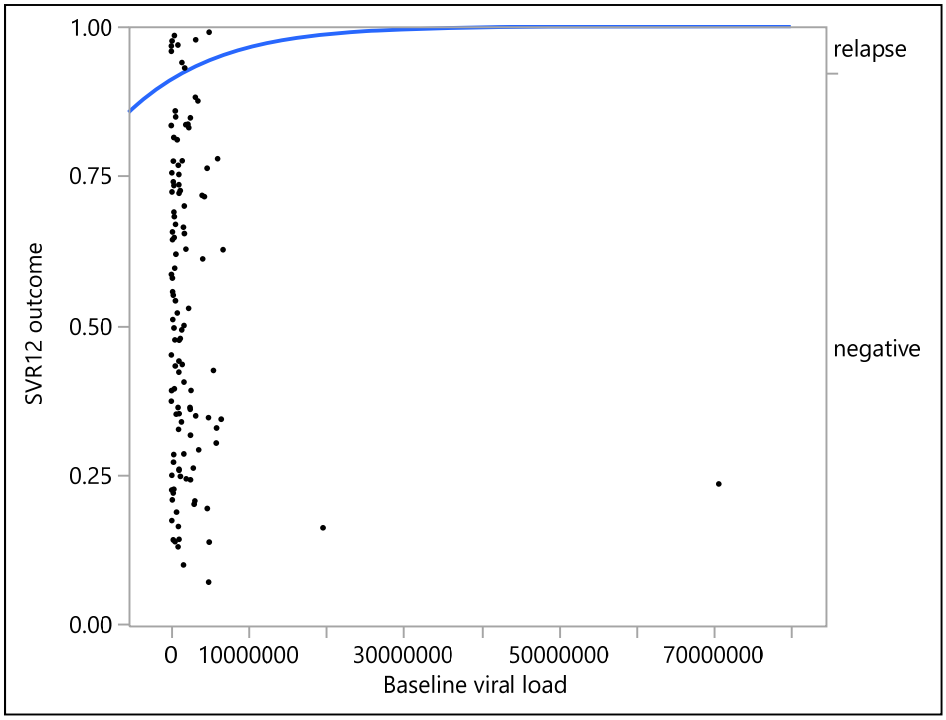

Fig. 84. Logistic fitting of SVR12 rate by baseline viral load in liver cirrhosis patients （P<0.001*）

