THE STUDY OF THE MECHANISMS WHICH INDUCE A LOW RESPONSE TO THE ANTIVIRAL THERAPY IN OBESE PATIENTS WITH INSULIN RESISTANCE IN CHRONIC VIRAL HEPATITIS C

Maria Moţa[1], Mihaela L Bîcu[2], Simona G Popa[1], R I Dinu[2], M Ioana[3], Mihaela I Vladu[1], P Mitruţ[4], E Moţa[5]

[1] Department of Diabetes Nutrition and Metabolic Diseases, UMF Craiova.
[2] Department of Diabetes Nutrition and Metabolic Diseases, Clinical Emergency County Hospital Craiova.
[3] Department of Genetics, UMF Craiova
[4] Department of Internal Medicine, UMF Craiova
[5] Department of Nephrology, UMF Craiova.

Abstract

Taking into consideration the fact that chronic hepatitis C (CHC) is associated with a high risk of developing hepatic cirrhosis and hepatocellular carcinoma, the success of the treatment of this infection is extremely important. In patients with CHC it was observed an increased obesity rate and insulin resistance (IR) and a diminution of sustained virologic response (SVR) to the treatment with Peginterferon (PegIFN) and Ribavirine. The researches have shown that IR interferes with the antiviral therapy, being an important predictor for a low SVR in CHC patients treated with PegIFN and Ribavirine. PegIFN can interfere with insulin path and inflammation mechanisms. Obesity modifies the secretor adipocytokines profile and determines inflammatory reaction and IR, reducing thus the SVR. Adipokines reduce SVR in obese patients with CHC, directly or through suppressor of cytokine signaling 3 (SOCS3). There was studied the polymorphisms (SNPs) of SOCS3 in non-responsive patients and in patients with SVR. Taking into consideration the relationship obesity - IR – SVR rate diminution, it is very important to identify the possibilities to reduce weight, to maintain ideal weight on the long term, to reduce IR, to modify adipocytokines level, with consecutive benefits for SVR.

Keywords: chronic hepatitis C (CHC), obesity, insulin resistance (IR), sustained viral response (SVR), suppressor of cytokine signaling 3 (SOCS3).

In the whole world there are almost 200 million people infected with hepatic C virus (HCV) [1]. Taking into consideration the fact that chronic hepatitis C (CHC) is associated with a high risk of developing hepatic cirrhosis and hepatocellular carcinoma, the success of the treatment of this infection is extremely important. In Romania the frequency of hepatitis C virus infection is of 4,5% (1 million people).

Sustained Virologic Response (SVR): Definition

Sustained virologic response (SVR) (defined as RNA HCV, undetectable 24 weeks after the antiviral treatment) is obtained in 54-55% cases for the HCV genotype non 1 and in 42-46% for the genotype 1 [2]. The current therapy in CHC consists in the administration of Peg Interferon (PegIFN) in combination with Ribavirine for a period of 24 weeks for the genotypes 2, 3, 5, while for the genotypes 1 and 4 the duration of antiviral therapy is of 48 weeks [3]. In Romania the combination treatment yielded a 51.5 percent SVR in patients with CHC [4]. It was observed an increased obesity rate in patients with CHC and a diminution of SVR to the treatment with PegIFN and Ribavirine in obese patients [5]. The researches have shown that insulin resistance (IR) interferes with the antiviral therapy, being an important predictor for a low SVR in CHC patients treated with PegIFN and Ribavirine [1]. Various recent studies have insisted on the correlation between SVR and age, sex, body mass index (BMI), abdominal circumference, HOMA-IR index (Homeostasis Model Assessment of Insulin Resistance), gamaglutamiltranspeptidase (GGT) and adipokines and insulinemia seric level [5]. SVR was associated with young age, genotype non 1 and with the absence of visceral obesity [6]. Old age, a high level of GGT, advanced fibrosis and moderate/severe steatosis are predictor independent factors for a low SVR [7].

The relationship between SVR and the viral factors

HCV Characteristics: there are HCV cvasispecies relatively related but genetically distinct and the continuous apparition of these versions allows HCV to hinder the carrier's defense against it and to determine the resistance to the antiviral therapy [10].

HCV genotype is the most important predictor of the response to the therapy with PegIFN. Presently there are 6 major known HCV genotypes with multiple subtypes, the subtypes 1a and 1b are the most frequently isolated in US [10]. Iacono et al. have shown that patients infected with HCV genotype 1 have a low SVR, compared to other genotypes [6]. Frie et al. suggest that HCV genotypes non 1 were significant independent predictors of SVR. Nguyen et al. have also considered a lower SVR rate to the therapy with PegIFN in patients infected with genotype 1 vs. genotypes 2 or 3 (13% vs. 61%) [10].

Viral load: patients with a high level of viral load before the treatment show a lower SVR on the long term than patients with low viral load. Jessner et al. have demonstrated that patients who responded to PegIFN and Ribavirine had a low initial viraemia, compared to non responsive patients [10]. In immunosuppressed patients (co-infected with HIV or with a hepatic transplant) the viral load can be very high. In these conditions the high viral level combined with a low tolerance to antiviral therapy for CHC can reduce SVR probability [11]. HCV itself can induce IR. This is possible through alteration of insulin signaling path, due to an increased TNFα level (tumor necrosis factor α) and/or SOCS expression (suppressor of cytokine signaling) [9], contributing thus to SVR diminution.

The relationship between SVR and the carrier's factors

Ethnic origin: the Afro-American patients infected with HCV had a lower SVR to the antiviral therapy than white non Hispanic population. A study which included

HCV infected patients with genotype 1 treated with PegIFN and Ribavirine yielded a 28 percent SVR in Afro-American patients and 52 percent in Caucasian patients [10]. Evaluating the correlation between IR and SVR in Asian and Caucasian patients, we can see that IR is significantly higher in Asians compared to Caucasians. In Asians the increased basic insulinemia level and the high HOMA-IR level have been associated with a low SVR to antiviral therapy [12].

