

RECURRENT AND *DE NOVO* NON-ALCOHOLIC STEATOHEPATITIS FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION

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ABSTRACT – *Background* – Non-alcoholic steatohepatitis was coined in 1980 to describe pathological and clinical features of non-alcoholic disease associated with pathological features, commonly seen in alcoholic-liver disease itself. It is now a well-recognised cause of end-stage liver disease and a rare cause of orthotopic liver transplantation. A small number of cases with recurrent non-alcoholic steatohepatitis following liver transplantation have been reported, however de novo non-alcoholic steatohepatitis in the liver allograft is not well recognised. *Aims/Results* - We report four cases of non-alcoholic steatohepatitis following orthotopic liver transplantation describing the factors related with the pathology. The recurrence of fatty infiltration occurred within 21 months and transition from mild steatosis to non-alcoholic steatohepatitis and early fibrosis was observed within 60 months post transplant in all four patients. All four cases had association with one or multiples risk factors (obesity, type 2 diabetes and/or hyperlipidemia). *Conclusions* - Management of this risk factors may play a therapeutic role in the prevention of recurrent and de novo non-alcoholic steatohepatitis following orthotopic liver transplantation.

HEADINGS – *Fatty liver. Hepatitis. Liver transplantation. Diabetes mellitus. Recurrence.*

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INTRODUCTION

Nonalcoholic steatohepatitis (NASH) is a clinicopathological entity characterised by the development of histological changes in the liver that are nearly identical to those induced by excessive alcohol intake, but in the absence of alcohol abuse⁽⁷⁾. NASH is a disease of emerging identity and importance, and is now considered as one of the commonest liver diseases in western countries. Establishing a diagnosis requires the secure exclusion of alcohol abuse and viral hepatitis as an alternate cause of liver injury. The gold standard of diagnosis is liver biopsy.

The cause remains unclear but it is frequently associated with severe obesity; especially abdominal adiposity. A substantial weight loss following gastroplasty⁽⁴⁾ is accompanied by a marked reduction in the prevalence and the severity of the various biological abnormalities of the metabolic syndrome and, concomitantly, by an important regression of liver steatosis in most obese patients. However, in some patients, this rapid and drastic weight loss may result in a mild increase in inflammatory lesions (hepatitis), which might result from the rapid mobilization of fatty acids or cytokines from adipose tissue. Non-insulin-dependent diabetes mellitus (NIDDM) is reported to occur in up to 50% to 75% of patients with NASH, although as few as 20% have had NIDDM in some series. Hyperlipidaemia is associated with NASH in up to 20% to 80% of patients, sometimes with concurrent diabetes mellitus⁽¹¹⁾. Other conditions implicated include short bowel syndrome, long-term administration of parenteral hyperalimentation, abetalipoproteinemia or hypobetalipoproteinemia and Weber-Christian disease.

Unfortunately, NASH, although a common entity, is without a satisfactory treatment. Weight reduction, optimum blood glucose control, and elimination of medications implicated in causing the disorder are sensible first steps in treatment. Drug therapy should not be used unless there is histologic evidence of necro-inflammatory injury and/or fibrosis. Ursodeoxycholic acid (UDCA) has been reported to be of benefit in non-controlled trials and anecdotal case reports⁽¹⁵⁾.

The natural history of the disease remains unknown, it is clear that some patients may follow an indolent course whereas some, 20% or more, may develop cirrhosis. A small number of patients progress to end stage liver disease and require orthotopic liver transplantation (OLT).

There is recent evidence to suggest that some cases of cirrhosis previously classified as cryptogenic may have developed as a consequence of NASH⁽⁵⁾. A small number of cases with recurrent NASH following liver transplantation have been reported⁽¹²⁾, however *de novo* NASH in the liver allograft is not well recognised.

We report four cases of NASH following OLT, where two patients had previous diagnosis of cryptogenic cirrhosis, one primary biliary cirrhosis and one with alpha-1 anti-trypsin deficiency and iron overload.

