

Stéatose hépatique: un problème émergent comprendre et agir

Lionel PIROTH
CHU Dijon
Strasbourg, 9 septembre 2008

Dear colleagues,

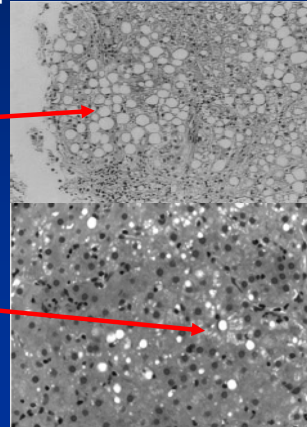
First of all I would like to thank the organizers for inviting me at this conference to talk about liver steatosis in HIV infected patients

QUESTIONS

- De quoi parle-t-on?
- Combien de patients concernés?
- Quels patients concernés?
- Pourquoi?
- Quelles conséquences?
- Comment dépister et diagnostiquer?
- Comment traiter?

De quoi parle-t-on?

- Stéatose = excès de graisse intra-hépatique (>5%?)
- Stéatohépatite = altérations hépatocellulaires
- Macrovésiculaire: goutte grasseuse simple large déplaçant le noyau
- Microvésiculaire: goutelettes intracellulaires multiples



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.

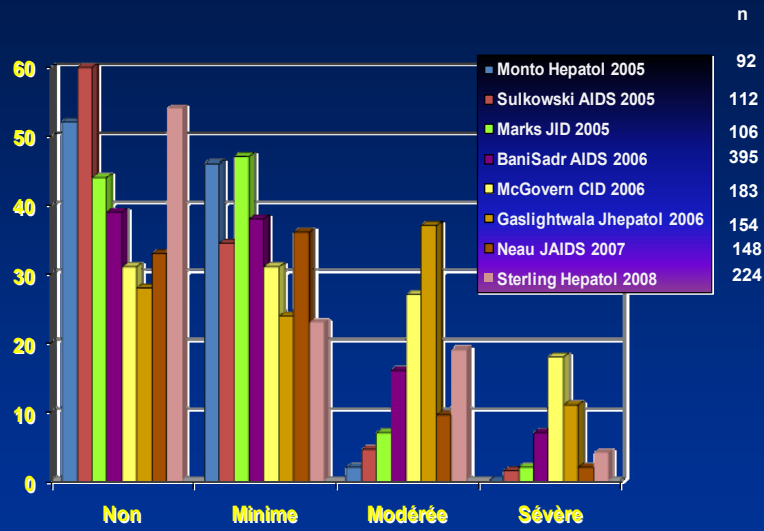
There are two patterns of liver fat accumulation:

-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.

