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Association of Serum Vitamin B_{12} Levels with Stage of Liver Fibrosis and Treatment Outcome in Patients with Chronic Hepatitis C Virus Genotype 1 Infection

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Hiermit erkläre ich, die Dissertation mit dem Titel "Association of Serum Vitamin B_{12} Levels with Stage of Liver Fibrosis and Treatment Outcome in Patients with Chronic Hepatitis C Virus Genotype 1 Infection" eigenständig angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

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List of Abbreviations

ALT	Alanineaminotransferase
Apo	Apoprotein
AST	Aspartate Aminotransferase
CHC	Chronic Hepatitis C
CI	Confidence Interval
CLDN1	Claudin-1
D	Day
DAA	Direct Antiviral Agents
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
IF	Intrinsic Factor
IFN	Interferon
IFN-λ3	Interferon Lambda 3
IFN-λ4	Interferon Lambda 4
IRES	Internal Ribosomal Entry Site
IL-28B	Interleukin 28B
IQR	Interquartile Range
LDL	Low Density Lipoprotein
LVP	Lipid Virus Particles
µg	Microgram
miR-122	Micro RNA-122
mL	Milliliter
Nm	Nanometer
NPV	Negative Predictive Value
NS	Non Structural
NVR	Non Sustained Virological Response
OCLN	Occludin
OR	Odds Ratio
Peg-IFN-α	Pegylated Interferon Alpha
Peg-IFN-λ1	Pegylated Interferon Lambda1
PPV	Positive Predictive Value
PTT	Partial Thromboplastin Time
RBV	Ribavirin
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristic
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RVR	Rapid Virological Response
SNP	Single Nucleotide Polymorphism
SRB1	Scavenger Receptor Part B1

Std. Error	Standard Error
SVR	Sustained Virological Response
TCII	Transcobalamin II
U/L	Units per Liter
UMG	University Medical Center Göttingen
UTR	Untranslated Region
VLDL	Very Low Density Lipoprotein
γ-GT	Gamma Glutamyl Transferase
χ^2	Chi Quadrat Test

1. INTRODUCTION

1.1. Hepatitis C

1.1.1. Epidemiology

Hepatitis C virus (HCV) infection is a global health problem. Approximately 170 million people are chronically infected with HCV worldwide. About 4 million people are yearly newly infected with HCV. Chronic hepatitis C (CHC) is estimated to be the cause of death for more than 350 thousand people each year (Marinho and Barreira 2013; Mohd Hanafiah et al. 2013). Due to a complex mechanism of persistence mainly in hepatocytes and a high genetic variability of HCV, no vaccine is available yet (Liang 2013).

Chronic HCV infection is responsible for 50% of liver cirrhosis and 25% of hepatocellular carcinoma in Western countries (Yano et al. 1996; Fattovich et al. 1997; Hu und Tong 1999). In the USA the populations at high risk of developing CHC were intravenous drug consumers or the people who have received blood transfusions until 1992 (Denniston et al. 2014).

The prevalence of HCV varies in Europe, between 2% in Southern Europe to 0.5% in Northern Europe (Cornberg et al. 2011). It is estimated that in Germany, every year 5000 people are newly infected with HCV, with a total number of approximately 350 000 persons. In developed countries, the majority of the HCV infections are caused by the intravenous drug abuse, represented mostly by a younger population. In less developed countries, a disrespect to the hygienic aspects during medical procedures represents an important cause for new HCV infections (Hatzakis et al. 2013; Wantuck et al. 2014). The HCV neonatal transmission risk is between 3 to 5 % from an HCV-positive

mother to her infant. This risk is positively correlated to the level of HCV-RNA copies in the mothers' blood, HIV coinfection and invasive procedures (Floreani 2013).

The populations at risk for the infection with HCV are represented by: intravenous drug consumers, blood transfusion receivers (risk had massively decreased after the introduction of HCV screening in 1990), sexual contacts with an intravenous drug user, people that have been stuck or cut with a bloody object, people that have piercings in ears or in other body parts, immunoglobulin injection receivers (Murphy et al. 2000). Other risks are represented by hemodialysis (massively decreased after HCV screening), organ transplantation, sexual or household contact with an HCV-positive patient or healthcare workers (Pereira et al. 1991; Geerlings et al. 1994; Stroffolini et al. 2001).

1.1.2. Hepatitis C Virus

HCV is a positive-sense, single-strand RNA virus belonging to the family of Flaviviridae. HCV was discovered in 1989 as the causative agent for posttransfusion or non-A-non-B hepatitis (Choo et al. 1989). Initially, HCV infection was diagnosed by the detection of anti-HCV specific antibodies using enzyme-linked immunosorbent assays (ELISA); later-on the reverse transcription polymerase chain reaction (RT-PCR) became established for the direct detection of viral genomes (Cha et al. 1991; Chien et al. 1992). Choo et al. identified the full nucleotide sequence of the HCV, using overlapping complementary DNA clones (Choo et al. 1989).

HCV has 7 genotypes (1 to 7) and approximately 100 subtypes (Scheel and Rice 2013). While HCV genotypes 1, 2 and 3 have a global distribution, the HCV genotype 4 and 5 is spread mostly in Central and South Africa, genotype 6 in South East Asia, genotype 7 in Central Africa (Magiorkinis et al. 2009; Scheel and Rice 2013). Genotype 1 is the

most common HCV genotype in Western countries and also in Germany (Scheel and Rice 2013).

The HCV genome has a length of approximately 9.600 nucleotides (Choo et al. 1989). The internal ribosomal entry site (IRES) is located at the 5' terminus and it is a highly conserved untranslated structure (Bartenschlager et al. 2011). The 3' end of the HCV genome contains an untranslated conserved region (the poly U sequence) (Tanaka et al. 1996) (Figure 1). As a result of the HCV genome translation a polyprotein is synthesized. From this HCV polyprotein three structure (core, E1, E2) and seven non-structure proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) are generated, mainly by signal peptidase and autocleavage procedures (Scheel and Rice 2013).

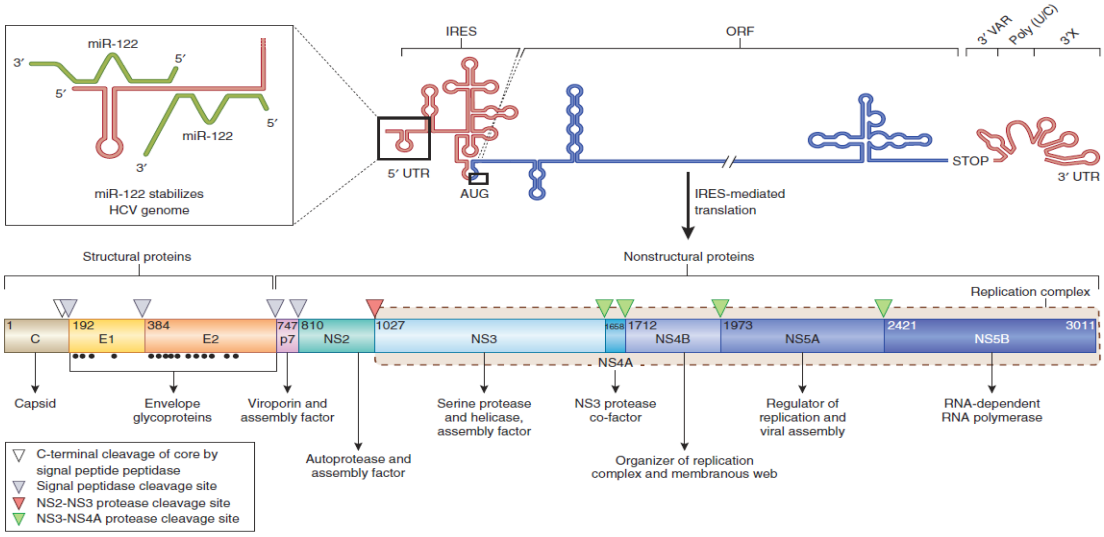


Figure 1 (modified from Scheel and Rice 2013 with Nature Publishing Group authorization) HCV genome and polyprotein processing. The HCV RNA contains one long translated region (blue) flanked by 5′ and 3′ UTRs (red). IRES-mediated translation of the translated region leads to a polyprotein (bottom) that prepared into viral structure and non-structure proteins. The development of the core protein requires the cleavage of a C-terminal signal peptide (white triangle). The E1 structure protein is cleaved due to a cellular signal peptidase. The sections E1, E2 and p7 are fragmented from the polyprotein (gray triangles) by the same cellular signal peptidase. The NS2-NS3 protease has a self-separation mechanism (red triangle). NS4A separates the other non-structural proteins (green triangle) (Scheel and Rice 2013).