MATERIALS AND METHODS

Medical records of all four patients were reviewed. Transplantation for all cases was performed between May 1992 and December 1995. Follow up was between 5 and 7 years. Six hundred seventeen orthotopic liver transplantation was performed at the liver unit from May 1992 to December 1995.

A complete pre and post transplant evaluation including laboratory and histological examinations had been undertaken in each patient. Medical records were reviewed with regard to patient demographics, height, weight, biochemical parameters, graft histology and major clinical events.

The diagnosis of NASH was unequivocally established in all patients based on the following criteria:

- a) persistent abnormal liver biochemistry for 3 months;
- b) liver histology: histological features relating to steatohepatitis (fatty change, Mallory's hyaline, parenchymal neutrophils, and pericellular fibrosis) were assessed and graded semi quantitatively on a scale of 0-3: 0 (absent), 1 (mild), 2 (moderate), 3 (severe).

For fatty change:

- 1 = fatty droplets <25% of hepatocytes
- 2 = fatty droplets in 25% to 50% of hepatocytes
- 3 = fatty droplets in >50% of hepatocytes

For fibrosis:

- 1 = zonal fibrosis
- 2 = bridging fibrosis (central-central or central-portal)
- 3 = nodule formation (cirrhosis)

- c) appropriate exclusion of other liver disease such as alcoholic liver disease, viral hepatitis, autoimmune hepatitis, drug induced liver disease and rejection.

Individuals with a body mass index (BMI) greater than 30, have been recognised to be obese. BMI was calculated using the formula: weight in kilograms/(height in meters)².

Immunosuppression therapy was based in tacrolimus (with azathioprine in one case and mycophenolate mofetil in another) in two cases and cyclosporin (neoral) plus azathioprine in other two cases. Glucocorticoid therapy was discontinued after 3 months according to the unit protocol.

RESULTS

Patients characteristics pre-transplantation

The group consisted in two males and two females with ages varying between 45 and 66, where both women had slightly elevated age (60/66) comparing with the two men (45/45). The characteristics of these

patients at the time of initial pre-transplant evaluation are summarised in Table 1. The presence of NASH was known before transplantation in one patient (case 4). Two patients (case 1 and 4) were obese (BMI >30). Two patients were diabetic previous OLT (case 2 and 4), and cases 2, 3 and 4 had mild to moderate hyperlipidaemia. Median time for the development steatohepatitis was 43.7 months.

Outcome of liver transplantation

The features post-OLT in all four patients is summarised in Table 2. All the patients are alive with no graft failure after being followed for up to 5 years (5 to 7 years).

With regard to the biochemical parameters, no characteristic pattern was identified for the development of recurrent or *de novo* NASH in the graft. The liver enzymes were abnormal in all patients at the time of the biopsy, with AST/ALT ratio >1 in 50% of them. Iron studies

were abnormal in the patient (case 1) with previous diagnosis of alpha-1 antitrypsin and iron overload (HFE negative), needing venesection after transplantation.

Three patients (cases 2, 3, 4) maintained high levels of cholesterol with one developing associated hypertriglyceridemia. Patient 1 developed high levels of triglyceride isolated. The two patients previously obese (1 and 4) maintained their body weight above ideal after OLT.

Diagnostic criteria for steatohepatitis post-OLT

In all cases, there was development of moderate to severe macrovesicular steatosis (Fig. 1) accompanied by ballooned hepatocytes and Mallory's hyaline (Fig. 2). All biopsies are summarised in Table 3. In some cases special techniques (Ubiquitin immunostaining) were required to demonstrate Mallory's hyaline (Fig. 3).

TABLE 1 – Patients characteristic pre-transplantation

Patient	Sex	Age	Indication for OLT	Pre OLT risk factors for NASH	Pre OLT histological evidence of NASH
1	Male	45	α-1 antitrypsin deficiency and iron overload	Obese	No
2	Male	45	Cryptogenic cirrhosis	Diabetic	No
3	Female	66	Primary biliary cirrhosis	Diabetic	Yes
4	Female	60	Cryptogenic cirrhosis hyperlipidemic	Morbid obesity	

TABLE 2 – Biochemistry pre and post-transplantation: post-transplant data at the time of the biopsy with NASH

