

# **The correlation of the hepatic expression of SOCS3 with the response to PegInterferon in patients with chronic hepatitis C and obesity induced insulin resistance**

Maria MOTA<sup>a</sup>, Ilie-Robert DINU<sup>b</sup>, Simona Georgiana POPA<sup>c</sup>, Iulian DIACONESCU<sup>d</sup>, Mihaela Ionela VLADU<sup>e</sup>, Mihaela Larisa BICU<sup>f</sup>, Eugen MOTA<sup>g</sup>

<sup>a</sup>Professor, Department of Diabetes Nutrition and Metabolic Diseases, UMF Craiova, Romania

<sup>b</sup>Resident Doctor, Department of Diabetes Nutrition and Metabolic Diseases, Clinical Emergency County Hospital Craiova, PhD candidate, Romania

<sup>c</sup>University Assistant, Department of Diabetes Nutrition and Metabolic Diseases, UMF Craiova, PhD candidate, Romania

<sup>d</sup>Professor, Department of Infectious Diseases, UMF Craiova, Romania

<sup>e</sup>University Assistant, Department of Diabetes Nutrition and Metabolic Diseases, UMF Craiova, Romania

<sup>f</sup>Resident Doctor, Department of Diabetes Nutrition and Metabolic Diseases, Clinical Emergency County Hospital Craiova, Romania

<sup>g</sup>Professor, Department of Nephrology, UMF Craiova, Romania

## **ABSTRACT**

*The infection with the hepatitis virus C (HVC) represents one of the major medical problems, because of its frequency, the limited therapy resources, and the severe chronic complications. The response rate depends on the viral and host's factors and many patients still fail to eradicate the virus. Three are the mechanisms proposed to explain the interference between obesity and treatment response. The first is based on the premise that obesity is an inflammatory condition which causes an abnormal immune response. Leptin, adiponectin, TNF- $\alpha$  have been proposed as heavily influencing non-responsiveness to the PegInterferon- $\alpha$  (PegIFN- $\alpha$ ) in patients with CHC and obesity. The second mechanism connects obesity with IR, fact which leads to fibrosis and the alteration of treatment response. It has been observed that HVC "core" proteins inhibit the phosphorylation induced by insulin upon the fostatidilinositol-3 kinase and of Akt which are downstream components of the insulin receptor substrate path in the liver. HVC mediates the dysfunction of insulin signaling paths by increasing the expression of the suppressor of cytokine signaling 3 (SOCS-3). Through SOCS3, HVC reduces the response to treatment, operating at the level of PegIFN- $\alpha$  action path by blocking the Jak/STAT path. From three SOCS3 genetic polymorphisms (8464 A/C, -4874 A/G, -1383 A/G), the lowest response rate to antiviral therapy was observe, in those with SOCS3 -4874 AA genotype. The third mechanism explains the low response rate in obese patients on the basis of the diminution of PegIFN- $\alpha$  biodisponibility.*

*The treatment of patients that have IR associated to obesity could induce higher rates of the sustained virologic response. The therapeutic option is represented by the supervised losing weight and physical exercise, adequate medication to sustain insulin sensibility, omega 3 fatty acids, and branched-chain aminoacids; in the future one can try to use drugs, which help to reduce SOCS3 expression, influence the adipokines secretor profile induced by obesity.*

**Key words:** chronic hepatitis C, obesity, host insulin resistance, sustained virological response, suppressor of cytokine signaling 3, adipokines.

Address for correspondence:

Maria Mota, MD, PhD, Clinical Emergency County Hospital, 1 Tabaci Street, Craiova, Zip Code 200642, Romania  
email address: mmota53@yahoo.com

## INTRODUCTION

**T**he infection with the hepatitis virus C (HVC) represents one of the major medical problems, because of its frequency, the limited therapy resources and the severe chronic complications. The total number of HVC carriers in the world is estimated at 170 millions, with a frequency of 3%. Out of these carriers 129 millions will develop chronic hepatitis, representing 2,3% (1). In Romania the frequency of HVC infection is of 4,5%, and there are almost 1 million people chronically infected with the HVC. Romania takes thus a leading position regarding the HVC infection in Europe.