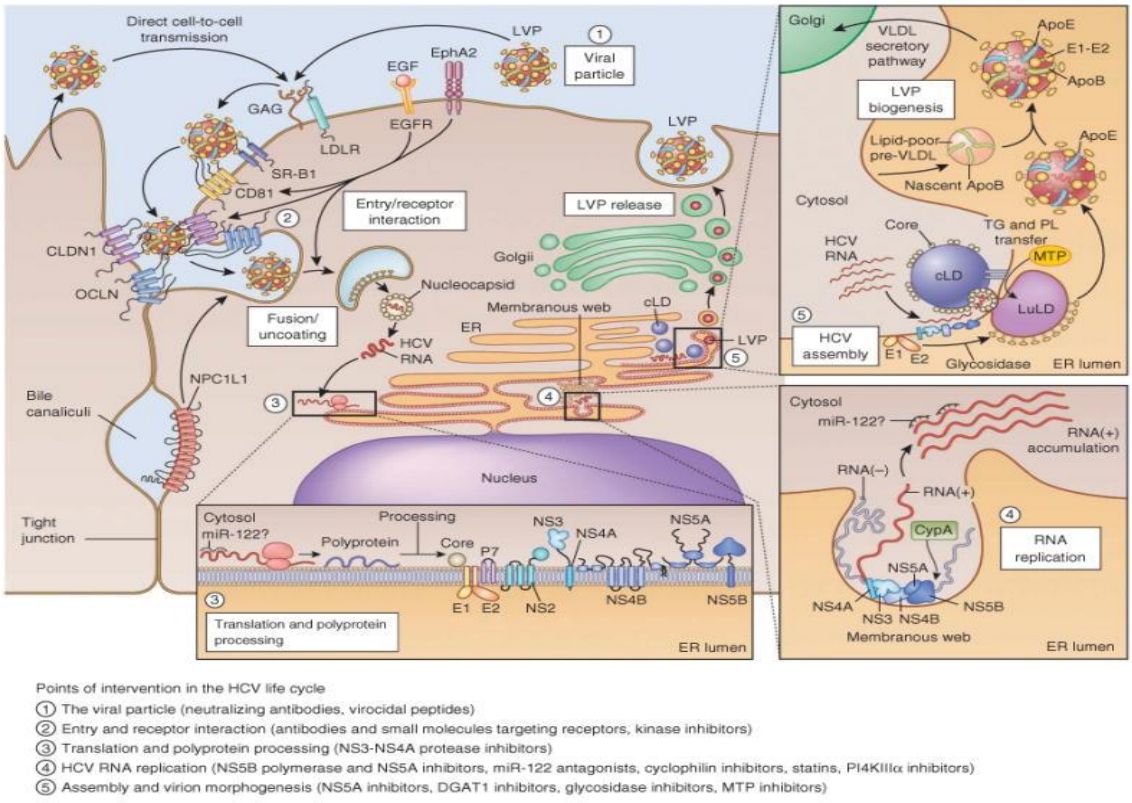
HCV virions are incorporated into lipid virus particles, together with Apoprotein E, B and C (Agnello et al. 1999; Barth 2003). The structure of the lipidviro-particles has similarities with the low density lipoprotein (LDL) and very low density lipoprotein (VLDL) (Thomssen et al. 1992; Monazahian et al. 1999). This aspect is considered as a characteristic mark of the HCV. At the hepatocytes membrane level, many receptors and proteins are involved in the entry of the virus into the cells, like: LDL-Receptor, glycosaminoglycans, Scavenger Receptor part B1(SRB1), Transpanin, CD81, Claudin-1 (CLDN1) and occluding (OCLN) (Scheel and Rice 2013). The intrusion of the virion particles into the hepatocytes is made via clathrin-mediated endocytosis, followed by an acid pH-dependent fusion and uncoating process (Blanchard et al. 2006; Tscherne et al. 2006).

The HCV genome will be released into the cytoplasm, where it will be translated. The infection with HCV might also occur transcellularly from an infected to a non-infected hepatocyte (Timpe et al. 2008).

The translation of the HCV genome takes place at the endoplasmatic reticulum level (Bartenschlager et al. 2004; Moradpour et al. 2007). This stage is initiated by the HCV IRES 5' terminal end (Hoffman und Liu 2011).

The HCV RNA replication takes place in the endoplasmatic reticulum-derived vesicles, which act as a membranous web (Romero-Brey et al. 2012). NS5B acts as RNA-dependent RNA-polymerase and together with other non-structural proteins and factors like Cyclophilin A and micro RNA (miR-122) catalyzes the HCV replication in the membranous web (Lesburg et al. 1999; Gosert et al. 2003; Lohmann 2013). After the replication, the resulted HCV genomes could be translated, replicated or incorporated into HCV particles (Miyanari et al. 2007).

The HCV assembly and release is a process that is associated with the lipid metabolism of the host cell. In the proximity of endoplasmatic reticulum, the nucleocapside is assembled, and E1, E2, P7, NS2 are attached to it (Lindenbach 2013). The nucleocapsid is transferred to the luminal lipid droplets following the VLDL pathway that will later form the lipid virus particles, which get enriched with Apo-B and Apo-E. The lipid virus particles follow then the cellular excretory pathways from Golgi (Gastaminza et al. 2008; Bartenschlager et al. 2011; Counihan et al. 2011; Coller et al. 2012) (Figure 2).



Points of intervention in the HCV life cycle
① The viral particle (neutralizing antibodies, virocidal peptides)
② Entry and receptor interaction (antibodies and small molecules targeting receptors, kinase inhibitors)
③ Translation and polyprotein processing (NS3-NS4A protease inhibitors)
④ HCV RNA replication (NS5B polymerase and NS5A inhibitors, miR-122 antagonists, cyclophilin inhibitors, statins, PI4KIIIα inhibitors)
⑤ Assembly and virion morphogenesis (NS5A inhibitors, DGAT1 inhibitors, glycosidase inhibitors, MTP inhibitors)

Figure 2 (modified from Scheel and Rice 2013 with Nature Publishing Group authorization) The HCV life cycle. Start of hepatocyte infection via interaction of extracellular HCV lipid virus particles (LVPs) (1) with cellular surface receptors (2). This procedure can also occur via cell-to-cell transmission. Fusion and uncoating of the HCV particles is pH-dependent and it is followed by translation and post-translational processes resulting in the HCV polyprotein (3). The replication takes place in the membranous web (4). HCV assembly and release follows the VLDL pathway, and LVPs are released from Golgi (Scheel and Rice 2013).

1.1.3. Hepatitis C infection symptoms and associated complications

Hepatitis C infection is usually asymptomatic. Flu-like symptoms appear only in 25% of the cases. An acute hepatitis C is not common, with about 80% becoming a chronic infection (persistence of HCV RNA and elevated ALT more than six months).

Patients with CHC infection have a higher risk of developing liver cirrhosis (20%), portal hypertension complications, liver failure and hepatocellular carcinoma (annual incidence 4-5% among the patients with CHC and liver cirrhosis) (El-Serag and Mason 1999; Di Bisceglie et al. 2003; Chen und Morgan 2006; Thomas et al. 2010).

In the USA, the annual costs of HCV therapy and HCV-infection associated complications are estimated at 5.5 billion $, most of them caused by the complications of chronic liver disease treatment and hepatocellular carcinoma treatments (Leigh et al. 2001). These facts set HCV therapy as an important player worldwide in the countries health budget. In the era of direct acting antivirals (DAA), the costs of CHC treatment are higher, but hopefully the new positive results in the elimination of the HCV will improve the overall costs of CHC patients in the health budgets (Mechie et al. 2015).

1.1.4. Hepatitis C Therapy

The therapy success is defined as a durable and sustained elimination of the virus at 24 weeks after the therapy. The elimination of the HCV is represented by undetectable levels of HCV-RNA utilizing a high sensitivity assay (limit of detection for HCV-RNA 12-15 IU/ml). A sustained virological response (SVR) is defined as undetectable HCV-RNA 24 weeks after the end of therapy and it defines a 99% of therapy success (Swain et al. 2010). A rapid virological response (RVR) is represented by an undetectable level of HCV-RNA in the first 4 weeks after initiation of therapy. A complete early virologi-

cal response is represented by the absence of HCV-RNA from serum in the first 12 weeks of the antiviral therapy. A breakthrough is represented by a 10 fold (1 log) or more rise of the HCV-RNA level under antiviral therapy or a new detection of HCV-RNA after initial success in the treatment. Non-response is represented by a decrease smaller than 10^2 until the 12^{th} week of the antiviral treatment or persistence of HCV-RNA in serum until the 24^{th}week.

In 1986, the first steps in the therapy for HCV (formally non-A-non-B associated hepatitis) were made. At this time point, the only treatment option was Interferon-α (IFN-α) (Hoofnagle et al. 1986). Even after HCV discovery IFN-α remained the therapy of choice for CHC, but only with a SVR rate of approximately 20% (Di Bisceglie et al. 1989; Causse et al. 1991). The mechanisms through which the IFN-α interacts with HCV replication are multiple. We could nominate here the suppression of the HCV translation, the degradation of the HCV-RNA, the activation of the immunological system for the recognition of the HCV-infected cells and the prevention of HCV-infection in the susceptible hepatocytes (Mihm et al. 2003; Mihm et al. 2004; Meier et al. 2008).

The most common side effects of the therapy with IFN-α are: flue-like symptoms (headaches, muscle pain, fever), hematological disorders (neutropenia, thrombocytopenia), neuropsychiatric effects (depression, irritability, mood disorder) and thyroid dysfunctions (Schaefer et al. 2005; Maan et al. 2014).

The next step in the CHC therapy was represented by the addition of RBV to the IFN-α therapy. RBV is a nucleotide analog with a large spectrum in the therapy of viral diseases. RBV is used in the therapy of both RNA and DNA viruses (Patterson and Fernandez-Larsson 1990). The clinical trials have proven that the combination of the two antiviral substances (IFN-α and RBV) had better SVR rate than IFN-α alone, becoming the standard therapy in the therapy of CHC (McHutchison et al. 1998; Reichard et al.

1998). With the combination of IFN-α and RBV a SVR rate of approximately 40% could be achieved in the cases of chronic HCV genotype 1 patients. Anemia and the teratogenic effect of RBV are one of the most common side effects of the therapy with this agent (McHutchison et al. 2006).

The next crucial development in the therapy of CHC was the introduction of Peg-IFN-α. With the attachment of the polyethylenglycol to the standard IFN-α, a much longer biological half-life was reached. For the combination of RBV and Peg-IFN-α, SVRs were reported between 45 to 80%, depending on the HCV genotype (Glue et al. 2000; Manns et al. 2001; Sulkowski et al. 2002). Another variant for the CHC therapy was the Peg-IFN-λ1. Peg-IFN-λ1 had in studies similar results to the Peg-IFN-α but with lower interferon specific side effects (Muir et al. 2010; Muir et al. 2014).

For the CHC patients, who did not respond to the antiviral treatment, therapeutic possibilities were limited to a re-exposure to the same category of therapeutics with modification of the regime of applying. These re-treatment strategies were associated with higher rates of side effects and usually with lower SVR rate (Jensen et al. 2009; Poynard et al. 2009; Mechie et al. 2015).

