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# Severe steatosis of transplanted liver in patient with genotype 3 hepatitis C virus infection

## Masywne stłuszczenie przeszczepionej wątroby u chorego zakażonego wirusem HCV o genotypie 3

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**Key words**

liver transplantation, HCV reactivation, liver steatosis, genotype 3

**Słowa kluczowe**

transplantacja wątroby, nawrót infekcji HCV, stłuszczenie wątroby, genotyp 3 HCV

S u m m a r y

**Introduction.** Hepatic steatosis is a common feature of CHC infection and can be related to both metabolic and viral specific factors. The prevalence of steatosis ranges from 40 to 86% and is more frequent and more severe in patients infected with HCV genotype 3. The recurrence of HCV infection after liver transplantation is universal but epidemiology of liver steatosis in patients infected with genotype 3 HCV after liver transplantation is poorly explored because of relatively small number of transplanted patients infected with HCV genotype 3.

**Aim.** The aim of the study was assessment of severity of steatosis of transplanted liver in patient infected with HCV genotype 3.

**Material and methods.** We present a patient in whom the liver transplantation was done due to decompensated liver cirrhosis with focus of hepatocellular carcinoma infected with genotype 3 HCV.

**Results.** Six months after orthotopic liver transplantation (OLTx), high HCV viremia in patient's serum – 2.4 x $10^7$ IU/ml and severe steatosis comprising 90% of hepatocytes with atypical fibrosis in liver biopsy was found.

**Conclusions.** We conclude that massive and rapid steatosis is connected in our patient primarily with genotype 3 infection and high HCV viremia as a result of intensive immunosuppression in the early time after LTx. The severity of steatosis was correlated with the liver fibrosis state. This observation has practical implication for management in patients after LTx. Patients infected with HCV genotype 3 and presenting with liver steatosis should be offered antiviral therapy, irrespective of other considerations, as quickly as possible.

S t r e s z c z e n i e

**Wstęp.** Stłuszczenie wątroby jest częstym zjawiskiem obserwowanym u chorych przewlekle zakażonych wirusem C zapalenia wątroby (HCV) i może być zależne zarówno od czynników metabolicznych, jak i związanych z samym wirusem. Częstość występowania stłuszczenia waha się w szerokich granicach 40-80% i jest bardziej nasilona u chorych zakażonych wirusem HCV o genotypie 3. Do nawrotu infekcji HCV w wątrobie przeszczepionej dochodzi w większości przypadków, ale epidemiologia stłuszczenia wątroby u chorych zakażonych wirusem HCV o genotypie 3 jest słabo poznana z uwagi na niewielką liczbę chorych przeszczepionych i równocześnie zakażonych tym genotypem wirusa HCV.

**Cel pracy.** Ocena nasilenia stłuszczenia w wątrobie przeszczepionej u chorego zakażonego wirusem HCV o genotypie 3.

**Materiał i metody.** Przedstawiamy przypadek chorego zakażonego wirusem HCV o genotypie 3, u którego przeszczepienie wątroby zostało wykonane z powodu niewyrównanej marskości tego narządu z ogniskiem raka wątrobowokomórkowego.

**Wyniki.** Sześć miesięcy po ortotopowym przeszczepieniu wątroby wykazano we krwi chorego wysoką wiremię HCV – 2,4 x $10^7$ IU/ml, a w badaniu histopatologicznym wątroby masywne stłuszczenie obejmujące 90% hepatocytów z obecnością nietypowego włóknienia.

**Wnioski.** Sądzimy, że w przedstawionym przypadku, nasilone i szybko postępujące stłuszczenie wątroby jest związane przede wszystkim z nawrotem zakażenia wirusem HCV o genotypie 3 w przeszczepionej wątrobie z wysoką wiremią będącą wynikiem intensywnego leczenia immunosupresyjnego we wczesnym okresie po transplantacji. Nasilenie stłuszczenia związane jest z pojawieniem się włóknienia w wątrobie. Ta obserwacja ma implikacje kliniczne u chorych po przeszczepieniu wątroby. Chorzy zakażeni wirusem HCV o genotypie 3, u których stwierdzono istotne stłuszczenie w wątrobie, powinni być kwalifikowani do pilnego rozpoczęcia terapii przeciwwirusowej, niezależnie od stopnia nasilenia włóknienia.