Discovered in 1989, the HVC belongs to the family Flaviviridae and contains 9500 nucleotides, which form a single gene that codes a viral polyprotein of cca. 3000 amino acids. Using the nucleotide sequencing, there have been identified at least 6 distinct HVC genotypes, named 1, 2, 3, 4, 5, 6, and also a number of 50 subtypes of genotypes. The ones that respond the least to the treatment are the types 1 and 4; the other types 2, 3, 5 and 6 show a better response to the treatment. In Romania the percentage of type 1, the 1b subtype of HVC is the most frequent and consequently only a very small number of patients can benefit from the conventional therapy (2).

After the acute infection, the probability to remain chronically infested is of 85-90%, and 20% of the patients develop hepatic cirrhosis after 10-20 years (3). The seriousness of HVC infection is due to the fact that the virus persists in the organism in most of the cases. After a silent period, there starts the fibrosis, then cirrhosis and all the other hepatic disease complications (4). The cause of fibrosis in the chronic HVC infection has been continuously studied. Some scientists consider that the virus does not have any cytopathic properties and the whole disaster is caused by the immune response of the carrier to the presence of the virus in the hepatic cells, while other specialists try to furnish proof for the direct destructive effect of the virus. The hepatic disease frequently appears in correlation with the HVC infection and Diabetes Mellitus type 2, along with other extra hepatic manifestations such as: mixed cryoglobulinemia, membranoproliferative glomerulonephritis, panarteritis nodosa,

Sjogren-like syndrome (sicca), autoimmune thyroiditis, arthritis, neuropathy, aplastic anemia, autoimmune thrombocytopenia, idiopathic pulmonary fibrosis (5). □

## THE THERAPY OF CHRONIC HEPATITIS C

**P**resently there are only two accepted therapies for the hepatitis HVC: Interferon- $\alpha$  (IFN- $\alpha$ ) in monotherapy or IFN- $\alpha$  plus ribavirin. The success rate to eradicate the virus is of 10-20% when using IFN- $\alpha$  monotherapy and of 30-40% in case of IFN- $\alpha$  + Ribavirin. Attaching one polyethylene glycol molecule (PEG) to the IFN molecule, PEG-Interferon was obtained and this reduces its renal elimination and causes the decrement of proteolysis and its antigenicity, increasing thus its action duration (6). The use of PEG-Interferon- $\alpha$  brings the response rate of a treatment close to 50% (in patients with HVC genotype non1) and 42-46% in patients with HVC genotype 1 (1,7). Presently there are 2 forms of PEG-IFN: PEG IFN  $\alpha$  2b of 12kd and PEG IFN  $\alpha$  2a of 40kd. The action mechanism of IFN is not fully understood but it is supposed that IFN induces in the infected hepatocytes some proteins that interact with the viral replication; IFN also has indirect immunomodulatory effects and anti-inflammatory effects (6).

Ribavirin is a nucleoside analogue with anti viral properties against many ARN viruses (it inhibits the viral reverse transcriptase). Its action mechanism is also not fully understood but it seems that it inhibits inosine monophosphate dehydrogenase, has immunomodulatory effects and, most probably, also mutagenic action for ARN viruses (6).

The present therapy consists in Ribavirin 800mg/day combined with PEG-IFN weekly for 24 weeks for the types 2, 3, 5 and 6, while for the genotypes 1 and 4 are necessary doses of Ribavirin of 1000mg/day for patients with a weight of <75kg and 1200mg/day, for patients whose weight is >75kg plus PEG-IFN for 48 weeks (6).