3.8.11 Treatment regimens of liver cirrhosis patients

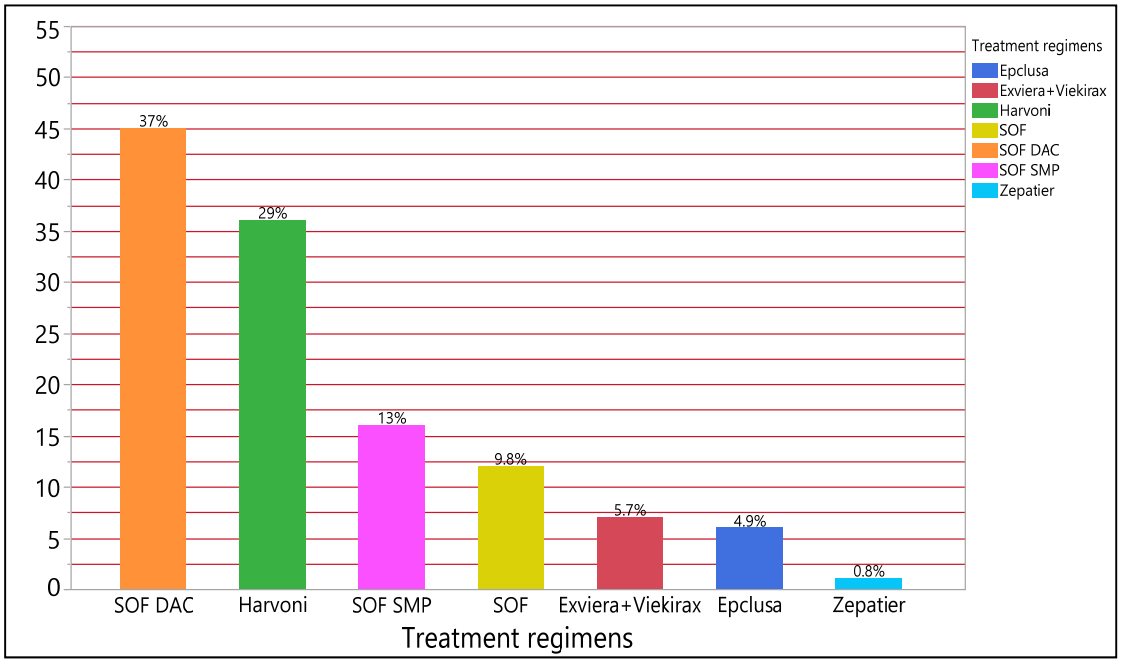

Fig. 85. Treatment regimens of liver cirrhosis patients

3.8.12 Analysis SVR12 outcome of different genotype in liver cirrhosis patients

(117 liver cirrhosis patients, exclude 5 patients of death, termination and lost to follow-up)

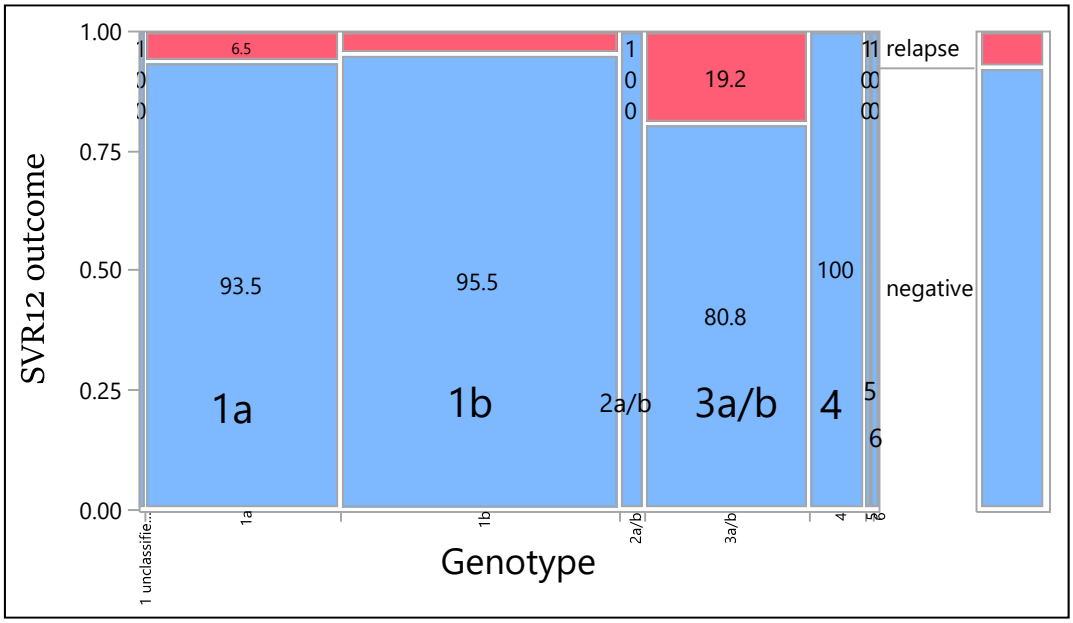

Fig. 86. Proportion of SVR12 negative vs. relapse by different genotype in liver cirrhosis patients

Count Row %	SVR 12 negative	Relapse	Total
1 unclassified	1 100.00	0 0.00	1
1a	29 93.55	2 6.45	31
1b	42 95.45	2 4.55	44
2a/b	4 100.00	0 0.00	4
3a/b	21 80.77	5 19.23 *	26
4	9 100.00	0 0.00	9
5	1 100.00	0 0.00	1
6	1 100.00	0 0.00	1
Total	108	9	117