Age: In old patients (>65 years), immunologic suppression, chronic diseases and combined medication have a negative impact upon the response to antiviral therapy and increase the probability of side effects in antiviral treatment [10]. The SVR realization probability decreases with almost 5 percent at every ten years [11].

The relationship between obesity - IR - steatosis – low SVR

Almost 20 to 37 percent of the patients infected with HCV are obese, the BMI being in inverse ratio to the SVR rate [10]. An explanation for the interaction between obesity and low response to antiviral therapy can be hepatic steatosis, obesity being an independent risk factor for hepatic steatosis [10, 13]. Adinolfi et al. have shown that HCV steatosis is strictly correlated with the abdominal adipocytes mass and not with the total adipocytes mass [13, 14]. The steatosis prevalence on hepatic biopsies in patients infected with HVC was of almost 50% [13]. The independent risk factors for steatosis were obesity induced IR for genotype 1 and viral load in patients with genotype 3 [15]. CHC patients with advanced steatosis and low SVR had low seric levels of adiponectin and increased levels of TNFα, histologic activity index (HAI) and of fibrosis score [16].

The relationship between obesity – inflammation - IR – low SVR

Obesity modifies the secretor adipocytokines profile and determines inflammatory reaction and IR, reducing thus the SVR[10]. The inflammation represents a major pathogenic IR mediator [17]. Both the increased level of proinflammatory cytokines, produced by white fatty tissue in obese animals and humans, and the anti-inflammatory adipokines deficiency can be involved in IR physiopathology [17]. The fatty tissue regulates insulin sensitivity through circulating adipocytokines (leptin, resistine, adiponectin, TNF-α, interleukin IL-6, IL-10, IL-1β) [5, 18, 19].

Modified adipocytokines in obesity and IR are significant factors of low SVR to PegIFN and Ribavirine in CHC [5, 19]. The carrier's genetic factors also influence SVR: the modified TNFα levels, PegIFN α receptor 1 (PegIFNAR1), IL 10, and MxA (myxovirus resistance protein A), and the functional genetic versions of these genes influence the response to the therapy with PegIFN [8]. In obesity, there are increased circulating levels of some proinflammatory molecules, including TNFα, IL-6, PCR (C reactive protein), MCP-1 (monocyte chemotactic protein-1), IL-1β, IL-8, IL-10, TGF-β (tumor growth factor), leptin, resistine. The proinflammatory molecules derived from adipocytes induce systemic IR and contribute to the pathogeny of many obesity complications, including Diabetes Mellitus

type 2 (type 2 DM) and atherosclerosis [5, 17, 21].

Potential mechanisms through which adipocytes hypertrophy disturbs their function, influencing the lipid and glucidic metabolism, include the SREBP-2 expression at adipocytary level (sterol regulatory element binding protein-2), the expression modification of SREBP-2 target genes and the modification of some adipocytokines secretion: adiponectin, resistine, leptin or TNF-α [5]. In obesity, the proinflammatory effects of adipocytokines through intracellular signaling paths involve the NF-kB (nuclear factor-kappa B**)** and JNK (c-Jun NH_2-terminal kinase) systems [17]. The IKK-ß path (inhibitor of κB kinase β) /NF-κB can influence the insulin sensibility by disturbing the adipocytokines synthesis, regulating at the fatty tissue and skeletal muscle level the secretion of TNF-α, IL-6, IL-8, but not the adiponectin, resistine and leptin synthesis [19]. Cua IH et al. indicated BMI, leptin seric level, (positive correlation) and adiponectin seric level (negative correlation), but not TNFα and IL-6 as independent predictors for IR in CHC [9].

The modification of secretor profile of adipocytes in patients with obesity and CHC can explain the negative influence of obesity associated IR upon responsiveness to antiviral therapy.

Leptin controls food ingestion and has pro-inflammatory properties *in vitro*, due to the increment of some cytokines such as IL-1β, IL-6, IL-10 IL-12 and TNF-α [22]. It has been demonstrated that leptin supports the activation of signaling paths in hepatic star cells (HSC), contributing to fibrosis aggravation and determining thus SVR diminution [23]. Leptin increases NF-kB activation and the expression of MCP-1 and of the VEGF (vascular endothelial growth factor) [14]. MCP-1 determines the diminution of adipocytes sensibility to insulin [5]. The leptin level is an independent risk factor for PegIFN resistance. Thus a high level of seric leptin reduces PegIFN effect in CHC patients with low viraemia, both men and women [24]. The seric leptin level and IR are independent predictive factors for SVR in CHC patients with antiviral therapy [25]. Giannini et al. didn't find any correlation between leptin level und steatosis severity [13]. In contradiction to this opinion, recent studies have shown that steatosis can be associated with leptin, BMI and visceral obesity [26]. The leptin level is significantly higher in obese patients and women and is correlated to HOMA-IR and resistine. IR correlates most strongly with leptin level [18, 13]. Leptin level depends significantly also on age and insulinemia [13].

Adiponectin is an antiinflammatory cytokine secreted exclusively by the fatty tissue and stays in an inverse ratio to obesity [27, 17] and SVR. The adiponectin level is negatively correlated to the total adipocytes mass percentage, fatty tissue central distribution and basic insulinemia [27]. Snehalatha C et al. have shown that there is an inverse ration between adiponectin level and IR, type 2 DM and atherosclerosis [28]. The variability of adiponectin codifying genes contributes to the modulation of adiponectin circulating level and to IR risk. The 11391G→A polymorphism of adiponectin gene is not associated with IR, while 276G→T version is a strong IR determinant [29]. The seric adiponectin levels are with 15 percent

