Stéatose hépatique: un problème émergent comprendre et agir

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CHU Dijon
Strasbourg, 9 septembre 2008

Dear colleagues,
First of all I would like to thank the organizers for inviting me at this conference to talk about liver steatosis in HIV infected patients
QUESTIONS

• De quoi parle-t-on?
• Combien de patients concernés?
• Quels patients concernés?
• Pourquoi?
• Quelles conséquences?
• Comment dépister et diagnostiquer?
• Comment traiter?
Hepatic steatosis is defined as collections of triglycerides within hepatocytes at an abnormal frequency. Indeed triglycerides can be observed in some hepatocytes in normal livers, at a frequency which is not clearly established. Thus the diagnosis of steatosis does not seem to be relevant when the proportion of fatty hepatocytes is low.

Steatohepatitis is defined by the presence of cytologic ballooning, scattered inflammation, perisinusoidal fibrosis and Mallory hyaline deposition in addition to steatosis.

There are two patterns of liver fat accumulation:
- **Macrovesicular steatosis** where a single large fat droplet displaces the nucleus in the hepatocyte,
- **Microvesicular steatosis** characterized by multiple intracellular droplets within the hepatocyte, assessable by using greater magnification, often associated with mitochondrial injury.

From a practical point of view, a semi-quantitative approach is used to assess the importance of steatosis. A universally accepted grading and staging system has not been established yet. Moreover, in most studies, either only macrovesicular steatosis is quantified and taken into account, or no difference is made between the two types. It has to be kept in mind when analyzing studies data, since these two types may be associated with different pathogenic mechanisms and different disease evolutions.
Combien de patients concernés?

Stéatose hépatique chez les sujets coïnfectés VIH-VHC
Several causes may be associated with liver steatosis in HIV infected patients. Toxic causes, in particular alcohol, are a well known and important risk factor of steatosis.
But, because of the limited time allowed to this presentation, we will focus on non-alcoholic fatty liver disease.
First, we are going to see the host related factors associated with liver steatosis.
Hepatic free fatty acids (or FFAs) have several origins:

1) They are synthesised de novo within the hepatocytes or
2) they are taken up by the liver from plasma FFAs released from adipose tissue, or
3) they are generated in the liver from the hydrolysis of intestinal chylomicrons

Then, hepatic FFAs
1) go into mitochondria to undergo mitochondrial beta-oxidation allowing ATP production, or
2) are esterified into triglycerides

These hepatic triglycerides either
1) are secreted as very low density lipoproteins (VLDL), corresponding to a droplet of triglycerides, cholesterol, phospholipids and a large protein termed apolipoprotein-B, or
2) accumulate as fat droplet within the cytoplasm of hepatocytes
Obesity increases plasmatic FFAs and also causes resistance to the action of insulin, which results in a decrease of glucose uptake and use by adipocytes and muscle cells. The increased load of FFAs within hepatocytes results from an increased uptake from plasma and an increased de novo synthesis. This leads to increased beta-oxidation, not sufficient to control the excess in FFAs, leading to increased formation of triglycerides, which are partly stored in the cytoplasm, causing macrovesicular steatosis.
In some patients, this steatosis remains isolated, but in other overweight patients necrosis and inflammatory infiltrate will develop associated to steatosis, defining steato-hepatitis. In these patients, the activity of respiratory chain complexes is decreased, with low ATP hepatic levels, and the accumulation of reactive oxygen species and lactate, leading to depletion of mitochondrial DNA. What makes steatosis evolve into steatohepatitis still needs to be demonstrated clearly, but it seems likely that the “two hits” hypothesis reflects what happens.
Another hit in the pathogenesis of steatosis and steatohepatitis may be HIV infection per se or antiretroviral treatment.
Quels patients et pourquoi?

VIH

- Données biopsiques et autopsiques à l’ère pré-HAART
  - Prévalence stéatose: 30 to 50%
  - Rôle propre du VIH?
  - Rôle des comorbidités
- Syndrome lipodystrophique
  - Lipoatrophie - INTI
  - Inhibition de l’ADN polymerase gamma (puissance inhibitrice ddc>ddI>d4t >> autres)
  - Augmentation du stress oxydatif
  - Lipohypertrophie centrale - IP – syndrome métabolique
    - Insulino- résistance
    - Interférence avec apo-B

In pre-treatment era, steatosis was found in thirty to fifty percent of HIV infected patients. However, it is likely that comorbidities such as alcohol, malnutrition and chronic illnesses played a significant part in these end stage or post-mortem evaluations.