New therapeutic options have been recently introduced such as direct acting antiviral agents (DAAs) which specifically inactivate HCV enzymes like NS3/4A, NS5A or NS5B. In 2011 the first two DAAs have started a new era in the treatment of CHC patients. Boceprevir and Telaprevir are specific NS3/4A protease inhibitors of HCV and were approved, in combination with Peg-IFN-α and RBV for the therapy of CHC. The SVR rate was raised to approximately 80% due to the first DAAs. Recently, a variety of DAAs such as Sofosbuvir, Simeprevir, Daclatasvir etc. were approved for treatment of CHC. These agents are being used either in combination with Peg-IFN-α and/or RBV or as IFN-α-free regimes, and obtain significant better SVR rates than Peg-IFN-α and RBV

alone (Jacobson et al. 2013; Kowdley et al. 2013; Lawitz et al. 2013; Afdhal et al. 2014a; Lawitz et al. 2014a; Sulkowski et al. 2014; Lawitz et al. 2014b).

The new IFN-free therapy regimen with Ombitasvir, Paritaprevir, Ritonavir (Viekirax®) and Dasabuvir (Exviera®) shows SVR rates of more than 95% with an acceptable side effect profile. Moreover, this therapy is also approved for patients that have received a liver transplantation (Hézode et al. 2015; Klibanov et al. 2015).

The costs of the DAA based antiviral therapy are very high, for example a three months therapy costs for Sofosbuvir approximately 60,000€. These aspects represent an important limiting factor for the CHC therapy (Petta et al. 2014; Mechie et al. 2015). On the other side the approval studies were performed with highly selected patients. The limiting factor regarding the costs of the treatment regime is important due to the fact that the majority of the CHC patients is treated in developing countries. The new DAAs (Sofosbuvir, Ledispasvir, Daclatasvir or Semiprevir) are not everywhere accessible for the therapy. This is an important reason to select the patients, who could achieve an SVR with Peg-IFN and RBV, to offer an affordable CHC therapy for these patients and to avoid new side effects (Mechie et al. 2015).

1.1.5. Predictors in the therapy of Hepatitis C

In order to identify specific predictors of the SVR, several studies were conducted. The predictors, which were identified, are categorized in virus or host related factors (Asselah et al. 2010; Khattab et al. 2010; Ramcharran et al. 2010; Amanzada et al. 2012b; Amanzada et al. 2013b; Bibert et al. 2013; Prokunina-Olsson et al. 2013; Mechie et al. 2015).

1.1.5.1. Virus-related predictors

HCV genotype 1 is considered to have the lowest treatment response rate to Peg-IFN-α and RBV (≈55%) compared to the other HCV genotypes. Studies for genotype 2 and 3 reported an SVR rate of about 80% (Asselah et al. 2010).

Low pretreatment serum HCV-RNA levels (< 800 000 IU/mL) showed a positive predictive value for therapy outcome with Peg-IFN-α and RBV (Asselah et al. 2010).

RVR is the most important predictive marker in the CHC therapy. Patients who achieved virus elimination at week 4 after the initiation of the therapy have showed higher SVR rates (Martinot-Peignoux et al. 2009).

1.1.5.2. Host-related predictors

Race (Afro-Americans have lower SVR rates compared to Caucasians and people from Asia and Japan have the best SVR rates) and age (higher age is associated with lower SVR rate) are two demographical factors that are associated with therapy outcome (Asselah et al. 2010).

Conditions of insulin resistance, obesity and cirrhosis have also a negative predictive value in the outcome of CHC therapy (Asselah 2006; Khattab et al. 2010).

Drug abuse, alcohol consumes and coinfections with HIV or HBV are also associated with a lower SVR rate (Alberti 2009).

Host laboratory parameter such as: low γ-GT, high ALT activity, low pretreatment γ-GT/ALT ratio, early anemia during therapy, high LDL and low HDL levels were significantly associated with therapy outcome (Mihm et al. 1996; Mihm et al. 1999; Gopal et

al. 2006; Shiffman et al. 2007; Economou et al. 2008; Harrison et al. 2010; Weich et al. 2011; Amanzada et al. 2012a).

In 2009, three genome-wide association studies identified polymorphisms in the neighborhood of IFN-λ3 gene, on chromosome 19, to be highly associated with therapy success in CHC patients infected with HCV genotype 1, who received an antiviral therapy consisting of Peg-IFN-α and RBV (Ge et al. 2009; Suppiah et al. 2009; Tanaka et al. 2009). IFN-λ3 rs12979860 is the most investigated polymorphism of IFN-λ3 in the western world. The molecular and immunological mechanism between the IFN-λ3 and HCV is still unclear. In 2013 a dinucleotide polymorphism ss469415590 (TT/ΔG) has been proven to create IFN-λ4 (ΔG). The absence of IFN-λ4 (TT) has been shown to be a better genetic predictor than IFN-λ3 for the success of the therapy with Peg-IFN-α and RBV (Amanzada et al. 2013a; Bibert et al. 2013; Prokunina-Olsson et al. 2013). IFN-λ3 polymorphism is recommended by The American Association for the Study of Liver Diseases, as an important predictive marker for the antiviral treatment with Peg-IFN-α and RBV or in combination with DAA. Recently we could show that the CC genotype of IFN-λ3 rs12979860 has a predictive value also in the combination of Peg-IFN-α, RBV and a DAA (Mechie et al. 2014). However, the IFN-λ3 predictive value is smaller in the case of the triple therapy (Peg-INF-α, RBV and a DAA) than in the case of Peg-IFN-α and RBV alone. The predictive value of IFN-λ3 is also variable between the DAAs (Mechie et al. 2014). Furthermore, in studies that investigated interferon-free based therapy regimens, the IFN-λ3 rs12979860 CC genotype was found to be correlated with a higher reduction of viral load (Chu et al. 2012).

1.2. Vitamin B_{12}

1.2.1. Vitamin B_{12}- physiology and pathophysiology

Vitamin B_{12} is one of the water soluble vitamins, which is also named cobalamine. Humans are not capable to synthesize Vitamin B_{12} and its only source is represented by meat and dairy products.

The Vitamin B_{12} chemical structure consists of a central cobalt in a tetra-pyrrole-ring (corrin-ring) (Figure 3).

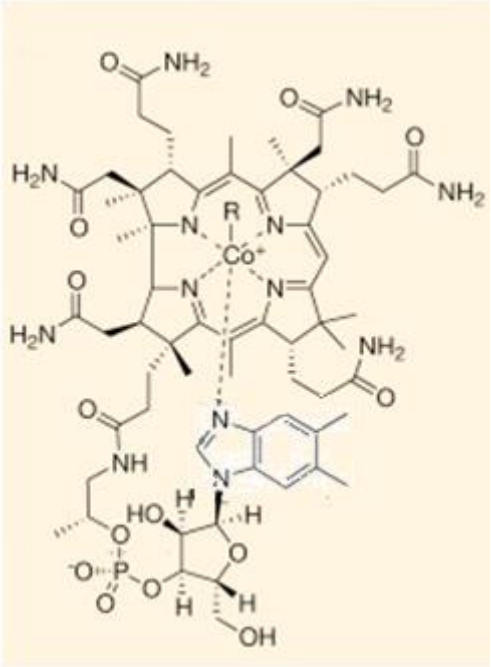


Figure 3. Chemical structure of Vitamin B_{12}, modified after Degnan et al. with permission from Elsevier (Degnan et al. 2014).

The recommended dietary intake for Vitamin B_{12} is for adults 2 µg/d and 2.6 µg/d for pregnant women. A normal diet provides a daily intake of approximately 5-7 µg. The Vitamin B_{12} reserves are estimated at 2 to 5 mg (Green and Kinsella 1995). A low consumption of Vitamin B_{12} leads therefore to Vitamin B_{12} deficiency years after stopping an adequate alimentary intake of Vitamin B_{12}. The excess of Vitamin B_{12} is mainly

eliminated through bile (part being reabsorbed by enterohepatic circulation) (Reizenstein 1959; Adams 1970; el Kholty et al. 1991).

Vitamin B_{12} is liberated in the stomach from the binding protein by the low pH of the gastric acid. R-Protein, which is secreted by the saliva, binds to Vitamin B_{12} in the stomach to prevent degradation of Vitamin B_{12} due to the acid milieu. In the duodenum it comes in contact with an alkaline milieu and with the pancreatic enzyme that releases Vitamin B_{12} from R-protein. Vitamin B_{12} combines with Intrinsic Factor (IF), a protein that is secreted in the stomach by the parietal cells, creating the Vitamin-B_{12}-IF-complex. In the terminal ileum, the Vitamin-B_{12}-IF-complex is recognized by the IF-receptor and the absorption of Vitamin B_{12} takes place. Vitamin B_{12} is absorbed into the portal system and is transferred to Transcobalamin II (TCII). The TCII-Vitamin B_{12}-complex binds to a receptor and is transferred intracellular via endocytosis, where TCII undergoes a lysosom-associated degradation following the liberation of Vitamin B_{12} into the cytoplasm (Tefferi and Pruthi 1994; Guyton and Hall 2006).

Vitamin B_{12} is physiologically stored in the liver tissue. Vitamin B_{12} deficiency could appear in several liver diseases such as: cirrhosis, hepatocellular carcinoma, hepatitis and liver metastasis (Nicolas and Guéant 1994; Mechie et al. 2015). This could be explained by the impaired liver storage, consequent to the high release during hepatic cytolysis or decreased clearance in the affected liver.

Vitamin B_{12} is metabolically involved, especially in the DNA synthesis, a fact that it confers a key role in the cellular metabolism (Guyton and Hall 2006).

Vitamin B_{12} deficiency is associated with hematological manifestations like macrocytic anemia, pernicious anemia or neurological manifestations (dementia, progressive weakness, ataxia, paraesthesia) (Hemmer et al. 1998).

Vegan patients (intake deficiency), patients on which a total gastrectomy, stomach by-pass or terminal ileum resection was performed (absorption deficiency), or with nitric oxide exposure (inactivation) are at risk to develop Vitamin B_{12} deficiency (Antony 2003; Balcı et al. 2014).