higher in women than in men [29]. Low adiponectin levels have been associated independently with steatosis and HOMA-IR level [30]. Steatosis was significantly associated age, HCV genotype 3 and the aspartataminotransferase level (ASAT) [13]. There was no connection found between adiponectin level and age, BMI, insulinemia, leptinemia or TNFR2 (type II TNF receptor). The TNFR2 level is significantly correlated to age, TNFR1 and ASAT. No correlation was found between TNFR2 and BMI [13]. Haluk Sargin et al. show an opposite correlation between adiponectin and BMI, insulinemia, HOMA-IR, proinsulinemia and triglycerides. Adiponectin level is correlated with alaninaminotransferase (ALAT) and GGT in healthy persons [31]. Haluk Sargin et al. showed a statistically significant correlation between adiponectinemia and ASAT, ALAT and GGT. Bajaj et al. have discovered for the first time a relationship between adiponectinemia on the one hand and insulin hepatic sensibility and hepatic fatty content on the other hand, in patients with type 2 DM [27]. Yamamoto et al. show that adiponectin is IR predictor in Japanese population [27]. HDL-cholesterol, sex, BMI and HOMA-IR were correlated independently with adiponectin seric level [32]. High levels of TNF-α were observed in patients with low adiponectin levels. TNF-α and IL-6 are inhibitors of adiponectin expression and secretion on human biopsies of white fatty tissue or adipocytes cultures [33]. The insulin exciting effect of adiponectin is mediated by lipogenesis inhibition through increment of fatty acids oxidation, through AMPK activation (AMP activated protein kinase) in skeletal muscle, similar to leptin action. Adiponectin activates also AMPK at the hepatic level, reducing the hepatic glucose production rate and increasing the glucose taking [33, 17]. Hypoadiponectinemia is strongly associated with steatosis presence in patients with CHC, irrespective of age, sex, virus characteristics, leptin and insulin levels and BMI [13]. The adiponectin level is significantly lower in obese patients compared to thin patients and higher in women, but it is not correlated with HOMA-IR [18].

Resistine can be a bridge between the fatty tissue, obesity and IR. In adipocytes cultures, resistine reduces the glucose transport stimulated by insulin. Recombined resistine determines hepatic IR and the resistine absence can determine AMPK activation and the decrement of the expression of the genes involved in hepatic neoglucogenesis [17]. Resistine inhibits the taking of glucose in L6 cells of skeletal muscle, as well as in adipocytes [19]. Most of the studies didn't find any correlation between resistine plasmatic level, BMI and IR [17]. In mice, resistine can determine IR, while its implication in insulin sensibility control in humans remains unclear [17]. Seric resistance increment seems to determine IR in mice, although some studies don't agree with that [34]. On the other hand, Haruhiko at al. showed that plasmatic resistine is associated with SNP 420 genotype, the most with G/G, then with C/G and C/C [40], correlating positively with HOMA-IR, low seric level of HDL- cholesterol and the high PCR level in Japanese population [35]. The increased expression of resistine gene in the liver increases seric resistine and IR [35, 18]. It was demonstrated that resistine has an intrahepatic cytokine role with proinflammatory action in

CSH, through Ca_2/NF-kB path, inducing thus hepatic fibrosis which reduces SVR [36].

Resistine doesn't correlate with BMI [18], but it correlates positively with HOMA-IR and negatively with seric HDL-Cholesterol, irrespective of age, sex and BMI [35, 18]. Resistine levels didn't significantly differ in obese and thin patients, but they are significantly higher in women than in men [35]. **TNF-α** is a proinflammatory cytokine, produced mainly by macrophages and lymphocytes and less by the fatty tissue. TNF-α is a link-molecule between inflammation and obesity. In persons with CHC there have been discovered increased TNF-α values in serum and monocyte, contributing thus to the SVR diminution. Some studies show that TNF-α inhibitory effect on SVR is mediated by SOCS3 [2]. Only TNF-α level is correlated with histologic activity (portal/periportal inflammation) [9]. TNF-α determines IR through the phosphorylation of the first sublayer of IRS-1 (insulin receptor substrate-1), hindering thus the interaction with β insulin receptor and blocking the insulin signaling path [17]. **IL-8:** Increased IL-8 values have been observed in patients who don't respond to classical antiviral therapy. IL-8 can inhibit the PegIFN-α effect. It was showed that 5A nonstructural protein of HVC induces IL-8 expression, blocking thus PegIFN. Bruun JM et al. showed that TNF-α increases adipocyte IL-8 secretion [19]. **IL-6:** is produced by fibroblasts, endothelial cells, monocytes and fatty tissue. IL-6 produced by fatty tissue is increased in obesity, playing a very important part in the relationship obesity-inflammation. ARNm-IL6 expression and secretion are more important in visceral fatty tissue than in subcutaneous fatty tissue. There is a close relationship between IL-6 level in the fatty tissue and the circulating levels of IL-6 and PCR, while IL-6 increases PCR hepatic production. IL-6 induces IR in hepatocytes [5]. IL-6 receptor belongs to the first class of receptors and involves JAK/STATs path (Janus kinases/signal transducers and activators of transcription). JAK activation determines STAT phosphorylation. In IR we can also speak about tyrosine phosphatase activation or the interaction between SOCS and insulin receptor [17]. Kern PA et al. showed a direct correlation between IR and the circulating level of IL-6 [19]. **IL-10** is a cytokine with antiinflammatory and immunosuppressant effects. IL-10 doesn't seem to have antiviral activity, but it can modulate the effects of PegIFN-α treatment, reducing its effects. IL-10 can reduce SVR by inhibiting PegIFN signaling through SOCS induction and T cells proliferation suppression. The circulating level of IL-10 is increased in obesity and there is a correlation between BMI and IL-10 secretion in subcutaneous fatty tissue, but not in the visceral one [37]. **IL-1β:** increased levels of IL-1β were detected in CHC and it was shown that they associate with PegIFN activity inhibition in fibroblasts and hepatocytes. It seems that the response inhibition to PegIFN by IL-1β is mediated through STAT1 activity suppression.