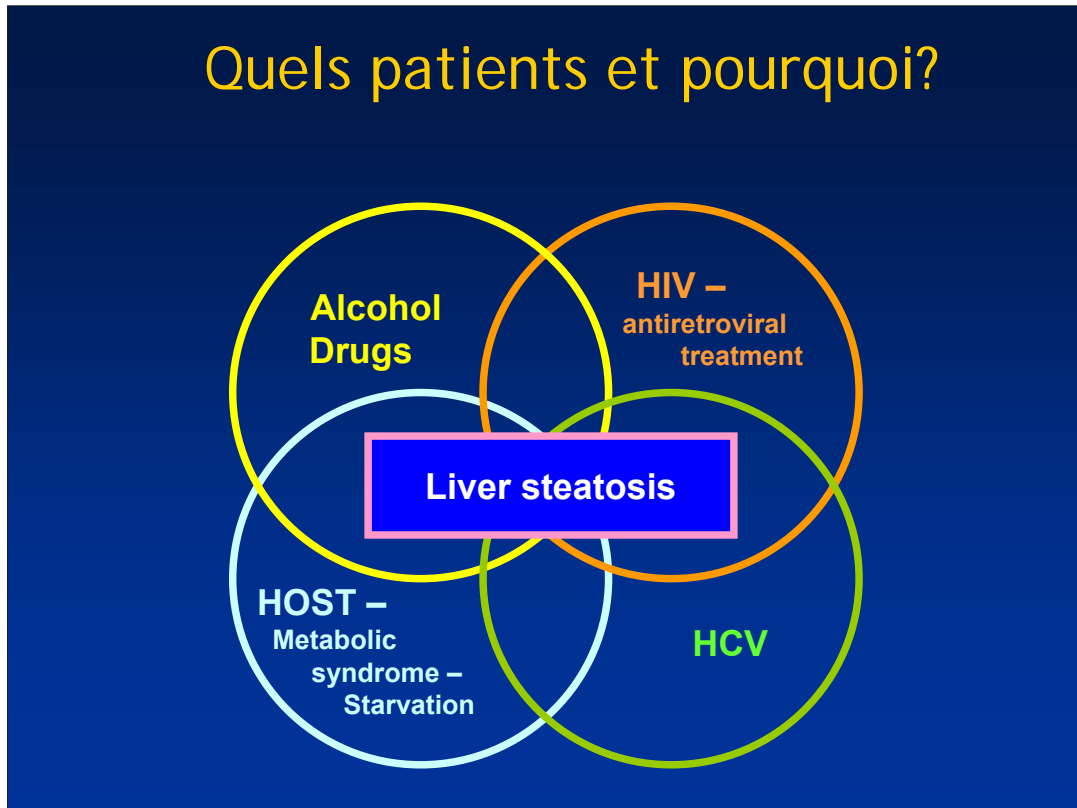
From a practical point of view, a semi-quantitative approach is used to assess the importance of steatosis. A universally accepted grading and staging system has not been established yet. Moreover, in most studies, either only macrovesicular steatosis is quantified and taken into account, or no difference 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?



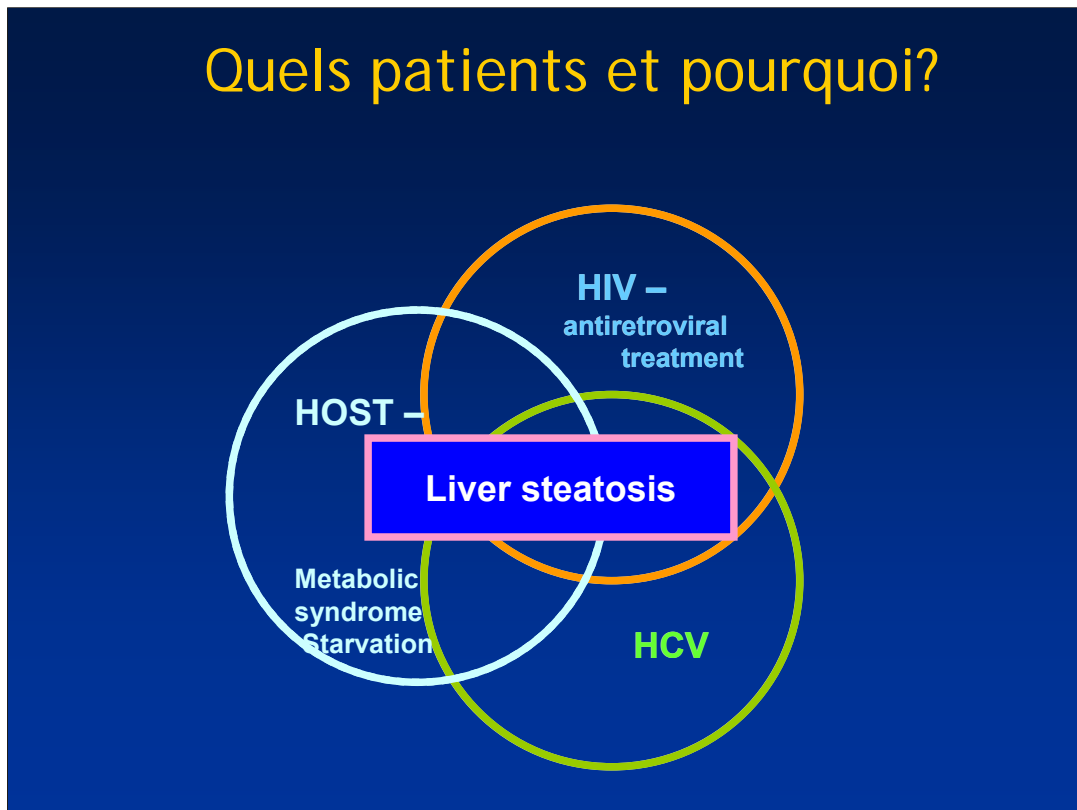
Stéatose hépatique chez les sujets coinfecteds VIH-VHC