High levels of serum Vitamin B_{12} were described in a series of diseases like: hypereosinophilic syndrome, myeloproliferative neoplasm, multiple myeloma (due to elevated TC levels of raised production of granulocytes and their precursors), liver diseases (cirrhosis, hepatitis, malignancies) due to impaired storage or decrease Vitamin B_{12} clearance, renal failure, inflammatory diseases (rheumatoid arthritis, systemic lupus reumatoides) (Andres et al. 2013).

1.2.2. Vitamin B_{12} and Hepatitis C Virus

Several studies have proven, in vitro conditions, that Vitamin B_{12} inhibits HCV IRES-dependent translation. It is supposed that the inhibition process is due to a direct interaction between Vitamin B_{12} and HCV IRES RNA (Lott et al. 2001; Takyar et al. 2002). Li et al. demonstrated that HCV IRES domain IV (Figure 4) is responsible for the inhibitory effect of Vitamin B_{12} for HCV translation (Li et al. 2004).

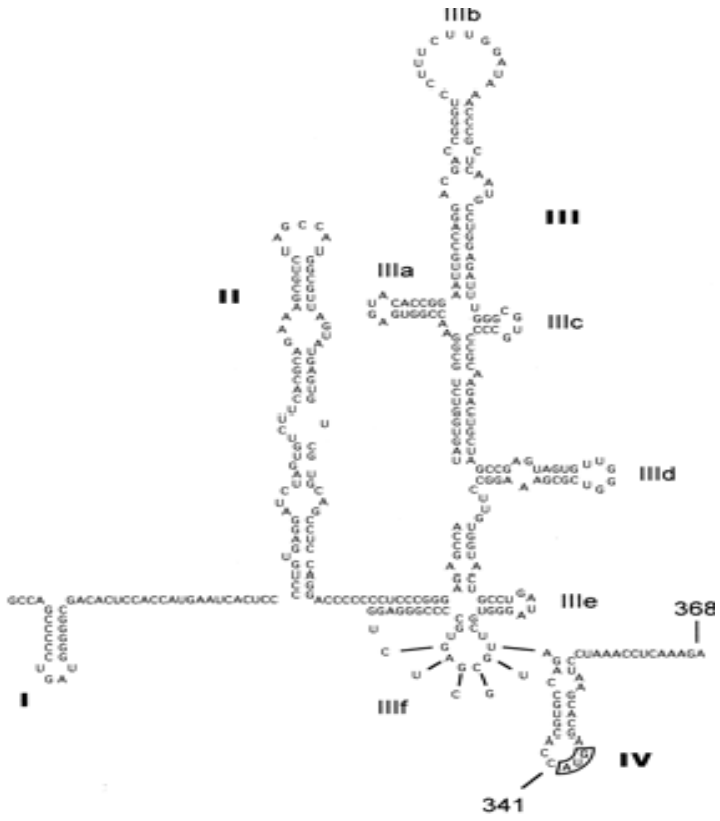


Figure 4. The Secondary structure of the HCV IRES, adapted from Honda et al. (Honda et al. 1996; Lott et al. 2001). © 2001National Academy of Sciences.

The main element of the HCV IRES is domain III, which during translation is attached to the 40S ribosomal subunit. We know that Vitamin B_{12} allows the assembling of the translation complex between the 80S ribosomal subunit and HCV IRES, but seems to stop the translation process (Takyar et al. 2002). As the hepatocytes represent the natural reservoir for Vitamin B_{12}, having high intracellular Vitamin B_{12} levels, the inhibition effect of Vitamin B_{12} on the HCV translation could be one of the mechanisms to which HCV became adapted causing chronic infection and very rare acute hepatitis (Li et al. 2004). Due to the short half-life (few hours) of the HCV virions, HCV-RNA is a good parameter for the quantification of the HCV replication. Elevated serum Vitamin B_{12} levels were shown to be associated with high viral load in CHC patients, a fact that sug-

gests a biological significance and interaction between Vitamin B_{12} in HCV life cycle (Lott et al. 2001). Only one study suggested that serum Vitamin B_{12} levels are associated with end-of-treatment response in CHC patients (Rosenberg and Hagen 2011). An open-label pilot study demonstrated that supplementation of Vitamin B_{12} to the therapy with Peg-IFN-α and RBV increases the SVR rates in treatment naïve CHC patients (Rocco et al. 2013).

1.3. Aim

The aim of this study was (I) to determine the relation between serum Vitamin B_{12} levels with regard to serum HCV-RNA, clinical and histological characteristics of CHC patients and (II) to evaluate its predictive value for treatment outcome in CHC patients that received an antiviral combination therapy with Peg-IFN-α and RBV (Mechie et al. 2015).

2. MATERIALS AND METHODS

2.1. Patients

The demographical, laboratory and histological data from a total of 116 CHC genotype 1-infected patients was analyzed in this study. All patients were from Germany, of Caucasian ethnicity and treated as out-patients in the Department of Gastroenterology at the University Medical Center Göttingen (UMG). CHC was regarded by the presence of HCV RNA in patients' sera for at least six months, the presence of anti-HCV antibodies and biochemical and/or histological signs of liver inflammation. Liver biopsy samples were acquired using standard techniques, ultrasound guided biopsy or mini laparoscopy, as a part of a routine clinical evaluation (Mechie et al. 2015). After taking liver biopsy, liver samples were fixed in formalin and embedded in paraffin. The necroinflammatory activity was graded according to Desmet et al. (grading score 1-3).The staging is represented by the grade of fibrosis or cirrhosis and was graded according to Desmet et al.: no fibrosis (score 0), mild (score 1, periportal fibrous expansion), moderate (score 2, portal-portal septa), marked (score 3, bridging fibrosis/ portocentral septa), or cirrhosis (score 4) (Desmet et al. 1994). The histopathological evaluation was performed without knowledge of patient clinical and biochemical parameters at the Institute of Pathology UMG, http://www.pathologie-umg.de.

All 116 patients with HCV genotype 1 received an antiviral combination therapy consisting of Peg-IFN-α and RBV. The patients were followed up during the therapy at the Department of Gastroenterology and Endocrinology, UMG (Mechie et al. 2015).

Exclusion criteria comprised: active HBV infection, HIV coinfection, continued alcohol or intravenous drug abuse, or immunosuppressive medication. None of the patients included in the present study had diseases that could be associated with high serum Vita-

min B_{12} levels like myeloproliferative disorders, acute fulminate hepatitis, hypereosinophilic syndrome or a renal failure (Mechie et al. 2015).

In order to participate in the study, all patients gave written informed consent in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. The study was initially approved by the ethics committee of the University Medical Center with the number 4/8/93 with a subsequent amendment from 2010. This dissertation was approved with the reference number DOK_101_2014.

Treatment was performed according to standard therapy regimens at that time: with a combination of Peg-IFN-α_{2b} in a dose of 1.5 µg/kg body weight and weight-based RBV (800 to 1400 mg per day) or a combination of 180 µg Peg-IFN-α_{2a} with weight-based RBV (1000 or 1200 mg per day). Depending upon the presence of the side effects and viral dynamic, both the dose and duration of therapy were adjusted. Viral load was monitored monthly, and achievement of RVR or SVR was documented (Mechie et al. 2015).

2.2. Laboratory analysis

The following laboratory analyses were performed using automated systems in the Central Laboratory at the Department of Clinical Chemistry, UMG: aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, γ-glutamyl-transferase (γ-GT) activity and baseline serum Vitamin B_{12} concentration (Mechie et al. 2015).

Serologic parameters of viral hepatitis due to HBV or HCV infection (anti-HBs, anti-HBc and anti-HBe antibodies, HBs antigen, anti-HCV antibodies) were determined in the Department's Virologic and Molecular Diagnostics, UMG.

2.3. Isolation of genomic DNA and IFN-λ3 rs12979860 single nucleotide polymorphism (SNP) genotyping

Isolation of genomic DNA and IFN-λ3 rs12979860 SNP genotyping were conducted as part of the Department's Virologic and Molecular Diagnostics at UMG as described previously by Amanzada et al. 2012b.

Peripheral blood mononuclear cells were taken as a source for the isolation of genomic DNA using the QIAamp DNA Mini Kit and following the manufacturer's protocol (Qiagen). Absorbance was measured at 260 and 280 nm to determine concentration and purity of the isolated nucleic acids. In order to assess the integrity of genomic DNA, agarose gel electrophoresis was applied. DNA extraction was performed with QIAamp DNA Blood Midi Kit from 2 mL serum when insufficient sanguine probes were available (Amanzada et al. 2012b).

Isolated DNA was amplified by real-time PCR using TaqMan Universal Master Mix (Applied Biosystems, Darmstadt, Germany). The primers used for IFN-λ3 rs12979860 SNP genotyping were: forward, 5′-GCCTGTCGTGTACTGAACCA-3′ and reverse 5′-GCGCGGAGTGCAATTCAAC-3′. Allelic discrimination of IFN-λ3 was performed with differentially fluorescent dye-labeled allele-specific minor groove binder probes for IFN-λ3 rs12979860 (VIC, 5′-TGGTTCGCGCCTTC-3′; FAM, 5′-CTGGTTCACGCCTTC-3′). Reactions and analyses were performed with ABI prism StepOne plus (Applied Biosystems) according to the suppliers' instructions as described previously by Amanzada et al. 2012b.

2.4. Detection and determination of serum HCV-specific RNA and HCV genotype

The determination of the serum HCV-specific RNA and HCV genotype was performed by the Department's Virologic and Molecular Diagnostic Unit as described previously by Amanzada et al. 2012b and by Mihm et al.1996.

Serum HCV-specific RNA was determined utilizing a nested RT-PCR approach; HCV genotypes were determined using a line-probe assay as described (Mihm et al. 1996; Amanzada et al. 2012b).