Table 79. Proportion of SVR12 negative vs. relapse in different genotype of liver cirrhosis patients

3.8.13 Analysis SVR12 negative vs. relapse of different treatment regimens

(117 liver cirrhosis patients, exclude 5 patients of death, termination and lost to follow-up)

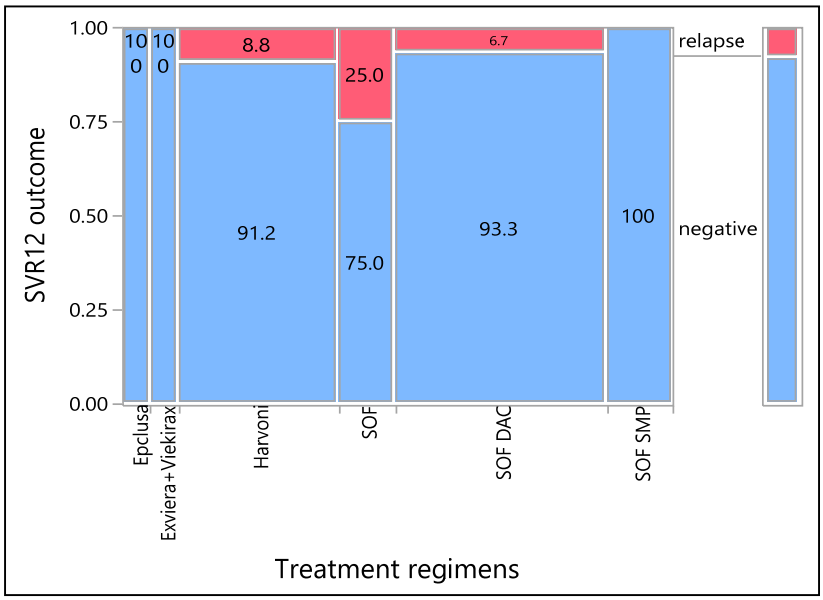

Fig. 87. SVR12 outcome of treatment regimens in liver cirrhosis patients

Count Row %	**Negative**	**Relapse**	**Total**
Epclusa	6 100.00	0 0.00	6
Exviera+Viekirax	6 100.00	0 0.00	6
Harvoni	31 91.18	3 8.82	34
SOF	9 75.00	3 25.00	12
SOF DAC	42 93.33	3 6.67	45
SOF SMP	14 100.00	0 0.00	14
Zepatier	0	0	0
Total	108	9	117

Table 80. Proportion of SVR12 negative vs. relapse of treatment regimens

(117 liver cirrhosis patients, exclude 5 patients of death, termination and lost to follow-up)

4. Discussion

Hepatitis C virus infections are world wild existing, and HCV is a major cause of liver cirrhosis and liver cancer. Therefore, to treat chronic HCV infection has a major impact on morbidity and mortality of liver diseases in human being. About two decades before, the standard treatment was based on ribavirin and interferon. But the success rate of treatment was less than 50%. From recent years, the direct-acting antivirals (DAAs) became available with much higher treatment success rate of more than 90%, and the treatment duration is much shorter.

4.1. Overall SVR12 rate

In this retrospective study of hepatitis C treatment with direct-acting antivirals, the overall outcome in the cohort shows SVR12 rate of total 437 patients is 95.95%. These data are similar to other study publications related DAA clinical research which the DAAs SVR12 rate achieved more than 90% (Asselah et al., 2016). The SVR12 rate statistical correlation with liver cirrhosis (P=0.0016*, liver transplantation (P=0.0166), coinfection with HBV (P=0.0004), baseline platelets (P=0.0068).