### The obesity effects on HVC infection and on the response to antiviral treatment

Besides the external factors (viral genotype) and the viral load at the beginning of the treatment, the response rate depends also on

the host's factors, such as the body mass index (BMI) which is in inverse ratio to the sustained viral response rate (SVR). The researchers study the connection between obesity, Diabetes Mellitus type 2 and the evolution of HVC infection. Three are three mechanisms proposed to explain the interference between obesity and treatment response (8). The first is based on the premise that obesity is an inflammatory condition which causes an abnormal immune therapy response. The second mechanism connects obesity with IR, fact which leads to fibrosis and the alteration of treatment response. The third one explains the low response rate in obese patients on the basis of the diminution of PEG-interferon biodisponibility.

### Obesity as an inflammatory state

The medical world has ceased to consider the fatty tissue a deposit, metabolically inactive tissue. It is known that adipocytes secrete several cytokines, some characteristic to the fatty tissue, which have various central and systemic effects. Some of these substances function as pro- or anti-inflammatory factors (9).

*Leptin*, one of the most studied adipokines, has a significant importance in obesity and alimentary ingestion control, but also pro inflammatory properties *in vitro*, by increasing some pro inflammatory cytokines such as IL-1 $\alpha$ , IL-6, IL-12 and TNF- $\alpha$  (tumor necrosis factor  $\alpha$ ) (10). It is known that fibrosis is a condition initiated by inflammation and that it appears as an exaggerated response to inflammation. On the other hand it seems that leptin plays an important part in activation the signal paths in hepatic stellate cells, contributing to intra-hepatic inflammation and fibro-genesis. There has been recently proved that leptin increases the nuclear factor kB and the expression of monocyte chemoattractant protein 1 (MCP-1) and of endothelial growth factor (VEGF). Leptin administration has caused in mice with chloroform induced hepatic disease a more significant growth of MCP-1 expression, inflammation and necrosis than in the case of those without leptin (11). Recent data suggest the probability that leptin resistance influences more the diminution of response rate to antiviral therapy than hyperleptinemia in obese patients (12).

*Adiponectin*, an anti-inflammatory cytokine, is involved in an inverse ratio with obesity. Thus

obesity increases fibrosis, independently of the increase of fibrosis caused by HVC presence. Fibrosis represents one of the causes of Interferon plus ribavirin therapy failure. The chronic inflammation caused by HVC, the increase of pro-inflammatory cytokines induced by obesity and the diminution of anti-inflammatory cytokines increases the oxidative stress and the inhibition of interferon action paths.

### Obesity – IR – combined therapy failure

The IR existence in obese patients is very well known. On the other hand the IR presence has been detected in patients chronically infected with HVC, Diabetes Mellitus type 2 being twice more frequent in comparison with patients non infested with HVC (13). Consequently one can speak about a vicious circle in which is trapped the patient chronically infected with HVC: HVC infection induces IR which causes hyperinsulinism and this induces then obesity.

Various studies have shown that IR associates with fibrosis extension in patients with HCC. Other studies bring more and more arguments to sustain the idea that HVC can cause IR. Insulin exercises its biologic effects through the substrate for the insulin receptor 1 (insulin receptor substrate 1, IRS1) and IRS2. The destruction of IRS1 leads to IR but not to Diabetes Mellitus, due to compensatory hyperinsulinemia. The destruction of IRS2 leads to Diabetes Mellitus, due to IR and disorder of insulin secretion. The mechanism which causes the HVC infection to lead to IR is not fully understood, but new studies intend to bring light in this issue. It has been observed that "core" HVC proteins inhibit the phosphorylation induced by insulin upon the subunit p85 of fostatidilinositol-3 kinase (PI3K) and of Akt which are downstream components of the IRS path in the lever (14). HVC mediates the dysfunction of insulin signaling paths by increasing the expression of the suppressor of cytokine signaling 3 (SOCS-3). The link between HVC and IR seems to be TNF alfa. The HVC "core" proteins increase the production of TNF alfa. This one phosphorylates IRS1 serine residues and increases SOCS3 production. Then SOCS3 inhibits Akt and PI3K phosphorylation (15). All these modifications block GLUT-4 translocation, hindering the cells to process