Serum samples (140 µL) were used in order to extract the HCV RNA. To perform this process we have used the QIAamp Viral RNA Kit (Qiagen, Hilden, Germany) according to the manufacturers' protocol. With the extracted HCV RNA a nested RT-PCR procedure was performed, using 2 tagged RT-PCR, as described by Mihm et al. 1996. To quantify the virus in human sera, an Abbott real-time HCV assay was used, with a detection limit of 12 IU/mL (Amanzada et al. 2012b).

In order to determine HCV genotypes, the Innolipa HCV II line probe assay (Innogenetics, Ghent, Belgium) was applied, according to the manufacturer's protocol as described previously by Amanzada et al. 2012b.

2.5. Statistical analyses

Logistic regression models were used to determine the association between serum Vitamin B_{12} levels and the continuous and dichotomous variables. The univariate and multivariate analyses were performed in R Program by A. D. Goralzcyk**.** In the first step a univariate analysis was performed for all variables. For the ones, which were found significant in the univariate analysis ($p< 0.05$), a multivariate analysis was conducted. A backward selection, with a p-value > 0.10 for the elimination from the statistical model was applied in the multivariate analysis. Quartiles and interquartile range were used be-

cause data regarding serum Vitamin B_{12} were found to be skewed. Spearman's correlation was used to determine the association between the continuous variables. By introducing different sets of independent variables into the regression models, best predictive formulas were generated for cirrhosis or marked fibrosis. Wilcoxon Mann-Whitney, χ^2 and Fisher's exact tests were used to compare between the variables with an SVR or without. The statistical analyses were made using the R program (http://www.r-project.org) and logistic regression calculators (http://statpages.org/logistic.html). In this study a p-value<0.05 was considered to be statistically significant. Hardy–Weinberg equilibrium calculations (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) were used for IFN-λ3 rs12979860 genotype distribution. GraphPad Prism 5 was used to create and calculate the receiver operating characteristics (ROC) curve and the area under the receiver operating characteristics (AUROC) parameters. The graphics were made using GraphPad Prism 5 and Microsoft Office Excel (Mechie et al. 2015).

3. RESULTS

3.1. Descriptive statistics

A total of 116 patients chronically infected with HCV genotype 1 were included into this study (Table 1). All patients were treatment naïve to Peg-IFN-α and RBV. 48 patients were female and 68 patients were male (Figure 5) with a median age of 51 years, and a minimum of 22 years and a maximum of 80 years (Table 1). The age distribution of the patients had a peak in the 6th decade, followed by the 5th and 7th one (Figure 6) (Mechie et al. 2015).

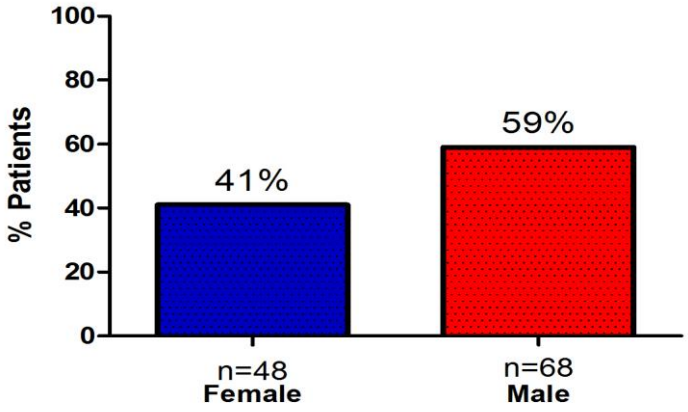


Figure 5. Gender proportions of the included patients.

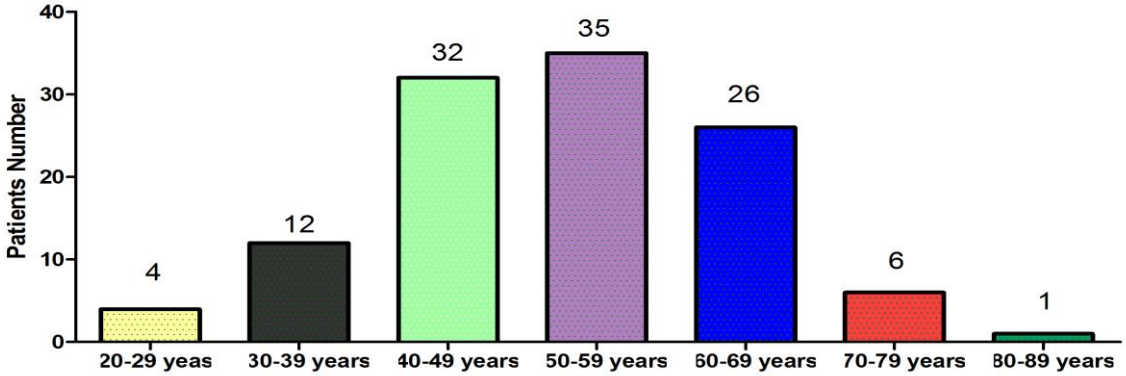


Figure 6. Age distribution of the patients included in this study on decades.

With regard to HCV subgenotype, 67% of the patients had the subtype 1b, 29% subtype 1a and 4% had a coinfection with both subtypes 1a and 1b (Figure 7).

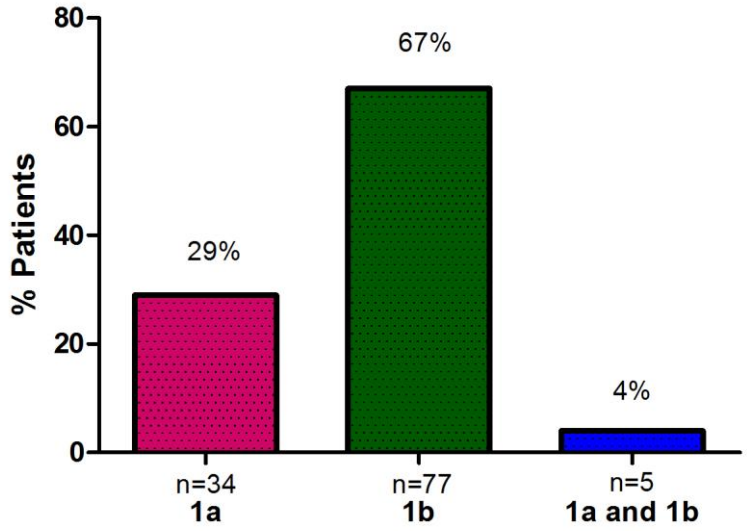


Figure 7. Subgenotype distribution of HCV genotype 1

The baseline median activity of AST was of 44 U/L with an IQR of 32-73 U/L. Baseline median ALT activity was 51 U/L with an IQR of 32-93 U/L. Both median transaminase activities were elevated when compared to the upper normal limits referenced by the UMG Diagnostic Facility Clinical Laboratory of the University Medical Center of Göttingen. γ-GT had a median baseline enzymatic activity of 50 U/L with an IQR of 28-100 U/L, which was also elevated when compared to the laboratory limits. Regarding Vitamin B_{12} serum concentration the baseline median was at 488 ng/L with an IQR of 339-727 ng/L. The median baseline level of HCV-RNA was 1.8 x 10^6 copies/ml with an IQR of $4.5x10^5$-$6.2x10^6$copies/ml. Patient characteristics are summarized in Table 1 (Mechie et al. 2015).

Table 1: Patient baseline characteristics

Age [median (range)] years	51 (22-80)
HCV-RNA level [median (IQR)] copies/mL	$1.8x10^6$ ($4.5x10^5$-$6.2x10^6$)
AST [median (IQR)] U/L	44 (32-73)
ALT [median (IQR)] U/L	51 (32-93)
γ-GT [median (IQR)] U/L	50 (28-100)
Vitamin B_{12} [median concentration (IQR)] ng/L	488 (339-727)
Hepatitis activity n (%) mild moderate/severe	 79 (68%) 37 (32%)
Fibrosis n (%) absent/mild/moderate severe/cirrhosis	 89 (77%) 27 (23%)
Steatosis n (%) 0%-5% 6%-100% Missing	 58 (50%) 57 (49%) 1 (1%)
IFN-λ3 rs12979860 genotypes n (%) CC CT TT Missing	 44 (38%) 54 (47%) 14 (12%) 4 (3%)
Laboratory data are presented as median (IQR); number of cases are given in total and as a percentage; baseline serum Vitamin B_{12} concentrations were available for 107 patients (Mechie et al. 2015).	

A baseline histological evaluation was available for all patients. 79 patients (68%) had mild hepatitis activity, and 37 patients (32%) had moderate to severe hepatitis. Regarding the fibrosis stage, 89 patients (77%) had absent, mild or moderate fibrosis and 27 patients (23%) had advanced fibrosis or cirrhosis. 58 (50%) patients had no signs of hepatic steatosis, 57 patients (49%) had hepatic steatosis, for 1 patient the hepatic steatosis grade was not determined in the histological evaluation (Mechie et al. 2015).

IFN-λ3 rs12979860 genotyping showed a genotype distribution of 44:54:14 patients for CC:CT:TT genotypes respectively, with a minor allele frequency of 0.37 (Figure 8).

The IFN-λ3 rs12979860 genotype distribution met the Hardy–Weinberg equilibrium with p=0.64 (Mechie et al. 2015).

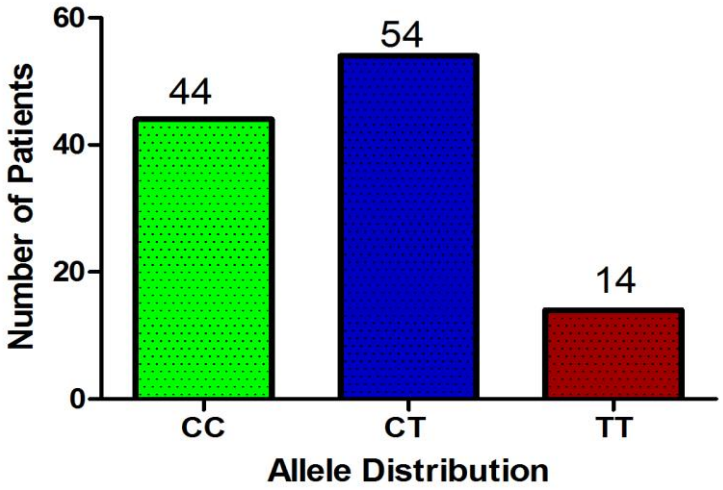


Figure 8. The distribution of IFN-λ3 rs12979860 genotypes

3.2. Quartile of baseline serum Vitamin B_{12} levels with regard to treatment response, laboratory, histological and IFN-λ3 rs12979860 genotypes

Baseline serum Vitamin B_{12} levels had a median value of 488 ng/L, with an IQR of 339-727 ng/L (Table 1). The minimum level of Vitamin B_{12} was 169 ng/L and the maximum at 2222 ng/L (Mechie et al. 2015).