Quels patients et pourquoi?



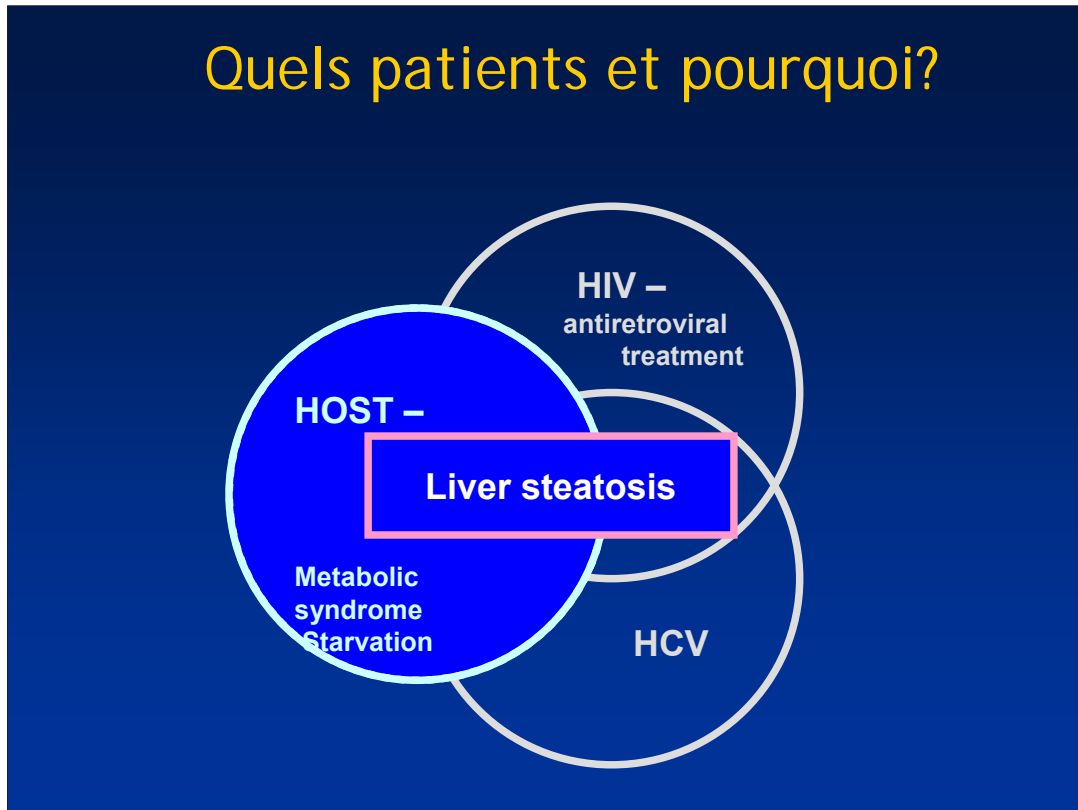
Several causes may be associated with liver steatosis in HIV infected patients. Toxic causes, in particular alcohol, are a well known and important risk factor of steatosis.

Quels patients et pourquoi?