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## INTRODUCTION

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease which induces end-stage liver disease and liver insufficiency due to cirrhosis or hepatocellular carcinoma. Currently it is the leading indication for liver transplantation (LTx) in most countries. Unfortunately, reinfection of new liver begins during reperfusion of the graft and accelerates liver disease and recurrent HCV disease is the most frequent cause of graft loss. First histological patterns of acute HCV appear usually between 4 and 12 week after transplantation. After 1 year features of chronic of chronic HCV appear in 70-90%, and after 5 years in 90-95% recipients. Chronic hepatitis C (CHC) can progress to cirrhosis quickly, affecting 10-30% of patients within 5 years after LTx (1, 2).

Steatosis, which is defined as increased fat content of the liver, is one of the factors exerting a significant impact on both liver fibrosis progression and response to antiviral therapy in native liver. There are two types of steatosis: micro- and macrosteatosis. Microsteatosis is defined as hepatocyte fat globules smaller than the size of the nucleus without macrovesicular deposit. Macrosteatosis occurs when large fat globules take up most of the cytoplasm, displacing the nucleus to the periphery. There is consensus that macrosteatosis affects liver graft function and survival more than microsteatosis. The clinical parameters used in classifying steatosis include less than 30% – mild steatosis, 31-60% – moderate steatosis and more than 60% – severe steatosis (3).

In patients with chronic hepatitis C, the prevalence of steatosis ranges from 40 to 86%. Almost 80% of patients with steatosis have mild steatosis affecting less than 30% of hepatocytes. Steatosis is more frequent and more severe in patients infected with HCV genotype 3 because this viral genotype, more often and more significantly than other genotypes, would be directly involved in the accumulation of triglycerides in hepatocytes (so called "viral" steatosis). Additionally, especially in patients with genotype 3, the severity of steatosis correlates with the level of HCV replication (4). In our paper we present a patient infected with genotype 3 HCV with severe steatosis of the liver graft as the form of HCV reinfection.

## AIM

The aim of the study was assessment of severity of steatosis of transplanted liver in patient infected with HCV genotype 3.

## MATERIAL AND METHODS

We present a patient, who was a 57 years old man, who underwent liver transplantation for end-stage liver cirrhosis and focus of hepatocellular carcinoma (HCC) infected with genotype 3 HCV (an unknown pre-LTx viral load). The native liver removed at OLTx showed active cirrhosis and HCC focus. Steatosis was not evident on multiple sections of the explanted, native liver (fig. 1a, b, c).

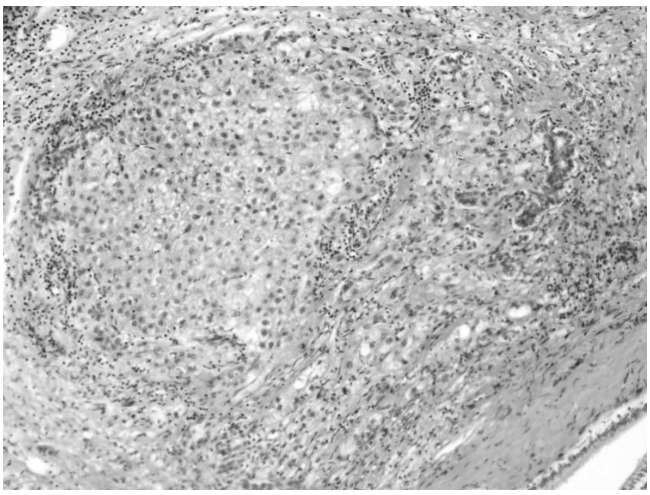

**Fig. 1a.** Histology of explanted liver. H&E x 200. Typical cirrhotic regenerative noduli separated by fibrous septa in explanted liver of HCV patient.