Patients who had successfully resolved chronic infection, i.e. those with a SVR, featured a lower median baseline serum Vitamin B_{12} concentration, 333 ng/L, than patients with a non-sustained virological response (NVR) with a median concentration of 616 ng/L. The difference between Vitamin B_{12} serum baseline levels in responding and non-responding patients was statistically significant ($p<0.0001$) (Figure 9) (Mechie et al. 2015).

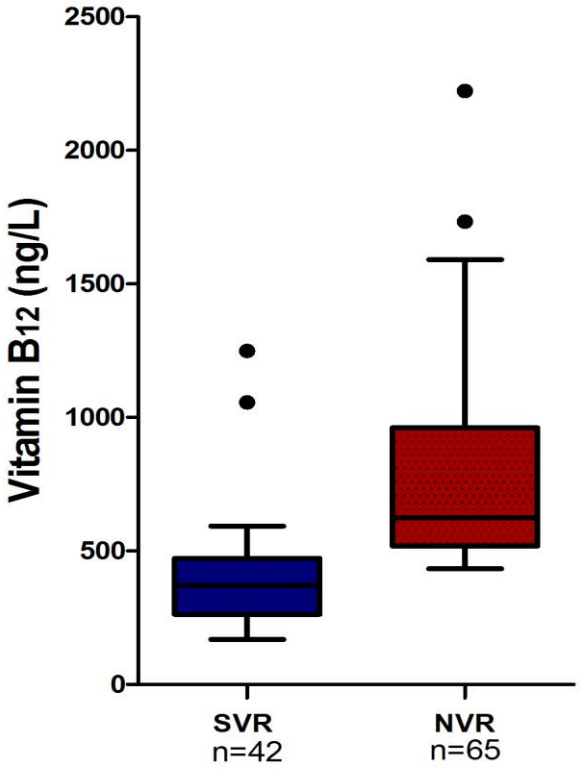


Figure 9. Association of baseline serum Vitamin B_{12} concentration and responsiveness to antiviral therapy.

Table 2 presents an analysis on the association between baseline serum Vitamin B_{12} concentration and several clinical, laboratory, histopatological and genetic variables. Baseline serum Vitamin B_{12} concentrations were classified into quartiles. Low baseline serum Vitamin B_{12} levels were associated with achieving a RVR ($p=0.001$) or a SVR ($p<0.001$), lower stages of fibrosis ($p=0.0001$), low serum activities of AST ($p=0.002$) and ALT ($p=0.04$), and the favorable IFN-λ3 rs12979860 genotype CC ($p=0.04$). Baseline levels of Vitamin B_{12} were not found to be associated with patient age, HCV subgenotypes, serum γ-GT activity, hepatitis activity and hepatic steatosis (Mechie et al. 2015).

Table2: Vitamin B_{12} quartile

Characteristics	<340 (n=27)	340-488 (n=27)	488-727 (n=26)	>727 (n=27)	*P* value
Sex [female/male] n	8/19	7/20	13/13	14/13	0.29
Age[median(range)]years	47 (23-77)	53 (22-70)	51 (32-73)	51 (23-71)	0.74
HCV [subtype] n					0.55
1a	8	12	7	6	
1b	18	15	18	19	
1a+b	1	0	1	2	
RVR n (%)	19 (70%)	12 (44%)	9 (35%)	5 (19%)	0.001
SVR n (%)	22 (81%)	12 (44%)	7 (27%)	2 (7%)	<0.001
AST [median (IQR)] U/L	39 (30-54)	42 (32-51)	45 (36-77)	73 (53-121)	0.002
ALT [median (IQR)] U/L	46 (24-94)	44 (27-64)	55 (36-85)	66 (49-150)	0.04
γ-GT [median (IQR)] U/L	38 (28-87)	52 (24-103)	63 (41-136)	68 (28-142)	0.12
Hepatitis activity n (%)					0.58
mild	21 (78%)	18 (67%)	19 (73%)	15 (56%)	
moderate/severe	6 (22%)	9 (33%)	7 (27%)	12 (44%)	
Fibrosis n (%)					0.0001
absent/mild/moderate	26 (96%)	22 (81%)	22 (85%)	11 (41%)	
severe/cirrhosis	1 (4%)	5 (19%)	4 (15%)	16 (59%)	
Steatosis grade n (%)					0.26
0%-5%	21 (78%)	19 (70%)	19 (73%)	15 (56%)	
6%-100%	6 (22%)	7 (26%)	7 (27%)	12 (44%)	
Missing	0	1 (4%)	0	0	
IFN-λ3 rs12979860 n (%)					0.04
CC	16 (60%)	11(41%)	5(19%)	9(33%)	
CT	8(28%)	15(55%)	15(58%)	12(44%)	
TT	2(8%)	1(4%)	5(19%)	5(19%)	
missing	1(4%)	0	1(4%)	1(4%)	

Baseline serum Vitamin B_{12} levels and the baseline viral load were positively and significantly correlated ($p<0.0001$, $r=0.46$) (Figure 10) (Mechie et al. 2015).

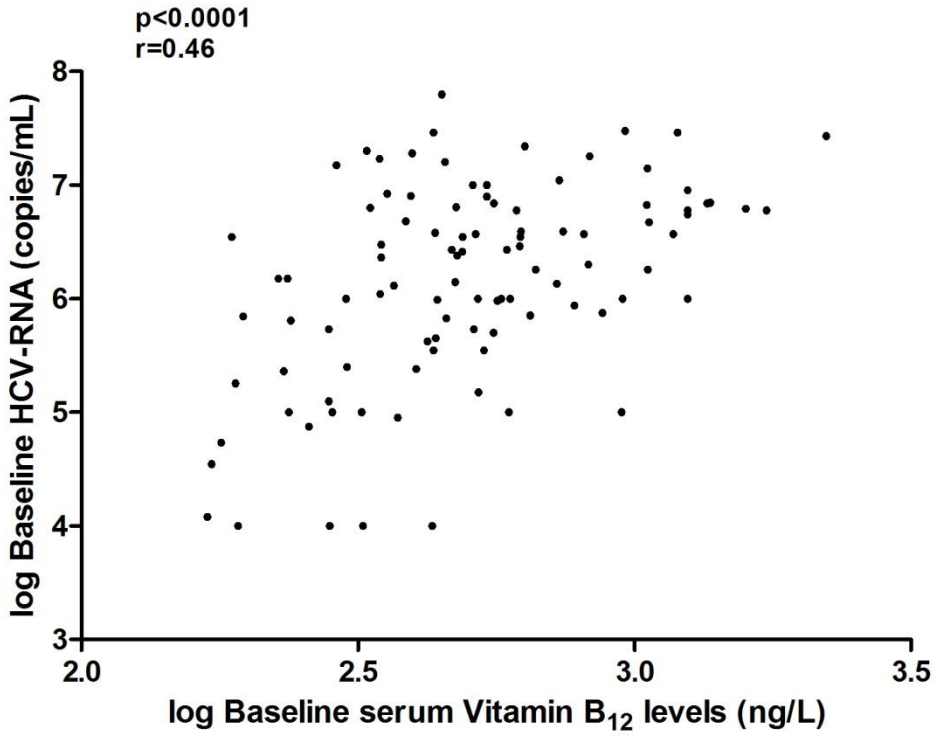


Figure 10. Spearman correlation analysis between serum baseline Vitamin B_{12} concentration and viral load (Mechie et al. 2015).

3.3. Treatment response with regard to baseline serum Vitamin B_{12} levels, histological features, baseline and on-treatment HCV-RNA levels and IFN-λ3 rs12979860 genotypes

The overall SVR rate among CHC genotype 1 patients was 41% (n=48) (Figure 11). We conducted a univariate analysis and found the following variables to be statistically associated with SVR: low baseline serum Vitamin B_{12} levels ($p<0.001$), RVR ($p<0.001$), low baseline viral load ($p<0.001$), IFN-λ3 rs12979860 CC genotype ($p=0.0001$), low stage of fibrosis ($p=0.01$) and low degree of steatosis ($p=0.02$) (Table 3). A multivariate

analysis revealed all these variables to be significantly and independently correlated with SVR (Table 3). Age and hepatitis activity were not related to treatment response (Mechie et al. 2015).