4. 2. Genotypes

In this study, the proportion of GT1 patients is the largest (310/437, 70.94%), which includes GT1a (30.66%) and GT1b (39.59%), followed by the GT3 (20.37%). Among the six genotypes, GT1a achieved the highest SVR12 rate (96.92%), followed by GT1b (95.78%). GT3 had the lowest SVR12 rate (93.02%). The relapse rate of GT3 (6.98%) is double times compared with of GT1 relapse rate (3.08% of GT1a, 3.01% of GT1b).

As recently published, the SVR12 rate of DAAs for GT3 is significantly less than for GT1, GT1a, GT1b. In this study, the SVR12 rate for GT3 was 90%, for GT1a 95%, for GT1b 93% (Werner et al., 2016).

It is necessary to further develop new direct antiviral combinations to improve the

success rate of GT3 HCV treatment.

Although the SVR12 rates of GT4, GT5, GT6, GT1 unclassified were up to 100%, the patient's numbers with these genotypes were small: GT4 (23/437), GT5 (2/437), GT6 (3/437), GT1 unclassified (3/437) respectively. So these genotype groups may not show the actual success rate of treatment.

Genotypes have a distinct geographical difference globally (Gower et al., 2014). The genotypes of European countries mainly consist with GT1, followed by GT3. The population of GT4, GT5, GT6 are rare (Gower et al., 2014).

There are five DAAs treatment options for GT1 suggested by EASL (European Association for the study of the liver): 1 SOF/LDV (sofosbuvir and ledipasvir); 2 SOF/VEL (sofosbuvir and velpatasvir); 3 GZR/EBR (grazoprevir and elbasvir); 4 SOF/DAC (sofosbuvir and daclatasvir); 5 ritonavir-boosted paritaprevir, ombitasvir and dasabuvir (EASL, 2017).

In this study, GT1a patients were treated with six treatment regimens, GT1b with seven treatment regimens. The main treatment regimens for GT1 is SOF/LDV (Harvoni) (157/310, 48.39%), followed by SOF/DAC (60/310, 19.35%) and Exviera/Viekirax (43/310, 13.87%). The SVR12 rate of SOF/LDV is: for GT1a 98.73%, for GT1b 95.31%. This result is similar to previous studies, three studies related GT1 using the SOF/LDV treatment regimen, the SVR12 rates were more than 95% (Afdhal et al., 2014a, Afdhal et al., 2014b, Kowdley et al., 2014).

There are two DAAs treatment options for GT3 suggested by EASL: 1 SOF/VEL (sofosbuvir and velpatasvir), 2 SOF/DAC (sofosbuvir and daclatasvir). In this study, six treatment regimens were used in GT3 patients. The main treatment regimen for GT3 is SOF/DAC (44/89, 49.44%), followed by SOF (27/89, 30.34%) and SOF/VEL (Eplusa) (13/89, 14.60%). The SVR12 rates for these three main treatment regimens of GT3 are SOF/DAC (93.18%), SOF (96.15%), SOF/VEL (Eplusa)(100%). A phase III study with SOF/DAC in GT3 shows: the SVR12 rate for noncirrhotic patients was 94% to 97%, for liver cirrhosis patients, it was 58% to 69% (Nelson et al., 2015). Another study showed 86% SVR12 rate in GT3 patients taking SOF/DAC (Leroy et al., 2016).

4.3. Treatment regimens

Seven treatment regimens were used for the six different genotypes. By comparing the SVR12 rate of the seven different direct-acting antivirals, SOF/LDV (Harvoni) achieved the highest SVR12 rate (96.71%), and also the largest proportion of all 437 hepatitis C patients (160/437, 36.61%) had taken it. The SVR12 rate for SOF/LDV in non-cirrhotic patients was 98.31% (116/118), for SOF/LDV with liver cirrhosis patients, it was 91.18% (31/34). This SVR12 rate is similar to the result of a previous study (Asselah et al., 2016). SOF/LDV SVR12 average rate of three studies is 97% (Asselah et al., 2016). In three studies of non-cirrhotic patients with SOF/LDV, the SVR12 rates were 99%, 95%, 94% respectively (Afdhal et al., 2014a, Afdhal et al., 2014b, Kowdley et al., 2014). The SVR12 rates of liver cirrhosis patients with SOF/LDV were 94%, 86%, showed in two studies (Afdhal et al., 2014a, Afdhal et al., 2014b).