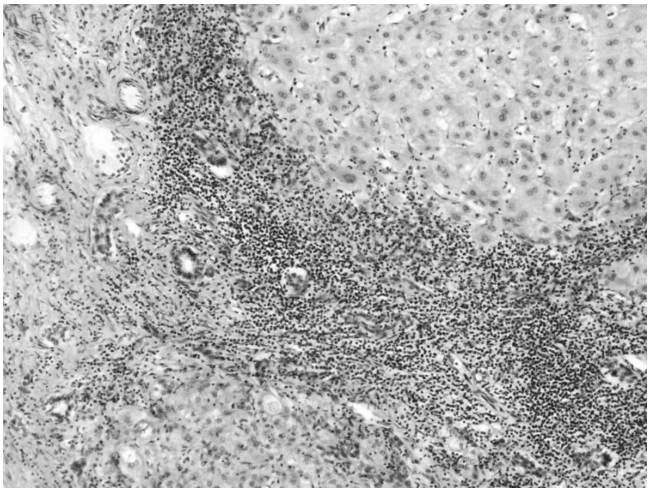

**Fig. 1b.** Histology of explanted liver. H&E x 200. Active cirrhosis. Dense mononuclear inflammatory infiltrate localized within and around the fibrous septa with interface hepatitis.

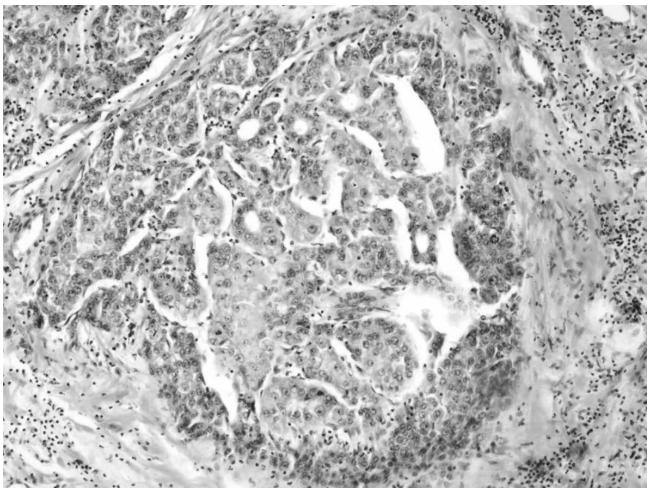

**Fig. 1c.** Histology of explanted liver. H&E x 200. Focus of hepatocellular carcinoma (trabecular-sinusoidal structure) in cirrhotic liver.

## RESULTS

The donor was a healthy 19-year old man without diabetes mellitus (DM), hyperlipidemia, or history of obesity and with a BMI of 24 and normal liver function tests. The donor wedge liver biopsy obtained after reperfusion was unremarkable (fig. 2a, 2b).

Post-LTx course of the patient was complicated with pneumonia and wound infection with prolonged healing but he was discharged on 45 post-OLTx day in very good condition with slighty elevated activity of alanine aminotransferase (ALT) (54 IU/l; normal range 10-40 IU/l) and bilirubin level (38.0 $\mu$mol/l; normal range 3.4-17.1 $\mu$mol/l) and normal activity of aspartate aminotransferase (AST), alkaline phosphatase, gammaglutamyl transpeptidase (GGT). During the subsequent weeks ALT and AST activity and bilirubin level more increased with maximum 6 months after LTx (ALT – 94 IU/l, AST – 50 IU/l, bilirubin – 49.6 $\mu$mol/l). In this time, high HCV viremia in patient's blood – 2.4 x $10^7$ IU/ml was found. Metabolic parameters: cholesterol, triglycerides, fasting glucose and insulin levels, as well as HOMA-IR index were normal. Patient was overweigh (BMI – 32 kg/m$^2$, his weigh increased by 9 kg after LTx). The liver allograft biopsy showed extensive fatty change (about 90%), predominantly of the macrovesicular type with mild inflammation (fig. 3a) and atypical fibrosis (fig. 3b). The patient was commenced on a 24-week course of peginterferon alfa-2a (Pegasys, Roche, Switzerland) and ribavirin (Copegus, Roche, Switzerland) which begun 10-months after LT. The ALT and bilirubin levels normalized after 20 weeks of therapy. Therapy was well-tolerated, without incidence of severe leucopenia or thrombocytopenia. Qualitative HCV RNA PCR after 24 (End Therapeutic Response – ETR) and 6 months after termination of therapy (Sustained Virological Response – SVR) was undetectable (lower limit of detection 15 IU/mL, Roche Amplicor assay, Switzerland) and ALT and bilirubin levels were still normal.