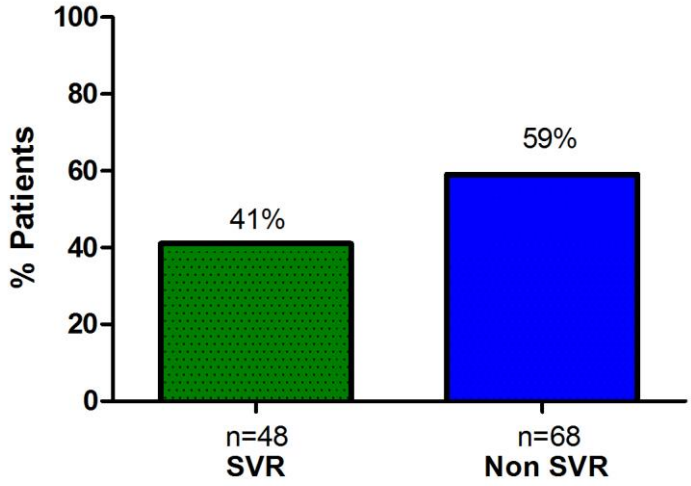


Figure 11. SVR rate in patients with HCV genotype 1 treated with Peg-IFN-α and RBV

Table 3: Uni- and multivariate analysis of factors associated with treatment response

		Univariate Analysis	Multivariate Analysis
		P value	*P* value
Male sex n (%)	68 (59%)	0.07	0.19
Age [median (range)]	51(22-80)	0.28	
RVR n (%)	40 (82%)	<0.001	<0.001
Vitamin B_{12}[median (IQR)] ng/L	488 (339-727)	<0.001	<0.001
HCV RNA level [median (IQR)]copies/mL	$1.8x10^6$ ($4.5x10^5$-$6.2x10^6$)	<0.001	<0.05
IFN-λ3 rs12979860 CC n (%)	28 (64%)	0.0001	<0.001
Hepatitis activity n (%) mild	79 (68%)	0.70	
Fibrosis n (%) Absent/mild/moderate	89 (77%)	0.01	<0.05
Steatosis n (%) 0-5%	58 (50%)	0.02	0.01
RVR: rapid virological response (Mechie et al. 2015)			

A cut-off value for serum Vitamin B_{12} of 570 ng/L was calculated using a ROC analysis with an AUROC of 0.83 (Std. Error 0.04, CI 0.75-9.91, p< 0.0001) (Figure 12). The cut-off level for serum Vitamin B_{12} of 570 ng/L showed a good sensitivity of 91% and an appropriate specificity of 58%. The positive and negative predictive values (PPV and NPV) were 59% and 90%, respectively (Mechie et al. 2015).

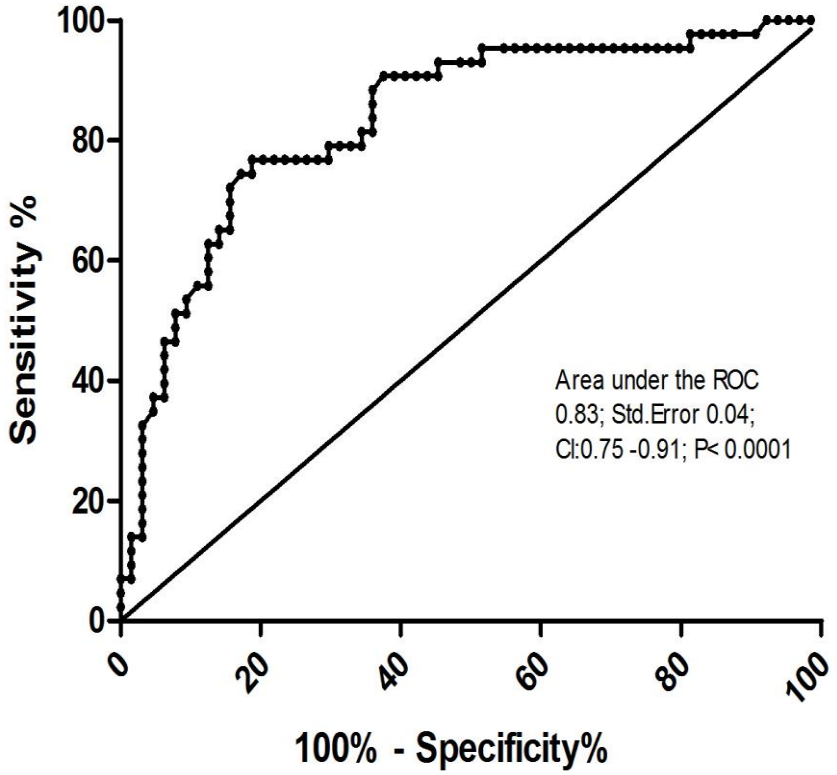


Figure 12. ROC curve of baseline serum Vitamin B_{12} levels and relation between sensitivity and specificity for baseline serum Vitamin B_{12} levels and SVR (Mechie et al. 2015)

The patients who had baseline serum Vitamin B_{12} levels below the cut-off level of 570 ng/L achieved an HCV therapy success rate of 59% and had an OR of 13.4 (CI: 4.3-41.9, p<0.0001) in comparison to the ones with levels above this cut-off value who had an SVR rate of only 10% (Figure 13) (Mechie et al. 2015).

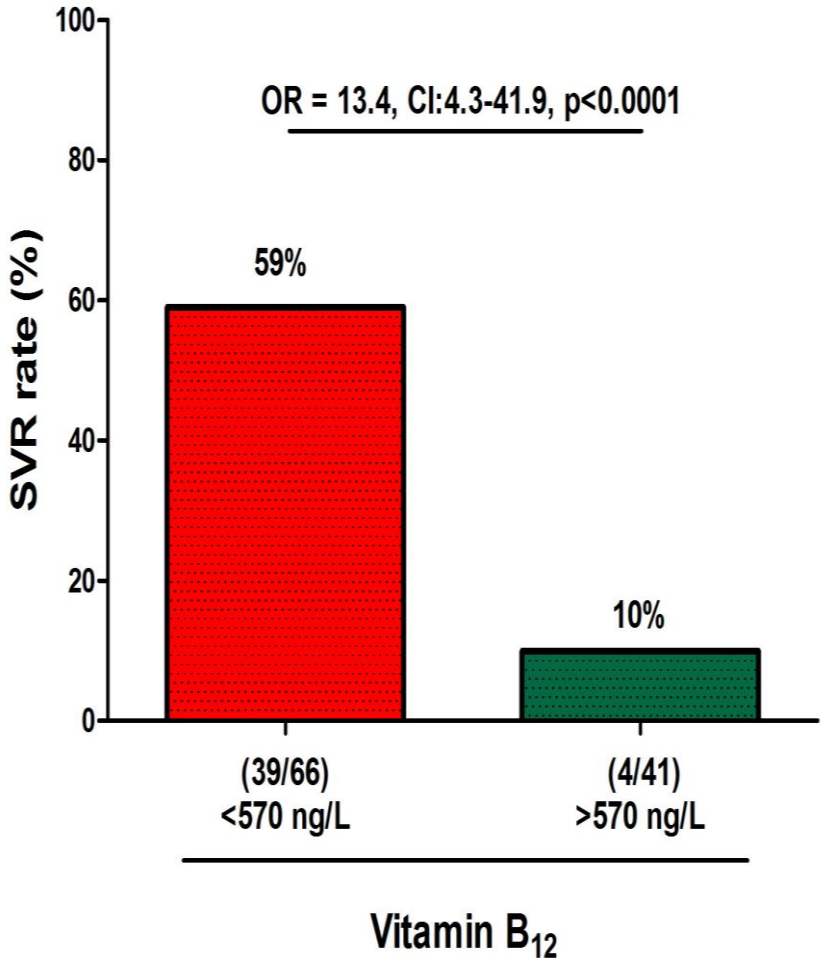


Figure 13. Relation between baseline serum Vitamin B_{12} levels using a cut-off value of 570 ng/L and the SVR rates. In brackets: number of patients with SVR/total number of patients in the respective group (Mechie et al. 2015)

By combining the baseline serum Vitamin B_{12} 570ng/L cut-off value and IFN-λ3 rs12979860 SNP genotype, patients who had baseline serum Vitamin B_{12} levels below this cut-off value and CC genotype reached an SVR rate of 80% with an OR of 54 (CI: 9.9-293, p<0.0001) in comparison to the ones with baseline serum Vitamin B_{12} above the cut-off level and having the CT or TT genotype (Figure 14) (Mechie et al. 2015).

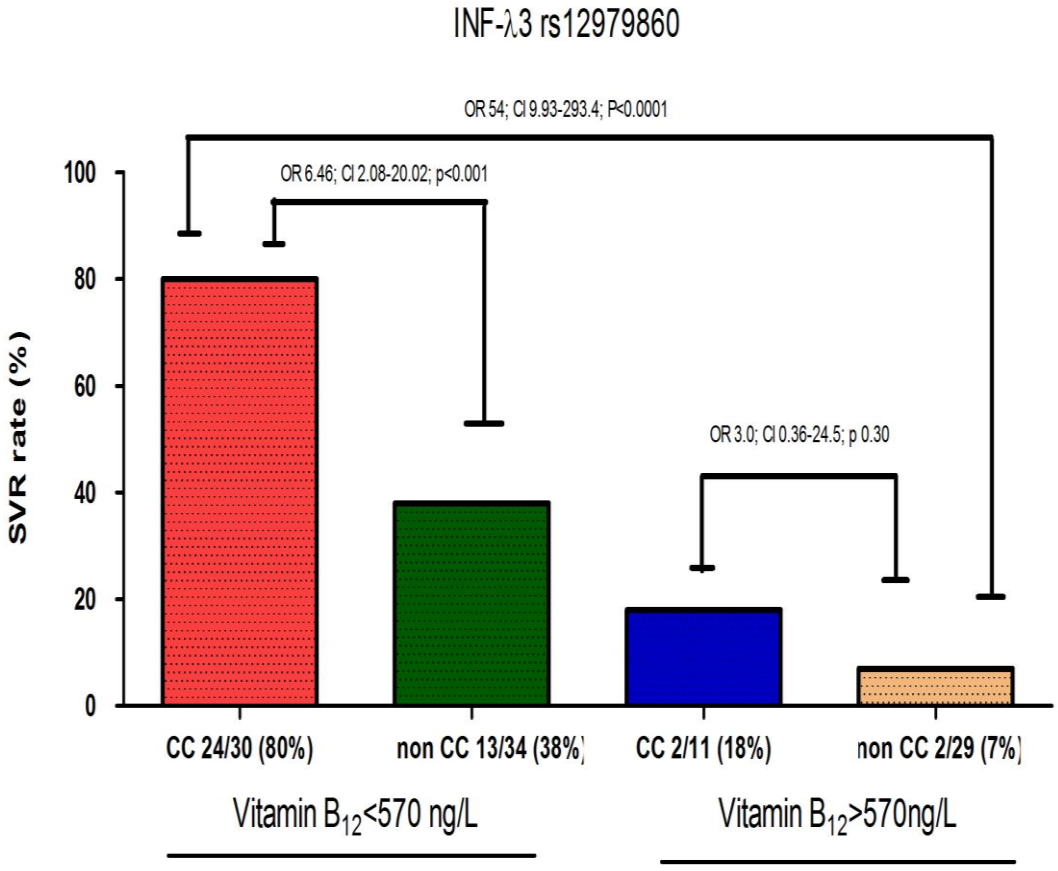


Figure 14. Relation between baseline serum Vitamin B_{12} levels using a cut-off value of 570 ng/L and IFN-λ3 rs12979860 genotypes with respect to SVR. In brackets are shown the number of patients with SVR/total number of patients in the respective group (Mechie et al. 2015)