In this study, the SVR12 rate for SOF/DAC was 95.28%, and for SOF it was 93.94%. The lowest SVR12 rate was seen for SOF/SMP (91.18%). The SVR12 rate for exviera/viekirax, epclusa (SOF/VEL), zepatier (GZR/EBR) achieved 100%. However, the number of patients taking SOF/SMP (34/437), exviera/viekirax (49/437), epclusa (SOF/VEL) (16/437), zepatier (GZR/EBR) (4/437) were small, so these DAAs groups may not be representative. In some studies, the SVR12 rates of GZR/EBR were 94% and 95% (Roth et al., 2015, Zeuzem et al., 2015). The SVR12 rate of SOF/VEL was up to 99% (Foster et al., 2015, Feld et al., 2015).

4.4. Viral load

In all 437 patients, the viral load declined to negative during treatment. The declining speed of viral load was very high. The majority of patients viral load had dropped to 0 or <15 in the fourth week. In some patients, the viral load rapidly declined to negative at the second week.

The speed of viral load decreasing in the first two weeks cannot be calculated accurately because parts of the data in the first two weeks were not available. So it is

possible, that more patients turned to negative already within two weeks, or even earlier.

The treatment duration of the previous standard treatments with ribavirin and interferon was 24 to 48 weeks, and the cure rate was about 50%. In comparison to the previous treatment, the DAAs treatment time is significantly reduced. The side effects of DAAs are also much milder than in previous treatment.

Interestingly, our results show that patients with high viral load (>800,000 IU/mL) have a higher SVR12 rate compared to patients with low viral load (<800,000 IU/mL). All the patients with high viral load (>6,000,000 IU/mL) and very high viral load (>25,000,000 IU/mL) achieved 100% SVR12 rates. The patients with very high viral load declined with faster speed and without relapse. This result is inconsistent with previous clinical studies. These studies of the old treatment with ribavirin and interferon have shown that patients with high baseline viral load levels are more difficult to treat and had lower SVR12 rates than the patients with low viral load (McHutchison et al., 1998).

4.5 Gender

Hepatitis C patients have a significant difference with gender. Analysing the proportion of all 437 hepatitis C infected patients, male patients (265/437, 60.6%) were obviously more than female patients (172/437, 39.4%). The SVR12 rate of male (94.53%) was lower than female (98.71%). The relapse proportion of male (12/15, 80%) was four times more compared to female relapse rate (3/15, 20%), with statistically significant differences ($P<0.05$). The liver cirrhosis patients consisted of 65.85% male (81/123) and 34.15% female (42/123). The liver transplantation patients consisted of 68.18% male (15/22) and 31.82% female (7/22). The two death during treatment were males.

The HCV infection rate shows lower in women was confirmed by previous studies (Kenny-Walsh, 1999, Wiese et al., 2000). A study about anti-D immune globulin contaminated with HCV in Germany, about nine hundred women had

received this kind of hepatitis C virus contaminants, The result of twenty years followed up showed HCV chronic infection rate of these women was only 55% (Wiese et al., 2000). Another similar study in Ireland, about seven hundred pregnant women who received anti-D immune globulin contaminated with HCV, after seventeen years followed up, also found 55% HCV RNA positive rate (Kenny-Walsh, 1999).

4.6. Relapse

There are 15 patients relapsed in a total of 437 patients; the relapse rate is 3.57%. Multiple factors were analyzed for the relapse correlation, such as genotypes, treatment regimens, treatment duration, baseline viral load, age, gender, liver cirrhosis, liver transplantation, coinfection with HBV or HIV, baseline platelet, pre-therapy. Among these factors, the liver cirrhosis, baseline PLT, and the treatment duration correlated with the relapse.