glucose (16). In transgenic mice that don't express SOCS3, the HVC infection did not lead to IR and this fact proves the SOCS3 involvement in IR. On the other hand the TNF alfa blocking with Infliximab can prevent IR apparition. Another mechanism which causes HVC to lead to IR could be the process in which an ubiquitin molecule is attached to IRS1 and IRS2 (process called "death kiss"), inducing their degradation by proteosomes action. It seems that the process of attaching the ubiquitin molecule is performed again through SOCS3, this protein directing IRS 1 and IRS 2 on the proteolysis path.

Besides the relation HVC – SOCS3 – IR – fibrosis – decreased IFN response, there is another way for HVC through SOCS3 to reduce the response to treatment, operating at the level of IFN action path.

IFN alfa acts through a receptor (IFNAR) made up of two subunits IFNAR1 and IFNAR2 (17). In men there have been identified an IFNAR1 form and three forms for the subunit IFNAR2: the long form IFNAR2c and two short forms IFNAR2a and IFNAR2b. Out of the three isoforms only IFNAR2c is involved in transmitting interferon signal, while the other short forms inhibit the IFN action by competing with IFNAR2c isoforms for IFN (18).

IFN- $\alpha$  attaches to the receptor and activates the IFNAR associated tirosinkinases, Janus kinase 1 (Jak1) and tirosinkinase 2 (Tyk2) which phosphorylate both IFNAR subunits. Their phosphorylation allows the attachment of STAT 1 and STAT 2 (signal transducer and activator of transcription factor 1 and 2), which are activated and freed as monomers in cytosol. They form here heterodimers and combine themselves with p48 protein to form the genetic factor 3 stimulated by IFN. This complex reaches the nucleus and attaches to the IFN-stimulated response element ISRE to initialize the target genes transcription including antiviral and immunoregulatory proteins (17). Through microarray techniques one was able to determine IFN target genes. Some of these code for Mxa proteins 2'-5' oligoadenylate synthetase (2'-5'OAS) and protein kinase R (PKR), which have important antiviral effects, or immunity modulating proteins (19).

On this IFN path there are some HVC stimulated substances which have a negative action. To this category belong the substances from SOCS family. There have been identified

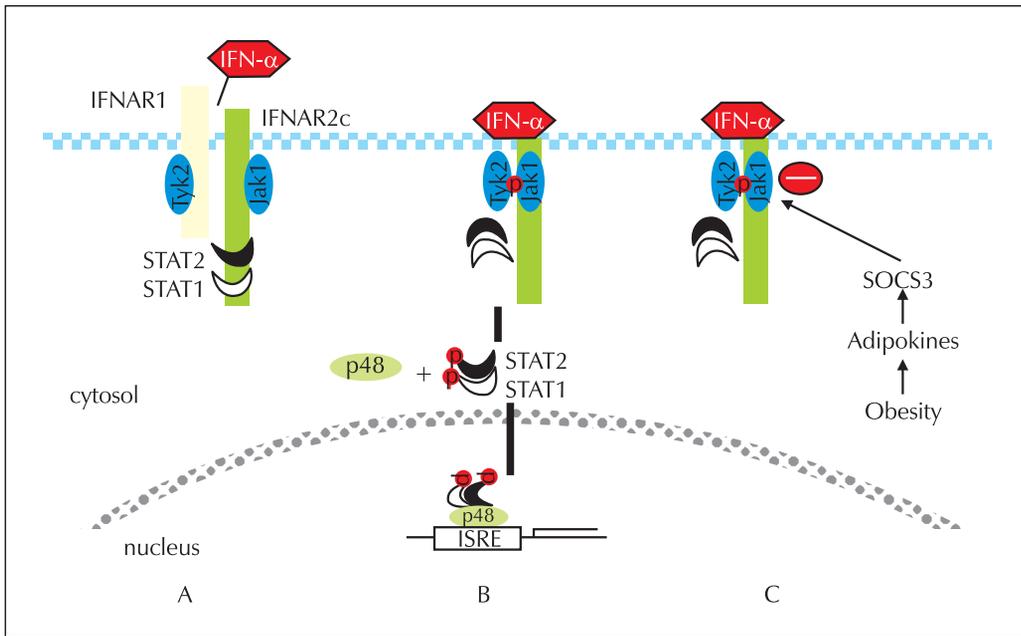
until now eight members of this family: SOCS1-7 and the cytokine-inducible Src-homology 2-containing protein CIS. SOCS 1-3 and CIS appear as a response to cytokine stimulation and operate through a negative feedback curve, inhibiting the initial cytokinic signal responsible of their own induction. The role of the others SOCS 4-7 remains still unknown (20).