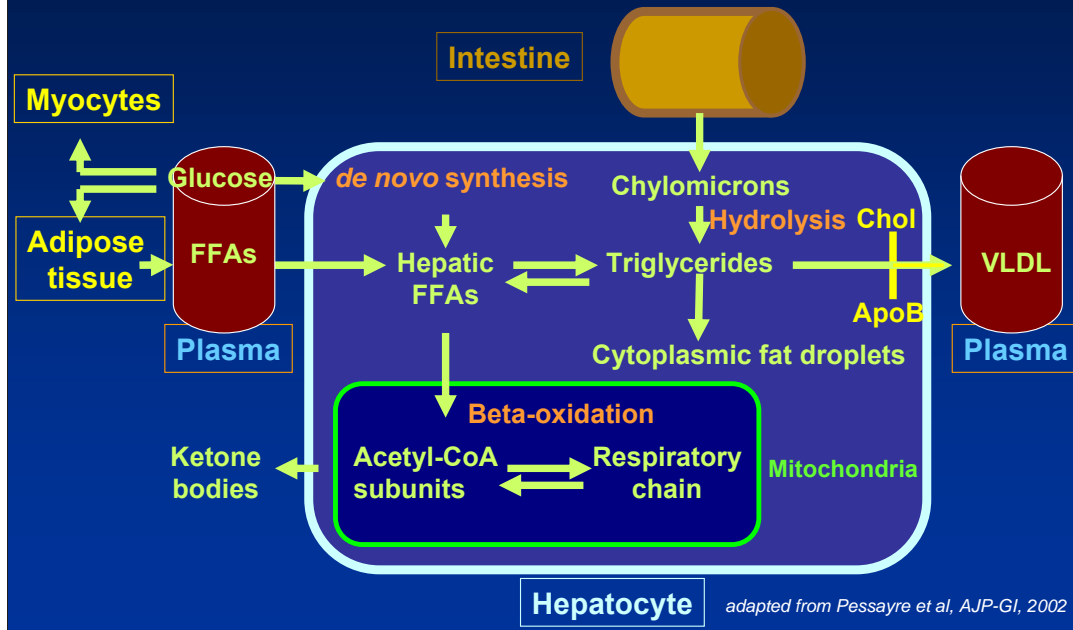
But, because of the limited time allowed to this presentation, we will focus on non-alcoholic fatty liver disease.

Quels patients et pourquoi?



First, we are going to see the host related factors associated with liver steatosis