The relapse rate in liver cirrhosis patients (7.32%) was significantly higher than the noncirrhotic patients (1.99%) (P<0.01). This result is consistent with other previous publications (Afdhal et al., 2014a, Afdhal et al., 2014b, Kowdley et al., 2014).

Interestingly, the results showed that the longer the treatment time, the higher the relapse rate. The relapse rate of patients with 24 weeks treatment duration (8.11%) was significantly higher than patients with 8 weeks (4.44%) and 12 weeks (2.33%). Some studies have shown that the shorter treatment duration with the more possibility of relapse. The sustained virological response is likely more stable with the longer treatment time. The result of this study is inconsistent with previous studies. However, some factors are leading to longer treatment time (24 weeks), such as difficult to treat patients with liver cirrhosis or liver transplantation. 24 weeks treatment duration group included 70.76% liver cirrhosis patients and 29.33% non-cirrhotic patients. About half (53/123, 43.09%) of cirrhosis patients and liver transplant patients (15/22, 68.18%) had 24 weeks treatment duration. The reason for higher relapse rate in the 24

weeks group is because of the patient's condition or comorbidity (liver cirrhosis), and not because of longer treatment time.

Relapse patients mainly distributed in the 45-60 yr age range. Usually, the older patients (>60 yr) be considered difficult to treat. But in this study, the results showed that younger patients had the higher relapse rate (4.43%) compared to the older age group (0.96%). Only one relapse patient could be seen in the >60 yr age group, and no relapse patient was older than 65 yr. Some studies showed elderly patients with similar SVR12 rate to young patients, probably because of the second generation direct-acting antivirals have good tolerance and low toxicity characteristics (Sulkowski et al., 2016).

Contrary to the previous studies，most of relapse patients were in the low baseline viral load range. The relapse rate of patients with low viral load (<800,000 IU/mL) was higher than the patients with high viral load (>800,000 IU/mL), and no relapse patient was in the range of very high viral load (>6,000,000 IU/mL). Most of the relapse patient’s viral load after treatment stop is much higher than the baseline viral load. The changing trend of viral load after treatment showed diversity for each relapse individual. Some viral load was continuously rising during 24 or 48 weeks after treatment, while others were rising first and then declined after relapse. Only one patient was positive at SVR12 but has dropped to negative at SVR24.

Low baseline platelet (PLT) is the significant statistical correlation with relapse. The relapse rate in low (<100/nL) PLT patients (9.59%) was much higher than the relapse rate in patients who had PLT >100/nL (2.43%) (P<0.01). The patients with low platelets are also considered difficult to treat successfully (Werner et al., 2016).

Analysis the relapse rate correlation with genotype in this study, the GT3 had the largest relapse rate (6.98%), the relapse rate for GT1 was 3.01%.

The primary reason for relapse in DAAs treatment is resistance-associated variants (RAVs), which lead to virological failure (Sarrazin, 2016, Pawlotsky, 2016). The virological failure of GT3, GT1, liver cirrhosis may be caused by viral variants

resistant to NS5A inhibitors (Pawlotsky, 2016). RAV of NS3-4A protease inhibitor can be eliminated in blood within weeks or months (Pawlotsky, 2016). However, NS5A inhibitor RAV can exist in much longer time, even for several years (Pawlotsky, 2016).

HCV kinetics are biphasic and include two stages (Neumann et al., 1998). In the first stage, the Hepatitis C virus RNA decline with significant speed and the virus replication is quickly prevented. The second stage is slower because virus decrease relies upon the immune system effectors (Neumann et al., 1998). In the treatment duration, the sensitive virus get eliminated quickly. However, the resistant variants still exist in the liver, and cannot be detected in peripheral blood during treatment, but replicate after treatment finished (Pawlotsky, 2016). Some re-treatment strategies were recommended for the few virological failures in DAA treatment (Pawlotsky, 2016).