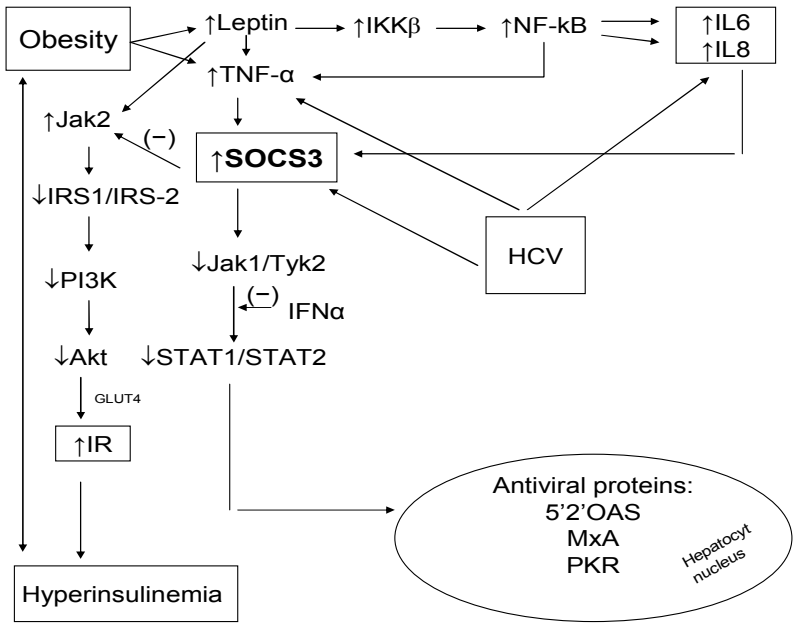

Fig .1 The relationship between obesity - SOCS3 - IR in CHC.

IFN α - Peginterferon; HCV - hepatic C virus; IR – insulin resistance; IRS-1(2) - insulin receptor substrate-1(2); TNFα - Tumor necrosis factor α; SOCS3 - suppressor of cytokine signalling3; IL – Interleukin; MxA - myxovirus resistance protein A; 5'2'OAS - 5'2' oligoadenylate synthetase; PKR - protein kinase R; NF-kB - nuclear factor-kappa B; IKK-ß - inhibitor of B kinase β; STAT1(2) - signal transducers and activators of transcription1(2); Jak1(2)- Janus kinase1(2) ; Tyk2 - tyrosine kinase2; Akt (PKB) - protein kinase B; PI3K - phosphoinositide 3-kinase; GLUT4-glucose transporter4.

Signaling paths of insulin and Peginterferon 2: PegIFN can interfere, irrespective of weight variation, with insulin path and inflammation mechanisms [38]. PegIFN action mechanism is not fully understood but it is supposed that it induces in infected hepatocytes the production of proteins which interact with viral replication; PegIFN has also indirect immunomodulating and anti-inflammatory effects [3]. In obesity induced IR, interfere both cytokines through pro-inflammatory effects and reactive oxygen species (ROS) and free fatty acids (FFAs), mediated through specific intracellular signaling paths which interfere with insulin signal through IRS phosphorylation inhibition, paths which involve NF-κB, IKK (inhibitor of B kinase), AP-1 (Activator Protein-1) and JNK [17]. PegIFN-α acts through a receptor (PegIFNAR) made up of two subunits: PegIFNAR1 and PegIFNAR2 [39]. In humans there has been identified only one PegIFNAR1 form, while for the subunit PegIFNAR2 there are 3 forms: the long form PegIFNAR2c and two trucked forms PegIFNAR2a and PegIFNAR2b. Out of the three isoforms, only PegIFNAR2c is involved in transmitting PegIFN signal, while the two short forms inhibit PegIFN action through competition with PegIFNAR2c isoforms for PegIFN (Pfeffer, 1997). Insulin activates through IRS-2 phosphatidilinositol-3-kinase (PI3K), blocking thus STAT1 translocation, and inhibiting PegIFN antiviral effect [40]. PegIFN induces antiviral activity by attaching to extra-cellular receptors. PegIFN signaling path determines Jak1 (Janus kinase1) and tyrosine kinase (Tyk2) activation, initiated

through PegIFN attachment to heterodimeric complex of PegIFN receptors (PegIFNAR1 / PegIFNAR2), leading to STAT1 and STAT2 signal activation and transcription activation. Activated STAT stimulates response elements in PegIFN stimulated genes [40]

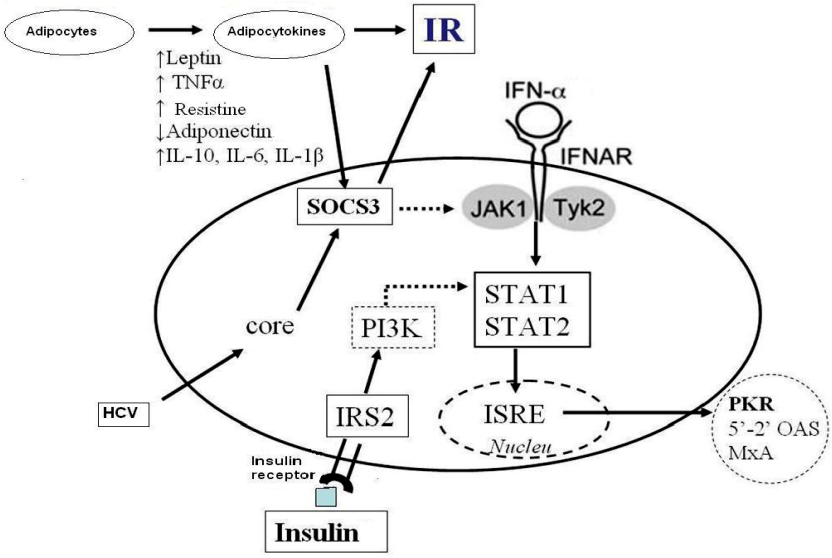

Fig. 2. Signaling paths of insulin and PegIFN 2
(Romero-Gomez M., Huang Y. – modified)

IFN α - Peginterferon ; HCV - hepatic C virus; IR – insulin resistance; IRS 2 - insulin receptor substrate 2; TNFα - Tumor necrosis factor α; SOCS3 - suppressor of cytokine signalling3; IL – Interleukin; MxA - myxovirus resistance protein A; 5'2'OAS – 5'2' oligoadenylate synthetase; PKR - protein kinase R; STAT1(2) - signal transducers and activators of transcription1(2); Jak1- Janus kinase1; Tyk2- tyrosine kinase2; PI3K - phosphoinositide 3-kinase; ISRE- IFN-sensitive response element.