Fat metabolism in hepatocytes



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

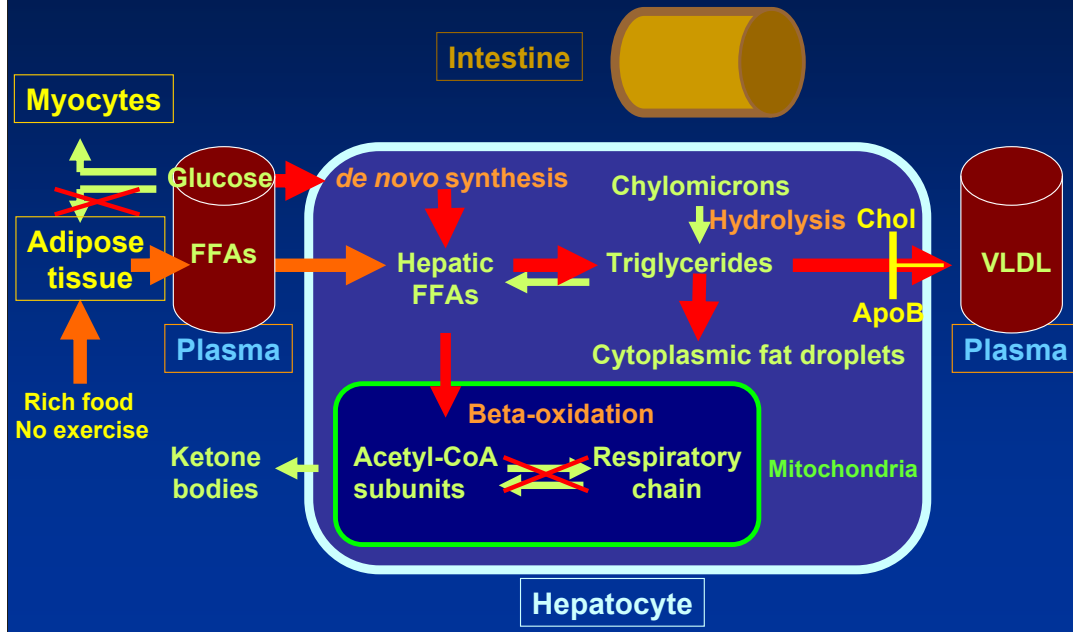
Then, hepatic FFAs

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

These hepatic triglycerides either

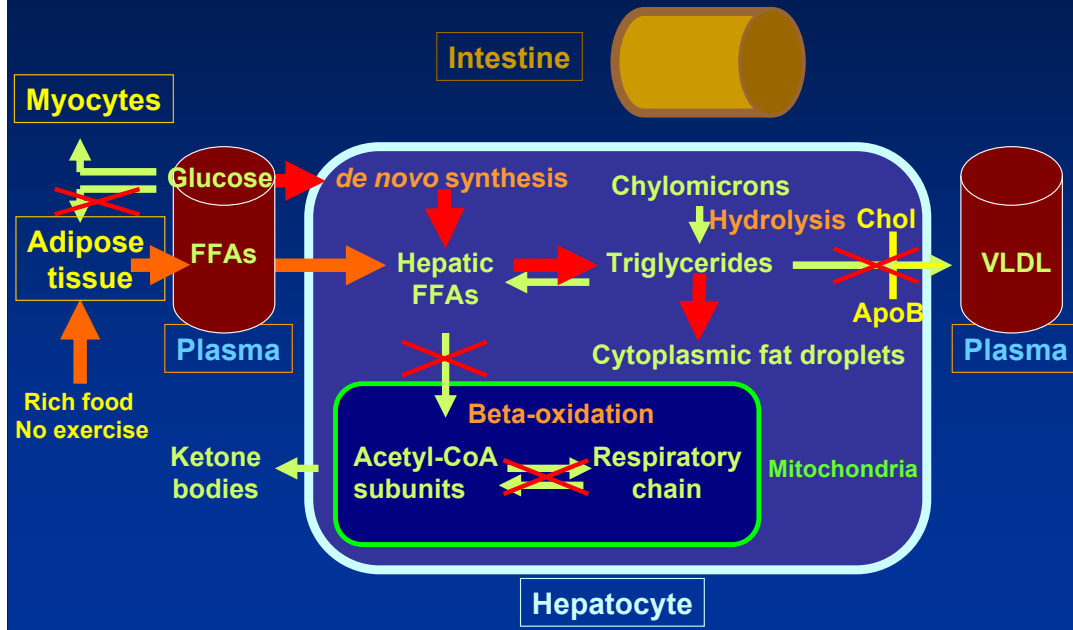
- 1) are secreted as very low density lipoproteins (VLDL), corresponding to a droplet of triglycerides, cholesterol, phospholipids and a large protein termed apolipoprotein-B, or
- 2) accumulate as fat droplet within the cytoplasm of hepatocytes