	CASE 1		CASE 2		CASE 3		CASE 4		TOTAL POS OLT	
	Pre OLT	Post OLT	Pre OLT	Post OLT	Pre OLT	Post OLT	Pre OLT	Post OLT	AVERAGE	RANGE
BMI ¹	30,2	35	28	29	25	26	43,5	38,4	32,1	25-43,5
AST ²	57	47	70	104	244	143	60	56	87,5	47-143
ALT ³	60	55	85	107	190	90	45	54	76,5	55-107
AST/ALT	0,95	0,85	0,82	0,97	1,2	1,5	1,3	1,03	1,08	0,85-1,5
BILIRUBIN ⁵	75	12	56	18	75	18	158	14	15,5	12-18
GGT ⁶	60	87	44	75	56	85	54	94	85	75-94
ALBUMIN ⁷	28	42	26	45	29	42	25	45	43,5	42-45
FERRITIN ⁸	2420	1754	170	120	170	149	270	245	567	120-1754
TRANSF.SAT	80%	64%	22%	20,7%	26%	25%	35%	24%	33,4%	20,7-64%
CHOLESTEROL ⁹	3,25	3,6	5,4	5,1	6,8	6,6	6,3	5,5	5,2	3,6-6,6
TRIGLYCERIDES ¹⁰	1,98	4,25	5,1	4,25	1,95	1,51	1,9	1,98	2,99	1,51-4,25
HDL ¹¹	1,2	0,97	0,9	0,74	0,98	1,36	1,46	1,35	1,1	0,75-1,36
HbA1c ¹²	5,2%	5,5%	7,2%	7,5%	5,3%	5,6%	7,7%	8,2%	6,7%	5,5-8,2%

¹BMI (Body Mass Index): normal BMI = 18.5 to 24.9 kg/m², overweight = 25.0 to 29.9 kg/m², obesity = 30.0 to 39.9 kg/m², and morbid obesity > 40 kg/m²

²AST = 5 – 43 U/L, ³ALT = 5-35 U/L, ⁴Bilirubin = 1 – 22 mmol/L, ⁶GGT = 9 – 50 U/L, ⁷Albumin = 34 – 51 g/L, ⁸Ferritin = 10 – 300 mmol/L, ⁹Cholesterol < 5 mmol/L, ¹⁰Triglycerides < 2,3 mmol/L, ¹¹HDL = men > 1,15 mmol/L, women > 1,4 mmol/L, ¹²HbA1c = 3,5 – 6,5%

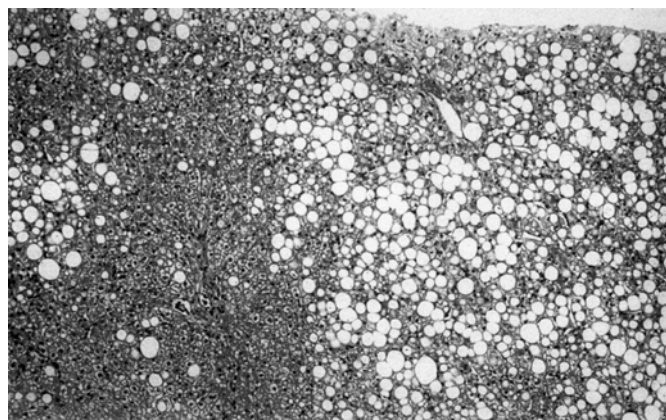


FIGURE 1 – Severe macronodular steatosis in a biopsy 22 months post-OLT (patient 1)

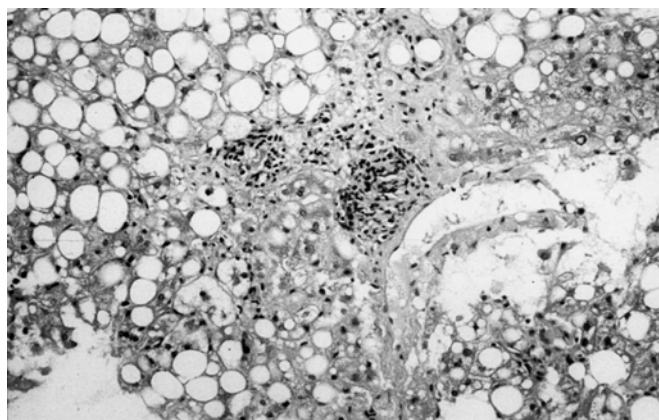


FIGURE 2 – Steatohepatitis in a biopsy 64 months post-OLT (patient 2). Ballooned hepatocytes contain Mallory's hyaline. There is an inflammatory infiltrate including neutrophils