4.7. Coinfection with HIV

Hepatitis C patients coinfected with HIV or HBV were increasingly concerned in recent years. There are about 2,278,400 HCV-HIV coinfected patients in the world, 2.4% prevalence in HIV-infected individuals (Platt et al., 2016). In this study, six of the 437 HCV positive patients were coinfected with HIV, the prevalence is 13.7%. Patients with HIV coinfection showed a good treatment result, SVR12 rate was up to 100% without relapse. Due to the small number of this group, it is hard to calculate the accurate SVR12 rate. One study showed similar SVR12 rate in hepatitis C patients coinfected with HIV vs. non-coinfected with HIV in patients with DAAs treatment regimens (Milazzo et al., 2017). Another study showed high cure rates of 92% SVR12 in a HCV-HIV coinfected cohort (Hawkins et al., 2016).

4.8. Coinfection with HBV

There were only two hepatitis C patients coinfected with HBV, and one of them relapsed. The 50% relapse rate cannot represent the real success rate because the

number of this group is too small. In last two years, some studies have reported that DAAs can lead to reactivation of Hepatitis B virus (Wang et al., 2017, Bersoff-Matcha et al., 2017, De Monte et al., 2016). In HCV patients coinfected with HBV, the HBV DNA usually fluctuates greatly and cannot be detected. The hepatitis activity in coinfection patients is primarily driven by HCV (EASL, 2017) The risk of HBV reactivation after HCV elimination is still existing but unpredictable (Saadoun et al., 2015). This kind of HBV reactivation may happen in both HBsAg-positive and HBsAg-negative patients. HCV coinfection with HBV may lead to hepatocellular carcinoma and serious liver diseases (Sagnelli et al., 2002). In coinfection cases, it is necessary to evaluate the extent of HBV reactivation and to consider whether to optimize the treatment regimens. (EASL, 2017)

5. Summary

There are approximately 185 million HCV infected people all over the world. A serious problem of HCV infection is the high chronicity rate of about 80% HCV-infected patients may develop liver cirrhosis or hepatocellular carcinoma.

The HCV standard therapy with interferon and ribavirin was used since1990s, the success rate of this treatment was only about 50%, and it was HCV genotype dependent. This changed significantly since the direct-acting antivirals (DAAs) became available from 2014 onwards. The success rate is about 95% and can be achieved in a very short time.

In this study, 437 patients with HCV infection who were treated at the University Hospital Tübingen in the period from Jan. 2014 to April 2017 were analyzed. In the cohort, six patients were coinfected with HIV and two patients were coinfected with HBV. The rate of coinfection was relatively low because of the specific patient population of the Tübingen University Clinic. In larger cities, the prevalence of HBV and especially HIV infection is higher. In this cohort, 15 patients relapsed after the treatment and the HCV RNA was detectable in the blood. These 15 patients are analyzed during treatment and after treatment with respect to multiple factors such as viral load.

Since different treatment regimens and treatment durations (8 weeks, 12 weeks, 24 weeks) were used, these were related to laboratory and clinical parameters such as HCV genotypes, age, and gender of the patients, coinfections, etc.

The most striking correlation was seen between relapse and liver cirrhosis. This also explained the unexpected finding that the patients with the longer treatment of 24 weeks treatment duration had a higher relapse rate than the patients with 8 weeks or 12 weeks duration. However, the 24 weeks treatment duration is mainly consisted by liver cirrhosis patients.

The analysis in this study shows impressively quick treatment success rate of 95.95% of the new direct-acting antivirals (DAAs) in HCV infected patients. The liver cirrhosis patients and the low PLT patients had the lower success rate.