IR and hyperinsulinemia can block the inhibition of HCV replication mediated by PegIFN through interaction with protein-kinase R (PKR) and PegIFN regulatory factor, preventing their activation through PegIFN [41]. Adipokines, directly or through SOCS3, reduce SVR in obese patients with CHC. SOCS3 belongs to the SOCS protein family, which has eight members: SOCS 1-7 and CIS protein (cytokine-inducible Src-homology 2-containing protein). SOCS 1-3 and CIS appear as response to cytokinic stimulation and action through a negative feed-back curve, inhibiting the initial cytokinic signal responsible of their own induction. The role of the other SOCS 4-7 is still unknown [42]. SOCS increased expression in liver induces hepatic IR, reducing also SVR [43]. SOCS directly inhibits insulin signal, through competition for insulin receptor phosphorylation situs, and indirectly through SREBP-1c induction, which reduces IRS-2 synthesis and increases fatty acids synthesis. The increment of acyl-CoA activates PKC-O, which determines IRS phosphorylation, and/or IKK-B which activates NF-kB with increment of IL-6 and SOCS induction [43]. At the hepatic level SOCS-1 specifically inhibits IRS-2, while

SOCS-3 inhibits both IRS-1 and IRS-2, interacting with insulin receptor. SOCS-1 and SOCS-3 can inhibit IRS through the diminution of JAK-STAT-3 activation [43]. In patients with increased SOCS1 hepatic expression there is a significantly lower SVR rate compared to patients with low SOCS1 levels [44]. Increased SOCS3 gene expression in the hepatic tissue is associated with a low SVR to antiviral treatment in patients with CHC genotype 1. To understand why patients with genotype 1 respond differently, scientists investigated SOCS3 genetic expression, IR and the therapy response in patients with HCV. 198 patients, out of them 108 with genotype 1 and 90 with genotype 2, treated with PegIFN and Ribavirine, were included in a study which determined SOCS3 expression in lymphoblastoid cells (virus Epstein Baar transformed) derived from peripheral lymphocytes in 130 patients. IR was more frequent in patients with genotype 1 than in patients with genotype 2. Increased SOCS3 levels were observed in patients with IR and genotype 1, who didn't respond to antiviral therapy. SOCS3 levels were higher in patients who didn't respond to treatment, irrespective of genotype. In the univariated analysis, genotype, age, SOCS3 and IR are significantly correlated with the response to therapy. In the multivariated analysis, SOCS3 was the only independent response predictor [45].

Polymorphisms SOCS3 – SVR: in order to strengthen the theory according to which SOCS3 is an useful marker in antiviral treatment efficiency prediction, scientists studied the **polymorphisms** (SNPs) of three isolated nucleotides of **SOCS3** in non-responsive patients and in patients with SVR: SOCS3 8464A/C, 4874 A/G and 1383 A/G. SOCS3 basic expression was significantly higher in non-responsive patients [8]. SOCS3-4874AA genotype was strongly associated with antiviral therapy failure. SOCS3 basic level – as inhibitor of PegIFN Janus kinase transduction signal and activator of transcription path – and its genetic polymorphisms influence antiviral treatment response. SOCS3 represents a new biomarker for the treatment response prediction [8]. The plasmatic basic levels of some genes were evaluated, including the inhibitors of transduction signal induced by Janus kinase of PegIFN α and the activator of the transcription path JAK STAT, SOCS1, SOCS2, SOCS3, PIAS1 (protein inhibitor of activated STAT1), PIAS3 and SUMO1 (small ubiquitin-like modifier 1), PegIFN α treatment failure associated genes (TNF α, IL10, PegIFNAR1, IL1β, IL6 and IL8) and antiviral PegIFN α induced effectors: MxA and 5'2'OAS (5'2' oligoadenylate synthetase), using two independent patient groups (one from Southern Italy and the other from the North) infected with HCV genotype 1 and treated with PegIFN and Ribavirine. Only SOCS3 gene had high levels in both patient groups with antiviral therapy failure [8]. QRT-PCR analysis showed that SOCS1, SOCS3 and PIAS3 ARNm levels are significantly increased in patients with antiviral therapy failure, compared to patients with SVR [8]. MxA and 5'-2' OAS are on the other hand significantly low in non-responsive patients [8]. PegIFN resistance in CHC can be explained by the fact that HCV determines endogen PegIFN production and increases SOCS3 expression, either directly, by means of viral protein (core), or indirectly, through PegIFN inhibitory factors (IL-6 or other

soluble factors) [46]. In adipocytes 3T3-L1, resistine diminishes various insulin effects such as insulin receptor phosphorylation, IRS-1 phosphorylation, PI3K activation, PI3P (phosphatidylinositol-3-phosphate) production and Akt/B protein kinase activation, necessary in glucose transport stimulation (GLUT4). The treatment with resistine induces SOCS3 genetic expression, insulin signal inhibitor. SOCS3 association with IR is increased by resistine. SOCS3 can be a cellular mediator of resistine capacity to reduce insulin action in adipocytes [47].