Now, the potential role of antiretroviral therapy appears to be important.

Nucleoside Reverse Transcriptase inhibitors are the backbone of most antiretroviral therapies. But they have been compromised by long term toxicities, in particular mitochondrial toxicity, with an estimated incidence ranging from 2 to 8 percent per year. Several cases of fatty liver disease with fatal outcome have been reported in HIV infected patients treated with NRTIs.

Mitochondrial toxicity seems to be related to the inhibition or the alteration of the human DNA polymerase gamma. This leads to liver mitochondrial DNA depletion and reduced respiratory chain enzyme activity. It occurs more frequently with D drugs (ddC, ddl, d4t) than with non-D drugs, as recently shown in eighty HIV-HCV coinfected subjects. Toxicity may be increased by concomitant use of other drugs such as ribavirin or hydroxyurea with ddl.

In addition, it is possible that NRTIs (in particular d4T) are associated to a higher risk of insulin resistance, and thus to a higher risk of liver steatosis via another way.

Protease inhibitors have been associated with central lipohypertrophy, dysplipidemia and insulin resistance. Indeed, they suppress proteasome-mediated breakdown of apolipoprotein B, and lower the uptake of glucose by adipocytes.

[Other mechanisms may be involved in such toxicity, such as incorporation in mitochondrial DNA or competitive inhibition of ATP/ADP translocation]
Among the factors involved in fatty liver disease onset which could be the second “hit”, we have to consider hepatitis C infection, since this infection is common in HIV infected patients, with nearly twenty to thirty percent patients coinfected with HCV.
Steatosis (whatever the level) is observed in 50% of liver biopsies. When using a threshold of thirty percent of fatty hepatocytes, the prevalence ranges from eight to twenty four percent. The few longitudinal data available seem to indicate that steatosis, once present, naturally persists and tends to increase with time.

Most studies indicate that the presence of steatosis is dependent on a complex interaction of viral and host related factors.

The role of obesity, insulin resistance, dyslipidemia has been clearly highlighted and appear to be of highest importance in patients infected with HCV genotype 1. Of interest, HCV has been associated with a higher risk of diabetes and could enhance the risk of steatosis by this way.

HCV (in particular genotype 1) could also be associated with mitochondrial impairment, causing a state of chronic oxidative stress. It has been recently shown in that HCV was found to independently cause mitochondrial DNA depletion in PBMCs of HIV-HCV co-infected patients.

HCV genotype 3 is independently and directly associated with steatosis, probably via the impairment of incorporation of triglycerides into VLDL by HCV core proteins.

Regarding the potential consequences of steatosis, an independent association between steatosis and the degree of fibrosis has also been shown. On the contrary, no clear relationship exists between the degree of necro-inflammation and steatosis.
Quels patients et pourquoi?

Host
- Genetics
- Visceral fat
- Insulin resistance
- Lipid metabolism
- Age?

Hepatitis viruses
- HBV (?)
- HCV

Alcohol and drugs

Antiretroviral drugs
- PIs
- NRTIs

HIV infection
per se

STEATOSIS

HIV-Positive Patients With Nonalcoholic Fatty Liver Disease Have a Lower Body Mass Index and Are More Physically Active Than HIV-Negative Patients
Quels patients et pourquoi?

HCV Genotypy 3 (n = 54)

<table>
<thead>
<tr>
<th>Less likely to have steatosis</th>
<th>More likely to have steatosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV RNA (per 1 log₂, increase)</td>
<td>3.48 (1.09-10.86) p = 0.034</td>
</tr>
<tr>
<td>Systolic blood pressure (per 10 mm Hg increase)</td>
<td>1.51 (0.65-2.33) p = 0.027</td>
</tr>
<tr>
<td>Glucocorticoid (per 1 unit, increase)</td>
<td>5.38 (1.89-14.66) p = 0.002</td>
</tr>
<tr>
<td>Non-alcoholic fatty liver grade (per 1 unit, increase)</td>
<td>0.17 (0.04-0.68) p = 0.011</td>
</tr>
</tbody>
</table>