TABLE 3 – Histological findings Post-OLT

Patient	Months post-OLT	Fatty change	Mallory's hyaline	Neutrophils	Fibrosis	Main diagnosis
1	22	3	1	1	1	1) Steatohepatitis
	49	3	1	0	1	1) Steatohepatitis
2	12	2	0	0	1	1) Fatty change? Cause
	24	2	0	0	1	1) Fatty change? Cause
	37	2	0	0	1	1) Fatty change? Cause
	64	3	2	1	2	1) Steatohepatitis/Fibrosis
	54	2	1	1	2-3	1) Steatohepatitis/Fibrosis with early cirrhosis
3	1	2	0	0	0	1) Moderate acute rejection
	4	1	0	0	0	1) Mild acute rejection
	24	3	0	0	1	1) Mild chronic hepatitis 2) Severe fatty change? Cause
	27	2	0	0	1	1) Moderate chronic hepatitis 2) Fatty change? Cause
	45	1	2	1	1	1) Mild chronic hepatitis 2) Steatohepatitis/Fibrosis
4	29	3	0	0	1	1) Fatty change? Cause
	44	2	1	1	2-3	1) Steatohepatitis/Fibrosis with early cirrhosis
	70	2	1	1	2-3	1) Steatohepatitis/Fibrosis with early cirrhosis

Development of fibrosis

In all biopsies there were features of pericellular fibrosis (Fig. 4). Pericellular fibrosis was also noted in some biopsies showing fatty changes without other diagnostic feature of steatohepatitis.

In two cases (patients 1 and 3) fibrosis persisted as a mild lesion confined to perivenular lesions (acinar zone 3).

In the other two cases (patients 2 and 4) there was a progressive fibrosis resulting in early micronodular cirrhosis (Fig. 5). The time for developing cirrhosis after OLT for these patients was 84 and 44 months, respectively.

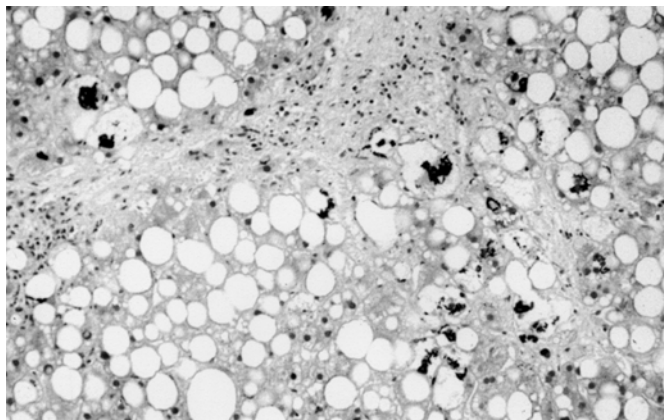


FIGURE 3 – Steatohepatitis in biopsy 64 months post-OLT (same biopsy as Figure 2) Ubiquitin immunostaining reveals abundant Mallory's hyaline like material in perivenular hepatocytes (zone 3)

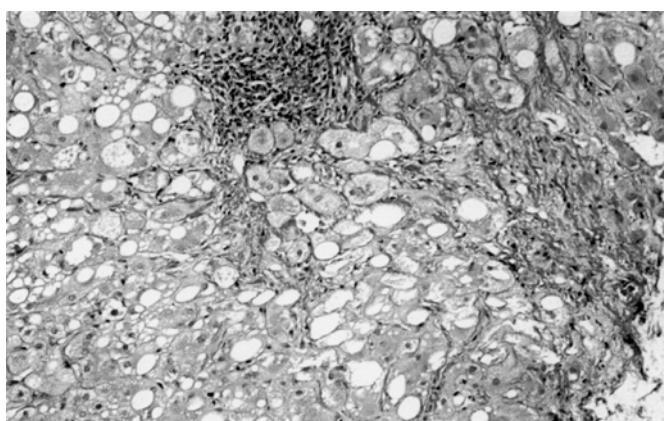


FIGURE 4 – Pericellular fibrosis in a biopsy 44 months post-OLT. Haematoxylin Van Gienson