Possibilities to improve SVR by modifying IR in obese patients with CHC

IR reduction in obese patients with CHC reduces hyperinsulinemia and can improve SVR in PegIFN and Ribavirine therapy, avoiding complications and hepatic disease progression [16, 40]. Taking into consideration the relationship obesity - IR – SVR rate diminution, it is very important to identify the possibilities to reduce weight, to maintain ideal weight on the long term, to reduce IR, to modify adipocytokines level, with consecutive benefits for SVR. Visceral obesity, phenotypic IR expression, is an important non-pharmacological target to increase the success rate of antiviral therapy in CHC [6]. Taking into account the fact that genotype 3 directly determines steatosis, while in non 3 genotypes (especially genotype 1) steatosis is mostly a consequence of carrier's risk factors, one could expect that weight reduction doesn't have benefits in patients infected with genotype 3. Opposed to this prediction, Hickman at al. [48] observed a diminution of steatosis degree in overweight or obese patients who didn't respond to antiviral therapy, not only with genotype 1 HCV, but also with genotype 3, after a losing weight program based on diet and physical exercise for a period of 15 months [49]. Global obesity epidemic led to studies dedicated to new therapies, including inhibitors of serotonin recapture, lipase inhibitors, leptin antagonists, PPAR agonists, melanocortin 3 agonists and selective CB1 receptor antagonists. The understanding of the influence of adipocytokines regulation through medication upon insulin sensibility is very important in IR and obesity treatment [19]. The blocking of TNFα production by means of anti TNF medication, such as Infliximab, can prevent IR [8]. The control of IKK-ß/NF-κB can represent a therapeutic strategy for cytokines eliberation regulation and, consequently, also for IR diminution [19]. Sulphasalasin and BAY 11-7082 - a selective inhibitor of IKK-ß action - increase insulin receptor ß expression, at the level of fatty tissue and skeletal muscle [19]. Yin M-J, Wahl C, Kim et al. showed that high salicylates doses can prevent IR in skeletal muscle by blocking IKK- ß activity and IRS-1 phosphorylation through IKK- ß and they reduce IRS-1 activation associated PI3K [19]. The Salicylates can reduce IR by interfering with the path of NF-κB [19, 17]. The involvement of inflammatory path in obese associated IR is sustained by the protector effect of some antiinflammatories [17]. Aspirin inhibits both IKK and JNK paths, but also other kinases involved in TNF-α induced IR. Through its antioxidant effects, aspirin reduces NF-κB activation and AP-1 as response to oxidative stress. Increased salicylates doses improve insulin sensibility in

patients with type 2 DM [17]. Selective inhibition of NF-kB function in liver and fatty tissue reduces IR [50]. Other medicines with antiinflammatory effect such as thiazolidinediones (TZD) and statins, interfere in IR: TZD increases insulin sensibility by decreasing the TNF-α adipocyte production, or the TNF-α effect upon target tissues, and by inducing adiponectin expression [51]. Statins modulate endothelial function and leucocytes transendothelial migration, inhibit proinflammatory cytokines secretion and interfere with NF-kB path [17]. The measures taken to increase adiponectinemia – such as weight decrement and TZD – can diminish steatosis in CHC [13]. Antagonist CB1 receptor (SR141716) modulates food ingestion, with great efficiency in diet induced weight reduction. SR141716 can increase SVR by reducing IR, leptinemia, insulinemia and FFAs level [52]. PUFA omega-3 acids can increase PegIFN therapy efficiency and the antifibrogenic effect in obese patients with CHC [53]. Some genes and polymorphisms such as HLA-B44 remain to be studied, in order to discover possible therapeutic resources [40].

Acknowledgements: This work was supported by grants "PNII-IDEI" CNCSIS, Director Maria Mota

REFERENCES

1. Decock S, Verslype C, Fevery J. Hepatitis C and insulin resistance: mutual interactions. A review. *Acta Clin Belg* 2007 ;62(2):111-119.

2. Hadziyannis SJ et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; 140(2):346-356.

3. Charlton MR, Pockros PJ, Harrison SA. Impact of Obesity on treatment of Chronic Hepatitis C. *Hepatology* 2006; 43:1177-1186.

4. Gheorghe L et al. Effectiveness and tolerability of pegylated Interferon alpha-2a and ribavirin combination therapy in Romanian patients with chronic hepatitis C: from clinical trials to clinical practice. *Rom J Gastroenterol*. 2005; 14(2):109-115.

5. Kahn C Ronald, King George L, Moses Alan C, Weir Gordon C, Jacobson Alan M, Smith Robert J. The Molecular Mechanism of Insulin Action and the Regulation of Glucose and Lipid Metabolism. *Joslin's Diabetes Mellitus,* 2005; 14th Edition.

6. O. Lo Iacono, G. Venezia, S. Petta, C. Mineo, S. De Lisi, V. Di Marco, V. Rodolico, M. Amato, D. Ferraro, C. Giordano, P.L. Almasio, A. Craxí. The Impact of Insulin Resistance, Serum Adipocytokines and Visceral Obesity on Steatosis and Fibrosis in Patients With Chronic Hepatitis C. *From Alimentary Pharmacology & Therapeutics* 2007; Posted 06/20/2007.

7. Cammà C, Bruno S, Di Marco V, Di Bona D, Rumi M, Vinci M, Rebucci C, Cividini A, Pizzolanti G, Minola E, Mondelli MU, Colombo M, Pinzello G, Craxì A. Insulin resistance is associated with steatosis in nondiabetic patients with genotype 1 chronic hepatitis C. *Hepatology* 2006; 43(1):64-71.

8. M Persico, M Capasso, R Russo, E Persico, L Crocè, C Tiribelli, A Iolascon. Elevated expression and polymorphisms of SOCS3 influence patient response to antiviral therapy in chronic hepatitis C. *Gut* 2008; 57:507-515.

9. Cua IH, Hui JM, Bandara P, Kench JG, Farrell GC, McCaughan GW, George J. Insulin resistance and liver injury in hepatitis C is not associated with virus-specific changes in adipocytokines. *Hepatology* 2007; 46(1):66-73.

10. Nyingi Kemmer, Guy W. Neff . Managing Chronic Hepatitis C in the Difficult To Treat Patient. *Liver International* 2007 ; Posted 12/11/2007.