HCV non-Genotype 3 (n = 106)

<table>
<thead>
<tr>
<th>Less likely to have steatosis</th>
<th>More likely to have steatosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolving fibrosis (per 1 unit, decrease)</td>
<td>9.20 (2.34-34.21) p = 0.002</td>
</tr>
<tr>
<td>Non-alcoholic fatty liver grade (per 1 unit, increase)</td>
<td>0.17 (0.04-0.68) p = 0.58</td>
</tr>
<tr>
<td>Triglycerides (per 1 unit, increase)</td>
<td>1.78 (1.34-2.31) p = 0.0001</td>
</tr>
<tr>
<td>BMI (per 1 unit increase)</td>
<td>5.31 (1.85-13.64) p = 0.003</td>
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</tbody>
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Indeed, it is likely that steatohepatitis, as opposed to steatosis, requires either a more severe and prolonged exposition to a causal factor or additional physiopathological abnormalities. A first hit (frequently insulin resistance) leads to the development of steatosis. The second hit is an intra-hepatic abnormality that develops as a response to the first hit, or is present independently, linked to other diseases.
Quelles conséquences?

Host
- Genetics
- Visceral fat
- Insulin resistance
- Lipid metabolism
- Age?

Hepatitis viruses
- HBV (?)
- HCV

Alcohol and drugs

Antiretroviral drugs
- PIs
- NRTIs

HIV infection
per se

STEATOSIS

Accelerated progression to cirrhosis / HCC
Response to anti-HCV treatment

Hepatitis viruses

Quelles conséquences?
Quelles conséquences?

VHC

INFLAMMATION NECROSE

STÉATOSE

FIBROSE

Hôte
Toxiques

Age
Sexe
Alcool
Coinfections

... 

F≥3

F<3

Mehta, Hepatology, 2005

Stéatose>30%

Stéatose<

Fartoux, Hepatology, 2005
Quelles conséquences?

- Corrélation degré stéatose/degré fibrose retrouvée dans 4 études chez patients co-infectés VIH-VHC

Mc Govern, CID, 2006
Sulkowski, AIDS, 2005
Mc Govern, CID, 2006
Lanternier CCB, 2007
Verma, BMCresnotes 2008

Figure 3
Box plot showing fibrosis progression rate/year in those with (yes) and without (no) hepatic steatosis.

Verma, BMCresnotes 2008
Combien de patients concernés?

Loulougué, J Acquir Immune Defic Syndr 2007;45:465

Hepatic Steatosis as an Emerging Cause of Cirrhosis in HIV-Infected Patients
Ce travail laisse penser qu’un traitement court est insuffisant en présence d’une stéatose.
Cependant, la stéatose semble associée à une prévalence plus élevée de cirrhose, à une charge virale plus élevée dans le génotype 3 et à une prépondérance du sexe masculin. L’effet indépendant de la stéatose reste donc à déterminer.
Il serait également important de déterminer l’effet confondant de l’insulino-résistance définie par le score de HOMA.
Quelles conséquences?

Fig. 3. Sustained virological response rates in patients with and without steatosis at baseline.

Comment dépister et diagnostiquer?

- Rechercher les facteurs de risque
  - Coinfection avec virus des hépatites
  - Alcoolisme
  - Evaluation métabolisme glucido-lipidique
  - Exposition aux INTI (d-drugs+++)

- Enzymes hépatiques élevées
  - avec tests négatifs pour des affections hépatiques virales, congénitales ou autoimmunes
  - mais nombreux cas décrits de stéatose hépatique avancée avec des enzymes hépatiques normales

- Stéatotest?? (A2M, ApoA1, Hapto, BiliT, ALT, GGT, chol, TG, glucose, âge, sexe, BMI) AUROC 0,8 pour seuil stéatose 5%)

- Echographie (sensibilité 60-90%, spécificité 90%), scanner, IRM
  - Uniquement pour les stéatoses modérées à sévères

- Biopsie hépatique = méthode de référence

Liver steatosis is often asymptomatic, or associated with mild gastrointestinal symptoms. The diagnosis is thus frequently made during the evaluation of ALT elevation up to 2-3 times the upper limit of normal, which is the most frequent lab abnormality encountered.