4. DISCUSSION

The CHC therapy was recently revolutionized, notably due to the entrance of the new DAAs such as Sofosbuvir, Semiprevir, Daclatasvir, Ledipasvir, Ombitasvir, Paritaprevir or Dasabuvir. CHC patients who were treated with the new IFN-free regime achieve SVR rates above 90%-95% (Forns et al. 2014; Sulkowski et al. 2014; Afdhal et al. 2014b). Regarding higher SVR rates for these patients, there is not necessarily a need for new predictors for CHC therapy. Nevertheless, the majority of CHC patients are living in developing countries. The budget of these countries usually does not permit such an expensive therapy, which, from a financial aspect represents a limiting factor of the new CHC therapies. Considering these aspects only a fraction of the CHC patients would have access to a DAA-based therapy. Therefore, these patients might receive a CHC therapy with Peg-IFN-α and RBV, which has a lower SVR rate and may generate serious side effects. Regarding these aspects, in order to predict the highest SVR probability, to avoid unnecessary therapy costs and side effects in the era of individualized medicine, more predictive factors have to be evaluated and confirmed in studies (Mechie et al. 2015).

Liver fibrosis is a histopathological statement, with a huge impact on the therapy that requires a liver specimen, invasively obtained with a percutaneous liver puncture, laparoscopy, mini-laparoscopy or transvenous liver biopsy (Denzer et al. 2007). These procedures can be associated with interventional complications such as post-interventional bleeding, organ perforation, pain and even death of the patients. They have also limitations in patients with impaired coagulation, obesity, post-interventional peritoneal adherences (Hilgard et al. 2014). Concerning these aspects the evaluation of new non-

invasive biomarkers for liver fibrosis as well as for therapeutic outcome plays a key role in the therapy of patients with liver diseases.

There are a number of demographics (black race, older age), laboratory (low λ-GT, high ALT activity, low λ-GT/ALT ratio, anemia under the therapy), histological stage (fibrosis grade, presence of steatosis hepatitis), genetical (IFN-λ3, IFN-λ4) and virological (genotype, baseline HCV RNA level, RVR) predictors for treatment outcome of CHC patients treated with a combination therapy consisting of Peg-IFN-α and RBV (Kau et al. 2008; Ge et al. 2009; Amanzada et al. 2012a; Amanzada et al. 2013b) or with Peg-IFN-α, RBV and one of the first-generation protease inhibitors (Mechie et al. 2014). These factors could predict very accurately an individuals' chance to achieve a SVR. By combining independent predictors, a better prediction can be achieved. One example for this is the combination of IFN-λ3 rs12979860 genotypes and the ratio of γ-GT/ALT. In this case, those patients with the IFN-λ3rs12979860 CC allele and low γ-GT/ALT quotient have a 26-fold higher chance to obtain SVR under therapy with Peg-IFN-α and RBV than patients with non-CC genotype and high γ-GT/ALT ratio (Mihm et al. 1999; Amanzada et al. 2012a). Simple and quickly measurable predictors are useful for medical doctors in countries with lower health budget to select the CHC patients who would achieve a high SVR rate undergoing a CHC antiviral treatment with Peg-IFN-α and RBV (Mechie et al. 2015).

It has been shown that in vitro conditions, Vitamin B_{12} binds the IRES-ribosomal structure inhibiting in a dose-dependent manner the HCV IRES-dependent translation (Lott et al. 2001; Li et al. 2004). On the other hand, in vivo, high levels of serum Vitamin B_{12} are associated with high serum HCV RNA in CHC patients (Lott et al. 2001). Through

these perspectives Vitamin B_{12}, might have opposing effects on HCV translation and replication.

It has been theorized that HCV could have evolved to use high Vitamin B_{12} levels in the hepatocytes in order to obtain high replication rates, or due to the blocking activity of the IRES-ribosomal complex, could be one of the mechanisms through which HCV promotes persistence (Lott et al. 2001; Li et al. 2004; Mechie et al. 2015).

Due to a raise in hepatic cytolysis and/or low clearance by the damaged hepatocytes a relative Vitamin B_{12} deficiency may result in some liver diseases like: hepatocellular carcinoma, liver metastasis, hepatitis, cirrhosis (Andres et al. 2013; Mechie et al. 2015). Considering these facts and with the inhibitor effect of Vitamin B_{12} on the HCV replication cycle (Lott et al. 2001; Li et al. 2004), it is reasonable to believe that supplementation of vitamin B_{12} to Peg-INF-α and RBV might improve the SVR rates in CHC patients (Rocco et al. 2013).

Our results confirm the findings of Lott et al. regarding to a positive correlation between serum Vitamin B_{12} levels and baseline serum HCV RNA in CHC patients. We were also able to show that baseline serum Vitamin B_{12} levels were associated with the liver fibrosis staging in CHC-genotype-1-infected patients, making Vitamin B_{12} one of the possible non-invasive biomarkers for liver fibrosis. Regarding these results, the concentration of Vitamin B_{12} in the hepatocytes would be interesting, and this would be probably reduced in patients with advanced liver fibrosis because of the lower number of hepatocytes. We also find that Vitamin B_{12} is inversely associated with RVR and SVR, a fact which could make the baseline serum Vitamin B_{12} in the future a simple, rapid measurable and affordable predictor for the therapeutic success of Peg-IFN-α and RBV (Mechie et al. 2015).

In the study of Rosenberg et al. elevated serum baseline levels of Vitamin B_{12} were statistically associated with End-of-Treatment Response but were not correlated with SVR. Nevertheless, Rosenberg et al. (Rosenberg and Hagen 2011) included in their study only 45 CHC patients infected with HCV-genotype-1. For this reason their study results may be statistically underpowered (Mechie et al. 2015).

In the only prospective study until this date, Rocco et al. showed that adding Vitamin B_{12} to the classical antiviral therapy with Peg-IFN-α and RBV, significantly increased the SVR rate in the group that received the additional Vitamin B_{12} as compared to the control group, which received only the classical antiviral therapy. The higher SVR rates in the patient group that received Peg-IFN-α, RBV and Vitamin B_{12} may be due to the inhibition of HCV IRES-dependent translation by Vitamin B_{12} and by a possible direct interaction with the HCV IRES RNA (Lott et al. 2001; Takyar et al. 2002; Mechie et al. 2015). On the other hand it may also be an effect of modulation that Vitamin B_{12} has on the immune system (Partearroyo et al. 2013; Mechie et al. 2015).

In conclusion, the serum baseline Vitamin B_{12} concentration was significantly associated with viral load and with liver fibrosis staging in CHC patients with HCV genotype 1 infection. Low serum vitamin B_{12} concentrations were found to be associated with SVR in a setting of a classical CHC antiviral combination therapy with Peg-IFN-α and RBV, predicting the therapy outcome. By combining serum baseline Vitamin B_{12} concentration with a second predictor, i.e. IFN-λ3 rs12979860 genotype, the OR to identify the responding patients reaches 54. According to our results, serum Vitamin B_{12} concentration may be used as an affordable, rapidly determinable, noninvasive surrogate parameter for liver fibrosis staging and may in addition help to predict the therapy responsiveness to Peg-IFN-α and RBV in CHC patients (Mechie et al. 2015).

SUMMARY

Chronic hepatitis C represents a worldwide health problem. In the last years new therapeutic strategies with a very good sustained virological response rate have been developed. These new therapeutic agents are mainly available in the economically developed countries. A problem in the treatment of the CHC is, globally, that the majority of patients is living in countries with health budgets unable to meet such costs. This is why predictors of therapeutic response under classical therapy (IFN und RBV) are still necessary. In vitro studies provided evidence for Vitamin B_{12} to interfere with HCV replication. Thus, we retrospectively analyzed the relationship between serum baseline Vitamin B_{12} concentrations and clinical data from a total of 116 CHC patients with HCV genotype 1 infection who received an antiviral combination therapy with Peg-IFN-α and RBV. The statistical approach covered uni- and multivariate analyses using logistic regression models. Serum baseline Vitamin B_{12} concentrations were found to be positively associated with serum transaminase activities (AST, p=0.002, ALT, p=0.04), baseline viral load (p<0.0001), stage of fibrosis (p=0.0001) and the favorable IFN-λ3 rs12979860 C allele (p=0.04). To the contrary, an inverse relationship was found between serum baseline Vitamin B_{12} concentrations and RVR and SVR rates (p=0.001 and p<0.001, respectively). By using a ROC analysis we defined a cut-off level for baseline serum Vitamin B_{12} of 570 ng/L. Patients with serum baseline Vitamin B_{12} concentrations below this level achieved an SVR rate of 59% compared to patients with higher Vitamin B_{12} concentrations achieving an SVR rate of only 10% (OR of 13.4 (CI: 4.3-41.9, p<0.0001). Notably, patients with serum baseline Vitamin B_{12} concentrations below the cut-off and a favorable IFN-λ3 rs12979860 CC genotype featured SVR rates of 80%, while patients with higher concentrations and carrying the unfavorable T allele reached a 7% SVR rate only (OR of 54 (CI: 9.9-293.4, p<0.0001)). Our data suggest

that serum baseline Vitamin B_{12} concentration may be used as a non-invasive marker for the CHC-related fibrosis. In addition, it might be taken as a useful predictor for therapy responsiveness by identifying patients with high chances to benefit from an antiviral Peg-IFN-α and Ribavirin combination therapy.

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