11. J. Heathcote, J. Main. Treatment of Hepatitis C. *Journal of Viral Hepatitis* 2005, Posted 04/29/2005.

12. R. D'Souza, C. A. Sabin, G. R. Foster. Insulin Resistance Plays a Significant Role in Liver Fibrosis in Chronic Hepatitis C and in the Response to Antiviral Therapy. *The American Journal of Gastroenterology* 2005; 100(7): 1509–1515.

13. Jean-Michel Petit, Anne Minello, Valerie Jooste, Jean Baptiste Bour, Francoise Galland,Laurence Duvillard, Bruno Verges, Niels Olivier Olsson, Philippe Gambert, and Patrick Hillon. Decreased Plasma Adiponectin Concentrations Are Closely Related to Steatosis in Hepatitis C Virus-Infected Patients. *The Journal of Clinical Endocrinology & Metabolism* 2005; 90(4):2240–2243.

14. Aleffi S, Petrai I, Bertolani C, Parola M, Colombatto S, Novo E, et al. Upregulation of proinflammatory and proangiogenic cytokines by leptin in human hepatic stellate cells. *Hepatology* 2005 ; 42:1339-1348

15. L Fartoux, A Poujol-Robert, J Guéchot, D Wendum, R Poupon, L Serfaty. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. *Gut* 2005**;** 54:1003-1008.

16. Parise ER, de Oliveira AC. Insulin resistance in chronic hepatitits C. *Arq Gastroenterol.* 2007*;* 44 (2):178-84.

17. Jean-Philippe Bastard, Mustapha Maachi, Claire Lagathu, Min Ji Kim, Martine Caron, Hubert Vidal, Jacqueline Capeau, Bruno Feve. Recent advances in the relationship between obesity, inflammation, and insulin resistance. European Cytokine Network 2006; 17(1): 4-12.

18. Josef V Silha, Michal Krsek, Jan V Skrha, Petr Sucharda, B L G Nyomba and Liam J Murphy. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. *European Journal of Endocrinology* 2003; 149: 331–335.

19. Martha Lappas, Kirin Yee, Michael Permezel and Gregory E. Rice. Sulfasalazine and BAY 11-7082 Interfere with the Nuclear Factor-κB and IκB Kinase Pathway to Regulate the Release of Proinflammatory Cytokines from Human Adipose Tissue and Skeletal Muscle in Vitro. *Endocrinology* **2004;** 146 (3): 1491-1497.

20. Ford ES. The metabolic syndrome and C-reactive protein, fibrinogen, and leukocyte count: findings from the Third National Health and Nutrition Examination Survey. *Atherosclerosis* 2003; 168: 351.

21. Vettor R, Milan G, Rossato M, Federspil G. Review article: adipocytokines and insulin resistance. *Aliment Pharmacol Ther.* 2005; 22(2): 3-10.

22. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, et al. Leptin regulates proinflammatory immune responses. *FASEB J* 1998 ; 12: 57-65.

23. Xiaokun Ding, Neeraj K. Saxena, Songbai Lin,Amin Xu, Shanthi Srinivasan, Frank A. Anania. The Roles of Leptin and Adiponectin-A Novel Paradigm in Adipocytokine Regulation of Liver Fibrosis and Stellate Cell Biology. *American Journal of Pathology* 2005; 166(6).

24. Eguchi Y, Mizuta T, Yasutake T, Hisatomi A, Iwakiri R, Ozaki I, Fujimoto K. High serum leptin is an independent risk factor for non-response patients with low viremia to antiviral treatment in chronic hepatitis C. *World J Gastroenterol.* 2006; 12 (4):556-560.

25. Zhu B, Zhang P, Wang XH, SunLH, Jiang ZY. A virological response study of serum leptin level and insulin resistance on the antivirus effect in patients with chronic hepatitis C. *Zhonghua Gan Zang Bing Za Zhi* 2006; 14(12):887-889.

26. Romero-Gomez M, Castellano-Megias VM, Grande L, Irles JA, Cruz M, Nogales MC, Alcon JC, Robles A. Serum leptin levels correlate with hepatic steatosis in chronic hepatitis C. *Am J Gastroenterol* 2003 ; 8:1135–1141.

27. Haluk Sargin, Mehmet Sargin, Hülya Gozu, Asuman Orcun, Gülcan Baloglu, Murat Ozisik, Mesut Seker, Oya Uygur-Bayramicli. Is adiponectin level a predictor of nonalcoholic fatty liver disease in nondiabetic male patients? *World J Gastroenterol* 2005; 11(37):5874-5877.

28. Snehalatha C, Mukesh B, Simon M, Viswanathan V, Haffner SM, Ramachandran A. Plasma adiponectin is an independent predictor of type

2 diabetes in Asian Indians. *Diabetes Care* 2003; 26:3226-3229.

29. Claudia Menzaghi; Vincenzo Trischitta; Alessandro Doria. Genetic Influences of Adiponectin on Insulin Resistance, Type 2 Diabetes, and Cardiovascular Disease. *From Diabetes* 2007; Posted 06/11/2007.

30. Durante-Mangoni E, Zampino R, Marrone A, Tripodi MF, Rinaldi L, Restivo L, Cioffi M, Ruggiero G, Adinolfi LE. Hepatic steatosis and insulin resistance are associated with serum imbalance of adiponectin/tumour necrosis factor-alpha in chronic hepatitis C patients. *Aliment Pharmacol Ther*. 2006 ; 24(9):1349-1357.

31. Bermejo AL, Botas P, Funahashi T, Delgado E, Kihara S, Ricart W, Fernandez-Real JM. Adiponectin, hepatocellular dysfunction and insulin sensitivity. *Clinical Endocrinol* 2004 ; 60: 256-263.