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## DISCUSSION

In the non transplant setting, HCV infection is recognized as an important risk factor for insulin resistance (IR) and hepatic steatosis. Steatosis is seen in 40-86 of liver biopsy samples from patients with chronic HCV infection (4). The development of hepatic steatosis can be viewed as a mechanism that promotes viral replication and HCV core protein is targeted to lipid droplets which act as intracellular storage organelles and are also required for virus assembly. Two main pathways for HCV-induced hepatitis steatosis have been proposed: metabolic and viral. The first involves a number of HCV-mediated mechanisms that result in IR and is seen in individuals infected with a non-3 genotype. The factors involved in the pathogenesis of HCV-induced IR are complex and include: direct effects on insulin signaling pathways, altered glucose uptake by hepatocytes, the production of proinflammatory cytokines and promote truncal obesity. In genotype 3-infected patients, there is a direct correlation between the HCV-RNA levels and the severity of steatosis which diminishes in patients who achieve a sustained virological response to antiviral

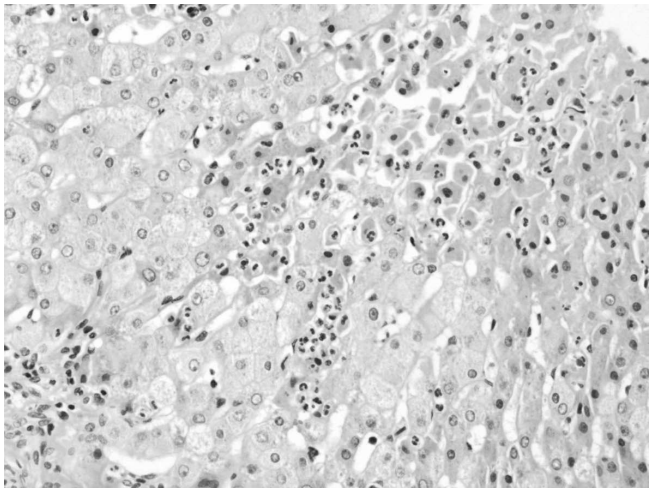

**Fig. 2a.** Histology of implanted liver. Postperfusion wedge liver donor biopsy. H&E x 200. One cell thick hepatocyte plates radiated out from the terminal venule. Note the accumulation of numerous neutrophils localized under the capsule within parenchyma, mark sites of hepatocellular necrosis. Typical features resulting from harvesting procedure.

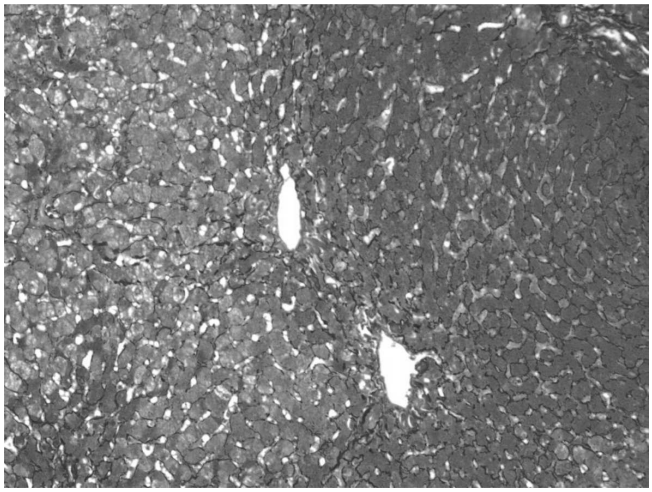

**Fig. 2b.** Histology of implanted liver. Wedge liver donor biopsy. Silver-methenamine x 200. Regular reticulin network between efferent hepatic venules.