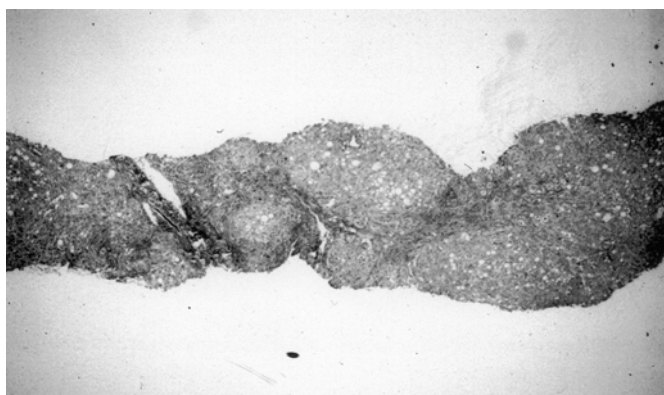


FIGURE 5 – Micronodular cirrhosis in a biopsy 44 months post-OLT (same biopsy as Figure 4). Haematoxylin Van Gienson

DISCUSSION

A small number of cases with recurrent NASH following liver transplantation have been reported⁽¹²⁾, however *de novo* NASH in the liver allograft has not been, so far described. Recurrences after liver transplantation suggest persistence of the pathogenic mechanisms responsible for the original disease or introduction of new mechanisms that produce the same histologic pattern.

According to CZAJA⁽⁶⁾, two hypotheses of pathogenesis are pertinent: the metabolic and free radical hypothesis. The metabolic hypothesis presumes that there is decreased tissue sensitivity to insulin^(3,17) reducing tissue responsiveness, most commonly occurring in conjunction with diabetes and/or obesity. This lack of responsiveness results in relative hyperinsulinism, which in turn impairs beta-oxidation of fatty acids and promotes hepatic steatosis⁽¹⁶⁾. Free fatty acids (especially the unsaturated variety) can impair hepatocytic membrane integrity, depress enzyme activity, directly injure blood vessels, cause mitochondrial swelling, and increase lysosomal fragility⁽¹⁾.

The free radical hypothesis presumes that endotoxemia is the basis for disturbances in hepatic lipid metabolism. Decreased hepatic clearance of endotoxin by the newly implanted liver and/or excessive endotoxin production from occult infection or intestinal bacterial overgrowth are unproven requisites for the hypothesis. Endotoxin can produce peroxisomal dysfunction, stimulate super oxide and hydrogen peroxide production, and release tumor necrosis factor from monocytes^(8, 10). These consequences can result in hepatic steatosis and liver cell necrosis. Indeed, depletions of glutathione, vitamin E, and superoxide dismutase occur commonly in systemic illness^(8, 10), and similar deficiencies of intrinsic antioxidants may be present before and after liver transplantation.

In this small series we report four patients with NASH post-transplantation, where in two cases NASH could be the cause of end-stage liver disease and in the other two NASH was a new event in the graft.

All four patients are alive with no graft failure after follow-up up to 5 years. The recurrence of fatty infiltration occurred within 21 months and transition from mild steatosis to NASH and early fibrosis was observed within 60 months post transplant in all four patients. This appears far more rapid and aggressive than the progression in the native liver reported in the literature, although the rate of progression of NASH is debated⁽²⁾. Glucocorticoids are frequently implicated in the pathogenesis of steatosis and steatohepatitis; however there is little evidence to support this association⁽¹⁴⁾ and all four patients were withdrawn from steroid within 3 months post-transplantation, following the unit policy.