32. Yamamoto Y, Hirose H, Saito I, Tomita M, Taniyama M, Matsubara K, Okazaki Y, Ishii T, Nishikai K, Saruta T. Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. *Clin Sci (Lond)*. 2002; 103 (2):137-142

33. M Guerre-Millo. Adipose tissue and adipokines: for better or worse. *Masson* 2004; 30(1): 13 – 19.

34. Way JM, Gorgun CZ, Tong Q, Uysal KT, Brown KK, Harrington WW, Oliver WR Jr, Willson TM, Kliewer SA, Hotamisligil GS. Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. *J Biol Chem* 2001; 276:25651–25653.

35. Haruhiko Osawa, Yasuharu Tabara, Ryuichi Kawamoto, Jun Ohashi, Masaaki Ochi,Hiroshi Onuma, Wataru Nishida, Kazuya Yamada, Jun Nakura, Katsuhiko Kohara, Tetsuro Miki, Hideichi Makino. Plasma Resistin, Associated With Single Nucleotide Polymorphism –420, Is Correlated With Insulin Resistance, Lower HDL Cholesterol, and High-Sensitivity C-Reactive Protein in the Japanese General Population. *Diabetes Care* **2007**; 30:1501-1506.

36. Cristiana Bertolani, Pau Sancho-Bru,Paola Failli, Ramon Bataller, Sara Aleffi, Raffaella DeFranco,Benedetta Mazzinghi, Paola Romagnani, Stefano Milani,Pere Gine´s, Jordi Colmenero, Maurizio Parola, Stefania Gelmini, Roberto Tarquini, Giacomo Laffi, Massimo Pinzani, Fabio Marra. Resistin as an Intrahepatic Cytokine Overexpression during Chronic Injury and Induction of Proinflammatory Actions in Hepatic Stellate Cells. *The American Journal of Pathology* 2006; 169 (6).

37. John N. Fain, Atul K. Madan, M. Lloyd Hiler, Paramjeet Cheema and Suleiman W. Bahouth. Comparison of the Release of Adipokines by Adipose Tissue, Adipose Tissue Matrix, and Adipocytes from Visceral and Subcutaneous Abdominal Adipose Tissues of Obese Humans. *Endocrinology* 2004; 145(5): 2273-2282.

38. Walsh MJ, Jonsson JR, Richardson MM, et al. Non-response to antiviral therapy is associated with obesity and increased hepatic expression of suppressor of cytokine signalling 3 (SOCS-3) in patients with chronic hepatitis C, viral genotype 1. *Gut* 2006; 55: 529-535.

39. Pestka S. The human interferon alpha species and receptors. *Biopolymers* 2000 ; 55: 254 –287.

40. M. Romero-Gómez. Hepatitis C and insulin resistance: steatosis, fibrosis and nonresponse. *Revista Espanola De Enfermedades Digestivas* 2006; 98(8): 605-615.

41. Yu Jian Wu, Li Shu Chen, Wang Gui Qiang. Effects of Fatty Liver and Related Factors on the Efficacy of Combination Antiviral Therapy in Patients With Chronic Hepatitis C. *Liver International* 2006; Posted 02/28/2006.

42. Hong F, Nguyen VA, Gao B. Tumor necrosis factor alpha attenuates interferon alpha signaling in the liver: involvement of SOCS3 and SHP2 and implication in resistance to interferon therapy. *FASEB J* 2001 ; 15: 1595–1597.

43. Claire Z. Larter, Geoffrey C. Farrell. Insulin resistance, adiponectin, cytokines in NASH: Which is the best target to treat? *Journal of Hepatology* 2006; 44(2):253-261.

44. Imanaka K; Tamura S; Fukui K; Ito N; Kiso S; Imai Y; Naka T; Kishimoto T ; Kawata S;

Shinomura Y. Enhanced expression of suppressor of cytokine signalling-1 in the liver of chronic hepatitis C: possible involvement in resistance to interferon therapy. *J Viral Hepat.* 2005; 12(2):130-138.

45. Persico M, Capasso M, Persico E, Svelto M, Russo R, Spano D, Crocè L, La Mura V, Moschella F, Masutti F, Torella R, Tiribelli C, Iolascon A. Suppressor of cytokine signaling 3 (SOCS3) expression and hepatitis C virus-related chronic hepatitis: Insulin resistance and response to antiviral therapy. *Hepatology* 2007; **46(4): 1009 – 1015.**

46. Huang Y., Feld. J., Sapp R., Nanda S., Lin J., Blatt L., Fried M., Murthy K., Liang T. Defective hepatic response to Interferone and activation of suppressor of Cytokine Signaling 3 in chronic hepatitis C. *Gastroenterology* 2007; 132(2): 733-744.

47. Claire M. Steppan,Juan Wang, Eileen L. Whiteman,Morris J. Birnbaum,and Mitchell A. Lazar. Activation of SOCS-3 by Resistin. *Molecular and Cellular Biology* 2005; 25(4):1569-1575.

48. Hickman IJ, Jonsson JR, Prins JB et al. Modest weight loss and physical activity in overweight patients with chronic liver disease results in sustained improvements in alanine aminotransferase, fasting insulin, and quality of life. *Gut* 2004; 53: 413-419.

49. A. Lonardo; P. Loria; L.E. Adinolfi; N. Carulli; G. Ruggiero. Hepatitis C and Steatosis: A Reappraisal. *Journal of Viral Hepatitis* 2006; Posted 03/16/2006

50. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 2005; 11: 183.

51. Moller DE, Berger JP. Role of PPARs in the regulation of obesity-related insulin sensitivity and inflammation. *Int J Obes* 2003; (Suppl 3): S17.

52. Christine Ravinet Trillou, Michèle Arnone, Claire Delgorge, Nadine Gonalons, Peter Keane, Jean-Pierre Maffrand, and Philippe Soubrié. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol*. 2003; 284(2):345-353.

53. Popa S.G., Mota M. Impact of obesity and omega-3 polyunsaturated fatty acids on fibrogenesis and responsiveness to antiviral therapy in chronic hepatitis C. *Rom J Intern Med*. 2007; 45(2):165-170.