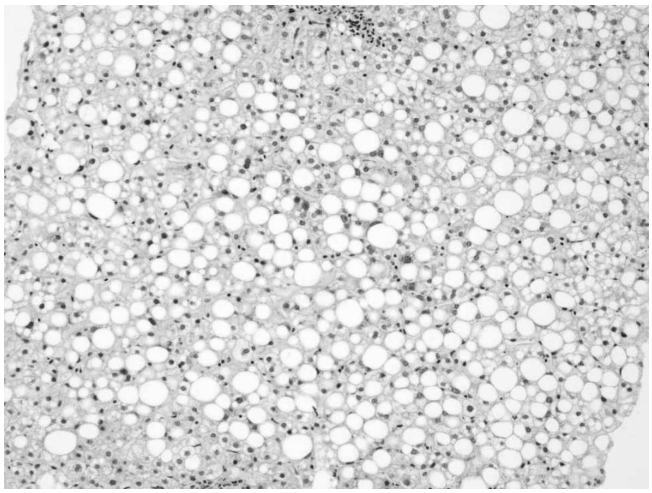

**Fig. 3a.** Histology of the liver 6 months after transplantation. Needle transplanted liver biopsy. H&E x 200. Macrovesicular steatosis. There are single large fat vacuoles displacing the nuclei to the edges of the cells present comprising as many as 90% of hepatocytes in particular fields with small foci of mononuclear inflammatory infiltrates.

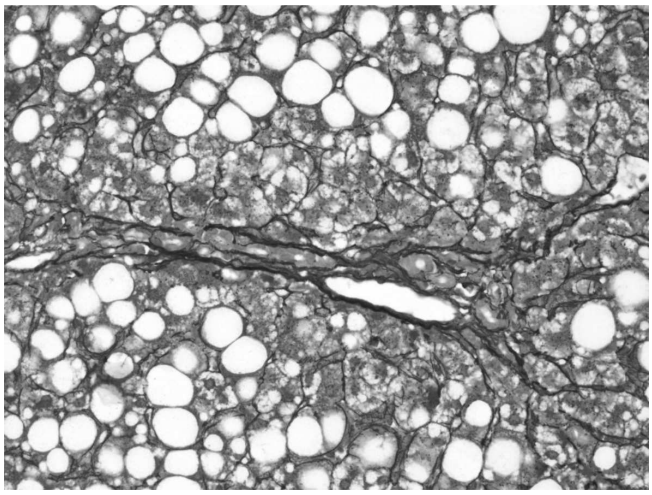

**Fig. 3b.** Histology of the liver 6 months after transplantation. Needle transplanted liver biopsy. Silver-methenamine x 400. Note the accentuation and atypical pattern of reticulin fibres. Slender bridging septa are present.

therapy; that suggest a direct effect of the virus on lipid metabolism: increased lipid production by the up-regulation of enzymes involved in lipid synthesis, reduced lipid breakdown by the down regulation of pathways involved in fatty acid oxidation and reduced lipid secretion by interference with the production of very low density lipoproteins (VLDL). Liver transplantation adds further factors to the relationship between HCV infection and steatosis because it has important effect on viral replication (5-7).

The epidemiology of liver steatosis in patients infected with genotype 3 HCV after liver transplantation is poorly explored because of relatively small number of transplanted patients infected with HCV genotype 3. In a paper by Gallegos-Orozco et al. (8) only 26 (13%) patients from 177 cohort of transplanted HCV patients were infected with genotype 3. The same percentage was observed in another study by Brandman et al. (9). In the same paper, steatosis (> 5%) was present in 29.6% of patients, but in 80% of patients the steatosis was grade I (mild steatosis). More intensive steatosis, moderate or severe was found in 13 and 7% of patients respectively. Interestingly, in this study, the proportion of obese individuals (BMI $\geq$ 30 kg/m$^2$) was lower in the steatosis group.