It has been recognised that some patients with NASH may follow a relatively benign course whereas some others progress to cirrhosis

and its inherent complications. The reason for different course of patients suffering apparently the same condition suggest that a less aggressive mechanism of liver injury operates in some patients whereas, in others, NASH seems to be potentiated by aggravating factors leading to increased fibrosis and cirrhosis. In a large series published by ANGULO et al.⁽²⁾ shown that some of these factors include older age, greater BMI (obesity) and presence of diabetes mellitus. In all four patients described, risk factors pre and post-OLT for developing NASH were identified. Two patients (cases 2 and 4) were diabetic pre-OLT, on insulin control. None of the patients has developed new-onset diabetes post-transplant.

The relation of body weight and recurrence or development of steatohepatitis suggests that the persistence of obesity and/or weight gain play a role in the pathogenesis of post-transplant NASH. Again, obesity was observed in two of four patients.

Hyperlipidaemia occurs frequently in the transplant setting and is attributed to the immunosuppression. All patients developed hyperlipidaemia post-OLT, three with hypercholesterolemia, one associated hypertriglyceridemia and one patient (case 1) developed high level of triglyceride alone.

Liver function tests were abnormal in all patients, at the time of the liver biopsy. With regard to biochemical parameters, no characteristic pattern was identified. The AST/ALT ratio less than 1 was found in two patients and more than 1 in the other two. The two patients presented with early cirrhosis had AST/ALT more and less

than 1 respectively, not reflecting some findings that change from <1.0 in uncomplicated NASH to > than 1 when cirrhosis is present. Iron studies were abnormal in one patient (with α -1 antitrypsin deficiency and iron overload).

In conclusion, all four cases of NASH post-transplantation demonstrate that recurrence and new onset of steatohepatitis in the liver allograft is possible. These cases-reports can suggest the hypothesis of perpetuation or creation of a pathogenic process that is specific for the host. Obesity, diabetes, drugs or alcohol abuse, chronic viral infection and bulimia are examples of host-dependent disorders that could afflict the native and implanted liver in a similar fashion.

The time of recurrence may be shorter than in the native liver and some patients develop hepatic fibrosis within 2 years. Indeed, NASH must be considered the cause of end-stage liver disease for which OLT was indicated in two cases. In those who were transplanted for other indications, the association with risk factors, such as obesity, diabetes and hyperlipidaemia, probably relates for the development of *de novo* NASH.

Although biochemical tests are not diagnostic, steatosis/steatohepatitis can be a cause for sustained abnormalities of liver function tests in patients post-transplantation. Management of obesity, diabetes mellitus and hyperlipidaemia may play a therapeutic role in the prevention of recurrent and *de novo* NASH following OLT.

Garcia RFL, Morales E, Garcia CE, Saksena S, Hübscher SG, Elias E. *Recorrência e "de novo" esteatohepatite não-alcoólica após transplante ortotópico de fígado. Arq Gastroenterol 2001;38(4):247-253.*

RESUMO – Racional – O termo NASH (esteatohepatite não-alcoólica) foi introduzida em 1980 para descrever "características patológicas e clínicas de doença não-alcoólica observadas comumente na própria doença alcoólica". Atualmente é causa reconhecida de doença hepática crônica e rara indicação de transplante hepático. Pequeno número de casos de recorrência de NASH pós-transplante foram descritos na literatura; entretanto, *de novo* NASH no enxerto jamais foi relatado. **Objetivos/Resultados** - Reportam-se quatro casos de NASH pós-transplante, descrevendo fatores associados a esta patologia. A média de recorrência da infiltração gordurosa foi de 21 meses com transição para esteatohepatite/fibrose aos 60 meses pós-transplante. Os quatro casos possuíam associação com um ou vários fatores de risco (obesidade, diabetes tipo 2 e/ou hiperlipidemia) no período que se seguiu ao transplante. **Conclusão** - Manejo destes fatores provavelmente exercem papel terapêutico na prevenção da recorrência e no aparecimento de NASH no pós-transplante.

DESCRITORES – Fígado gorduroso. Hepatite. Transplante de fígado. Diabetes mellitus. Recidiva.

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