5. Zusammenfassung

Hepatitis C Viren sind weltweit verbreitet und es werden 185 Millionen infizierte Menschen geschätzt. Ein wesentliches Problem der HCV Infektion ergibt sich durch die hohe Chronizitätsrate von ungefähr 80%, auf deren Boden Leberzirrhose oder auch Leberzellkarzinome entstehen können. Zunächst gab es in den 1990er Jahren Therapie mit Interferon kombiniert mit Ribavirin. Die Therapieerfolge lagen jedoch nur bei 50 % und waren HCV Genotyp abhängig. Dies änderte sich wesentlich seitdem die direkt wirksamen antiviralen Substanzen (DAAs) ab 2014 verfügbar wurden. Mit diesen DAAs werden Therapieerfolge/Heilung von ungefähr 95 % in sehr kurzer Zeit erreicht.

In dieser Arbeit wird die Auswertung von 437 Patienten mit chronischer HCV-Infektion, die am Universitätsklinikum Tübingen in der Zeit von Jan. 2014 bis April 2017 behandelt wurden, analysiert. Bei sechs Patienten lag eine Koinfektion mit HIV vor und bei zwei Patienten eine Koinfektion mit HBV. Die Koninfektionsraten waren relativ niedrig, welches an der Patientenkohorte des Universitätsklinikums Tübingen liegen dürfte. In größeren Ballungsräumen ist die Prävalenz von HBV und insbesondere HIV Infektionen deutlich höher. In 15 Patienten gab es einen Relapse und nach Beendigung der Therapie wurde die HCV RNA wieder im Blut nachweisbar. Diese 15 Patienten sind unter Therapie und nach Therapieende bezüglich der HCV Viruslast detailliert dargestellt. Da verschiedene Therapiekombinationen und insbesondere auch Therapiezeiten (8 Wochen, 12 Wochen, 24 Wochen) zur Anwendung kamen, wurden diese mit anderen Parametern wie HCV Genotypen, Alter und Geschlecht der Patienten, Koinfektionen und andere klinische Daten in

Bezug gesetzt.

Die auffälligste Korrelation zwischen Relapse und anderen Parametern ergab sich mit der Diagnose Leberzirrhose. Dies erklärte auch den zunächst überraschenden Befund, dass die Patienten mit den beiden längeren Therapiezeiten eine höhere Relapse Rate hatten als die Patienten mit 8 Wochen Therapie. Die 24-Wochen-Behandlungsdauergruppe bestand hauptsächlich aus Leberzirrhose-Patienten.

Die Resultalte dieser Arbeit zeigen eindrucksvoll den raschen Therapieerfolg und die hohe Rate von 95.95 % des Therapieerfolges mit den neuen direkt wirksamen antiviralen Substanzen (DAAs) bei chronischen HCV Infektionen in den letzten drei Jahren. Deutlich wird auch, dass es gehäufter zu Therapieversagen kommt, wenn im Rahmen der chronischen Hepatitis C schon eine Leberzirrhose oder geringe PLT (Thrombozytenzahl) vorliegen.

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7 Declaration of Contributions

The dissertation work was carried out at the Institute of Medical Virology and Epidemiology of Viral Diseases under the supervision of Prof. Dr. Gerhard Jahn.

The study was designed by Prof. Dr. Gerhard Jahn.

The hepatitis C patients clinical data was provided by Prof. Dr. Christoph Berg.

Statistical analysis was carried out under the supervision of Prof. Dr. Martin Eichner.

I confirm that I wrote the manuscript myself and that any additional sources of information have been duly cited.

Signed _____

on [date]_____ in Tuebingen

8. Acknowledgment

I especially appreciate my supervisor Prof. Dr. Gerhard Jahn for giving me the opportunity to perform this study.

Furthermore, I would like to thank Prof. Dr. Christoph Berg providing clinical data of hepatitis C patients and giving clinical suggestions.

Additionally, I am grateful for Prof. Dr. Martin Eichner helping me with the statistical methods and experimental analysis concept.

9 Curriculum Vitae

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