Graft steatosis leads to IL-17-mediated T-helper cell responses and activation of humoral immune responses to liver-associated self-antigens, which may contribute to allograft fibrosis in HCV recurrence. Graft steatosis is also associated with reduced production of interferon gamma, an important antiviral cytokine with a key role in HCV immune defenses, as well as an important inhibitor of hepatic stellate cells, which are the main fibrogenic cell type within the liver (1, 10).

Fibrosis progression with HCV recurrence is determined by interaction of donor, viral, transplant and recipient factors. Well-defined donor and post-LT factors for fibrosis progression and graft loss include advanced donor age, corticosteroid bolus treatment for acute cellular rejection (ACR), cytomegalovirus infection and preservation injury (11). In presented case, donor was young man, any incident of ACR was noted, as well as CMV reinfection, and cold ischemic time (CIT) was low (4.42 h). Recipient factors of more rapid fibrosis such as insulin resistance, gender (female) in presented case were absent. But, is well known, that patients who undergo LT for HCV have variable susceptibility for severity of HCV recurrence independent of donor or transplant factors (12).

The inflammatory grade of the explanted organ in patients undergoing liver transplantation for HCV was a significant predictive factor for progression to advanced fibrosis with HCV recurrence (13). In the second study presence of periseptal or interface hepatitis in explant were associated with significant HCV recurrence after LT (14).

Summarize, genotype 3 HCV, pre-LT hypertension and donor age proved to be independent predictors of postransplant steatosis which is connected with fibrosis progression. The patients with viral load over 150 000 IU/ml before LT (13) with inflammatory process in native liver had a greater risk of fibrosis progression. In our case the composition of that factors: genotype 3 infection, native liver inflammation and probably high viremia before LT caused severe steatosis with fibrosis and qualification for the rapid initiation of treatment. After therapy, SVR was achieved with patients with normalization of aminotransferase activity and bilirubin level normalization.

Several small studies have reported that treatment of recurrent HCV infection after orthotopic liver transplantation (OLT) with peginterferon (PEG-IFN) or conventional IFN plus ribavirin resulted in SVR rates ranging from 7 to 48%, with most studies reporting rates between 20 and 37%. A recent systematic review has indicated that 30% of patients treated with PEG-IFN

plus ribavirin will attain SVR; however, treatment discontinuation was also frequently recorded, with over 25% of patients failing to complete therapy. Common reasons for treatment discontinuation include fatigue, depression and cytopenias (12). In the recent study by Gordon et al. (12), the SVR ratio after LTx on peginterferon/ribavirin therapy regimen was also low and depended on HCV genotype. Total SVR for 125 treated patients was 28.8%, but it achived 23.8% in genotype 1 and 55.0% in genotype 2/3. Patients received peginterferon and ribavirin in dose-escalating regimen and LTx was to occur at least 3 months, but not longer than 3 years before enrollment. In other paper (13), SVR rates in patients starting antiviral therapy within 6 months of LTx, 6-12 months and > 1 month post-LT were 15, 30 and 30% respectively. This could be related to higher levels of immunosuppression and limited tolerance in the early post-LT-period. In our case, therapy was started in optimal time – ten months after LT and patients received full dose of treatment from the start of therapy. Despite the acute steatosis, the treatment was ended with success – SVR achievement.

## CONCLUSIONS

In our Department, six months after LTx, viremia and protocol liver biopsy are performed in all patients with HCV infection. Up to now, we did not found features of so significant steatosis in our chronic hepatitis C patients even used a similar pattern of immunosuppressive therapy. We conclude that massive and rapid steatosis is connected in our patient primarily with genotype 3 infection and high HCV viremia as a result of intensive immunosuppression in the early time after LTx. The intensity of steatosis was correlated with the liver fibrosis state. This observation has practical implication for management in patients after LTx. Patients infected with HCV genotype 3 and presenting with liver steatosis should be offered antiviral therapy, irrespective of other considerations, as quickly as possible.

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