A moderate or severe degree of liver steatosis may be seen on ultrasound, CT or MRI, whereas mild steatosis are often undetectable with imaging techniques. None of these 3 exams can discern inflammatory or fibrotic changes suggestive of steatohepatitis. Thus, the gold standard remains liver biopsy.

[The estimated sensitivity of ultrasound ranges from 60 to 94%, with a specificity of 84 to 95%].
Comment traiter?

- Exercice – perte de poids
- Traitement du diabète et des dyslipidémies
  - metformine - Thioglitazone?
  - statine- fibrates
- Traiter l’infection VHC (génotype 3+++)
- Contrôler l’infection par le VIH (= anti-TNF)
- Adapter le traitement antirétroviral
  - switch IP → INNTI?
  - Ne pas utiliser les d-drugs (ddI = don’t do it)
  - !!!!! Interactions (ddi+TDF, ddi+ribavirine)

When steatosis is suspected or proved, exercise and weight loss, when needed, seem to be mandatory. If needed, diabetes and hyperlipidemia have to be treated, with dietary first and specific pharmacologic therapies.

Regarding antiretroviral treatment, it could be advocated that PIs have to be replaced by NNRTIs.

Another point is to avoid the use of d4T, ddC and ddI. In the case of associated hyperlactatemia, early discontinuation of all NRTIs is needed.

HCV infection has to be treated, in particular for genotype 3. For patients infected with HCV genotype 1, it may be interesting to focus first on other factors, since that steatosis may be predictive for decreased virologic response.

Last, different drugs have been used for the specific treatment of non alcoholic fatty liver disease, but their interest is still to be established.

[such as ursodeoxycholic acid, vitamin E, and N acetyl cysteine]
Voir commentaire diapositive précédente.
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In this large study on five hundred and seventy four HCV mono-infected patients with paired liver biopsies, steatosis was markedly improved in genotype 3 infected patients who achieved sustained virologic response. In contrast, patients without sustained response had no significant changes in steatosis following treatment, as were patients infected with genotype 1, whatever the response to treatment. Similar results were also observed in HIV-HCV coinfected patients, in the recent therapeutic trial RIBAVIC.

[This argues for the responsibility of host-related factors in non-3 genotype infection, and for the interest of anti-HCV therapy in treating steatosis in patients infected with genotype 3]
Comment traiter?

Fig. 4. Prevalence of steatosis at baseline and end of follow-up in patients with (top) and without (bottom) a sustained virological response.

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Comment traiter?

• Eviter les médicaments toxiques pour la mitochondrie
  – Ibuprofène, acide valproïque, aspirine peuvent interférer avec métabolisme mitochondrial hépatique des acides gras
  – Amiodarone et tamoxifène (diminution production ATP)
  – Calcium bloqueurs
  – Aminosides, chloramphénicol (pourraient inhiber la transcription des peptides mitochondriaux), cyclines
  – Adefovir, cidofovir inhibiteurs de la polymerase gamma

• Utiliser des antioxydants?
  – Coenzyme Q10, vitamine C, thiamine (B1), riboflavine (B2), pyridoxine (B6), L-carnitine

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Many questions are still to be answered in HIV infected patients:

- What is the real prevalence of liver steatosis? Of micro-vesicular steatosis? Of steato-hepatitis? Such a differentiation is needed for further comprehension of the importance of steatosis in HIV infected patients.
- Is there a genetic background which could facilitate the onset of steatosis?
- Is there a clinical impact of liver steatosis, when assessed prospectively?
- If so, do we need to assess the reality of steatosis by liver biopsy? Since fibrosis tends to be assessed via non invasive tests, do we need a steatotest including for example adipokines?
- Is steatosis influencing the response to anti-HCV treatment? Is the lower response rate observed in HIV-HCV coinfected patients partly related to higher prevalence of steatosis?
- What is the impact of withdrawal of NRTIs on the evolution of steatosis, in particular on micro-vesicular steatosis and steato-hepatitis?

[All in all, it is not possible to draw definite conclusions, considering the limitations of histological evaluation, and since the potential impact of immune status, antiretroviral and anti-HCV treatments, for example, are difficult to assess in cross sectional and retrospective studies.]