The SOCS3 inhibitory effect is achieved by blocking the Jak/STAT path (1). To be more precise, SOCS3 and probably also other members of SOCS family inhibit IFNAR phosphorylation induced by Jak1. The whole IFN path is thus blocked (21) (Figure 1). In a recent research, Persico et al (2008) (22) study three SOCS3 genetic polymorphisms and discover significant differences between their effects. From these, SOCS3 -8464 A/C (rs12952093), -4874 A/G (rs4969170) and -1383 A/G, (rs4969168), the lowest response rate to antiviral therapy was observed in those with SOCS3 -4874 AA genotype. We can thus conclude that, besides the viral genotype, there is also the carrier genotype, which plays an important part, and patients respond differently to the same type of virus, due to their genetic heritage. Detailed researches are to be made in this domain in order to clarify the differences between the responses of the various SOCS3 genotypes and the possibility to counteract these effects. This discovery will certainly open new research possibilities for SOCS3 polymorphisms and will bring us close both to answers and to new questions.

Numerous experimental studies show that the overexpression of SOCS3 inhibits the antiviral activity and IFN- $\alpha$  induced signaling (23). Besides the SOCS family members there might be also other substances which have negative influence upon IFN path, such as protein tyrosine phosphatases (PTP) which dephosphorylate the activated tyrosine residues and tyrosine phosphatases 1 and 2.

### **IFN disponibility decrease in obese patients.**

According to this theory, the PEG-IFN subcutaneous injection leads in patients with obesity to the absorption of a small quantity. When the substances with a molecular weight higher than 15kd are injected subcutaneously, they are initially absorbed in the lymphatic system (24). In obese persons, the lymphatic



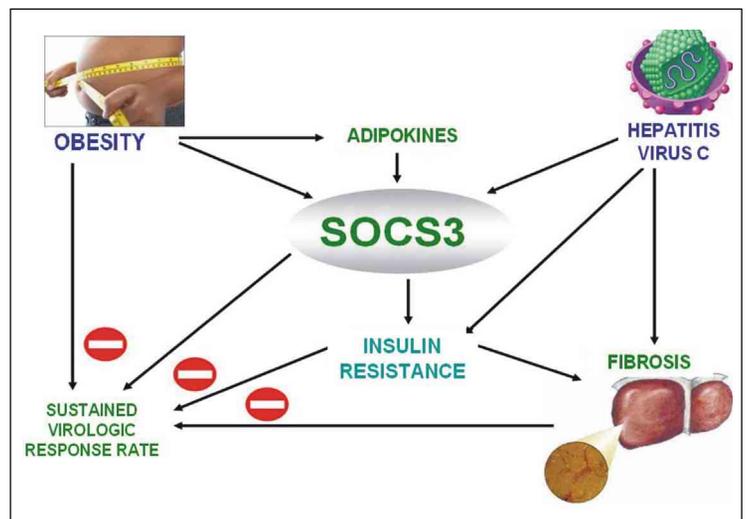
**FIGURE 1.** Schematic representation of IFN action molecular mechanism  
**A.** IFNAR receptor with its two subunits.  
**B.** IFN- $\alpha$  attachment to the receptor activates the path which leads to the transcription of several antiviral and immunomodulatory proteins.  
**C.** SOCS3 blocks Jak1 action and implicitly blocks interferon action (modified after Gao and colab. 2004).

drainage is disturbed and the lymphatic absorption of PEG-IFN is decreased. Obesity has different effects on the two PEG-IFN  $\alpha$  forms. The PEG-IFN  $\alpha$  2a distribution level is 8-12L/kg and it is not significantly influenced by body weight, while PEG-IFN  $\alpha$  2b has a distribution level of almost 1L/kg and varies with the weight (25). PEG-IFN  $\alpha$  2b is administrated depending on weight (1,5 $\mu$ g/kg/week), and PEG-IFN  $\alpha$  2a is taken in a fixed dose of 180 $\mu$ g/week (26).

Usually the medicines administrated depending on weight have a narrower therapeutic index. The dose is increased in conformity with body weight in order to obtain a certain concentration able to induce the desired therapeutic effect and decrease the individual variability.

**New interventional therapies in patients with obesity and viral hepatitis C (VHC)**

The discoveries regarding the way in which obesity influences the therapy response should be used to develop treatment strategies. Taking into account the fact that obesity is accompanied by a higher risk of reducing the treatment efficiency, one must consider losing weight before starting the antiviral treatment.



**FIGURE 2.** Schematic presentation of the way in which obesity decreases interferon treatment response.

The weight decrease in patients chronically infested with VHC has been associated with a decrease of steatosis, fibrosis and other activated hepatic star cells (27). Weight decrease has benefic effects also upon other associated diseases, such as: hypertriglyceridemia, arterial hypertension and IR (28).

Another strategy consists in IR treatment before or during the antiviral treatment. Collected data report a benefic effect of using metformin or thiazolidinediones (pioglitazone). The benefic effect is observed in the decrease of transaminases, histological features of hepatic fibrosis, inflammation, steatosis and the increase of insulin sensibility. Nevertheless further studies are needed to confirm these data. Another therapeutic option is represented by the omega 3 fatty acids. The benefic effects of polyunsaturated omega 3 fatty acids upon weight decrease and VHC replication inhibition as well as their insignificant negative reactions constitute strong arguments for recommending them to patients with CHC as food items and medicines (29-34).

We must also take into consideration the increment of antiviral treatment duration, of IFN  $\alpha$  doses and Ribavirin in conformity with IMC or HOMA-IR score, especially in patients infected with VHC type 1 (35).  $\square$

## CONCLUSION

The HVC infection remains one of the major medical problems, because of its high frequency, the limited therapy resources, the severe chronic complications and its association with Diabetes Mellitus type 2 and other severe extra hepatic manifestations; although the present therapy is expensive, the success rate

to eradicate the virus is only of de 10-50%; that is why there are necessary, besides the antiviral treatment, also other therapies which can influence the endogen factors (such as the carrier IR); It is thus compulsory to evaluate the anthropometric parameters in patients with HVC infection and quantify IR, in order to prevent type 2 diabetes mellitus and to slow down the hepatic injury progression; we can reduce IR by losing weight (under strict medical supervision), supervised physical exercise, adequate medication to sustain insulin sensibility (metformin, thiazolidinedione – need further studies), increased intake of omega 3 fatty acids and branched-chain aminoacids (leucine, isoleucine, valine); in the future one can try to use medicines which help to reduce SOCS3 expression, influence the adipokines secretor profile induced by obesity, or other ways through which HVC succeeds to harm the immunity system. Knowing the influence of obesity associated IR upon antiviral therapy response, hepatic star cell proliferation and fibrosis seriousness, we will be able to develop new therapeutic strategies in patients with obesity and HCC and also new criteria of initializing an antiviral therapy (presently there are no clear criteria for obese patients), including body mass index, abdominal circumference, HOMA-IR, adipokines doses, decreasing thus the long term costs.

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