Obesity and insulin resistance



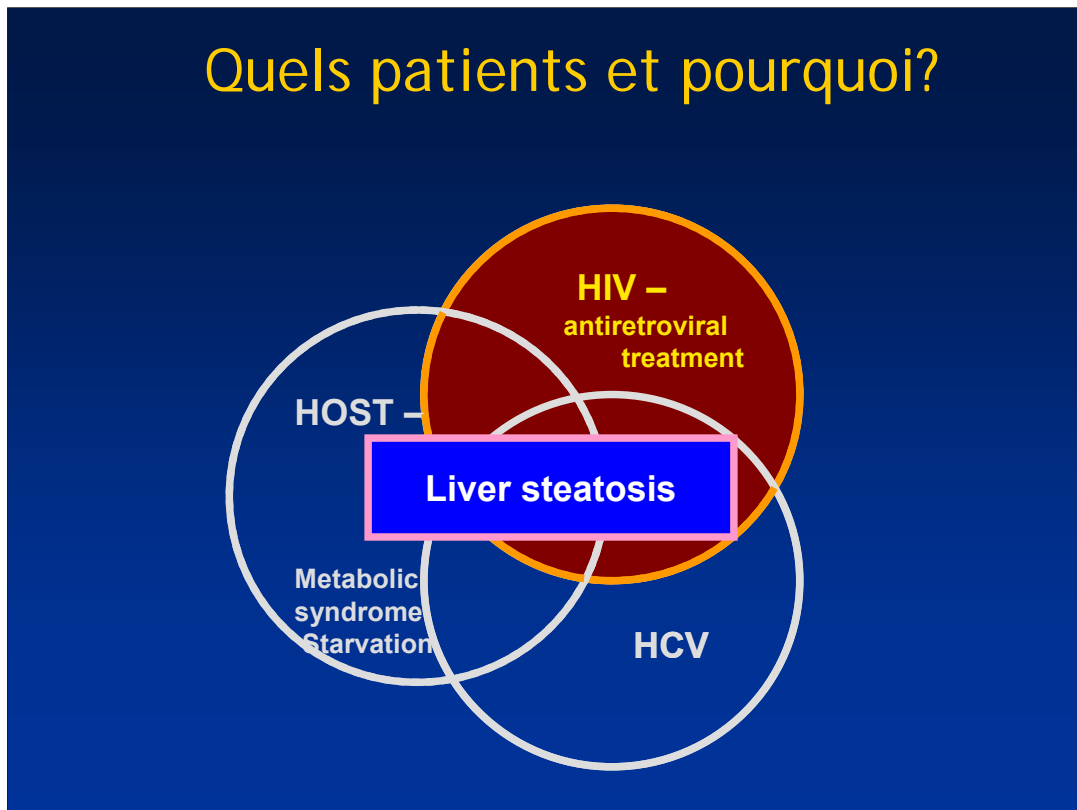
Obesity increases plasmatic FFAs and also causes resistance to the action of insulin, which results in a decrease of glucose uptake and use by adipocytes and muscle cells. The increased load of FFAs within hepatocytes results from an increased uptake from plasma and an increased de novo synthesis. This 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.

Obesity and insulin resistance



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.

Quels patients et pourquoi?



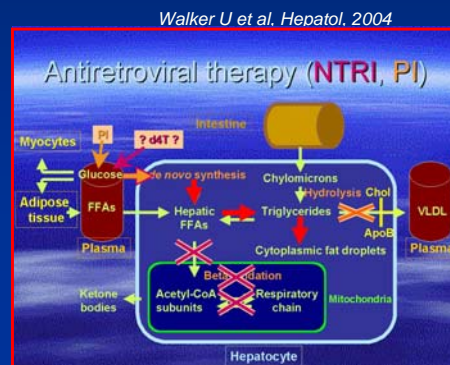
Another hit in the pathogenesis of steatosis and steatohepatitis may be HIV infection per se or antiretroviral treatment.

Quels patients et pourquoi?

VIH

- Données biopsiques et autopsiques à l'ère pré-HAART
 - Prévalence stéatose: 30 to 50%
(Glasgow, Am J Clin Pathol, 1985 – Lebovics, Hepatol, 1985)
 - Rôle propre du VIH?
 - Rôle des comorbidités
- Syndrome lipodystrophique
 - Lipoatrophie - INTI
 - Inhibition de l'ADN polymérase gamma (puissance inhibitrice ddc>ddl>d4t >> autres)
 - Augmentation du stress oxydatif
 - Lipohypertrophie centrale - IP – syndrome métabolique
 - Insulino- résistance
 - Interférence avec apo-B

Sulkowski, AIDS, 2005 Mc Govern, CID, 2006



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.

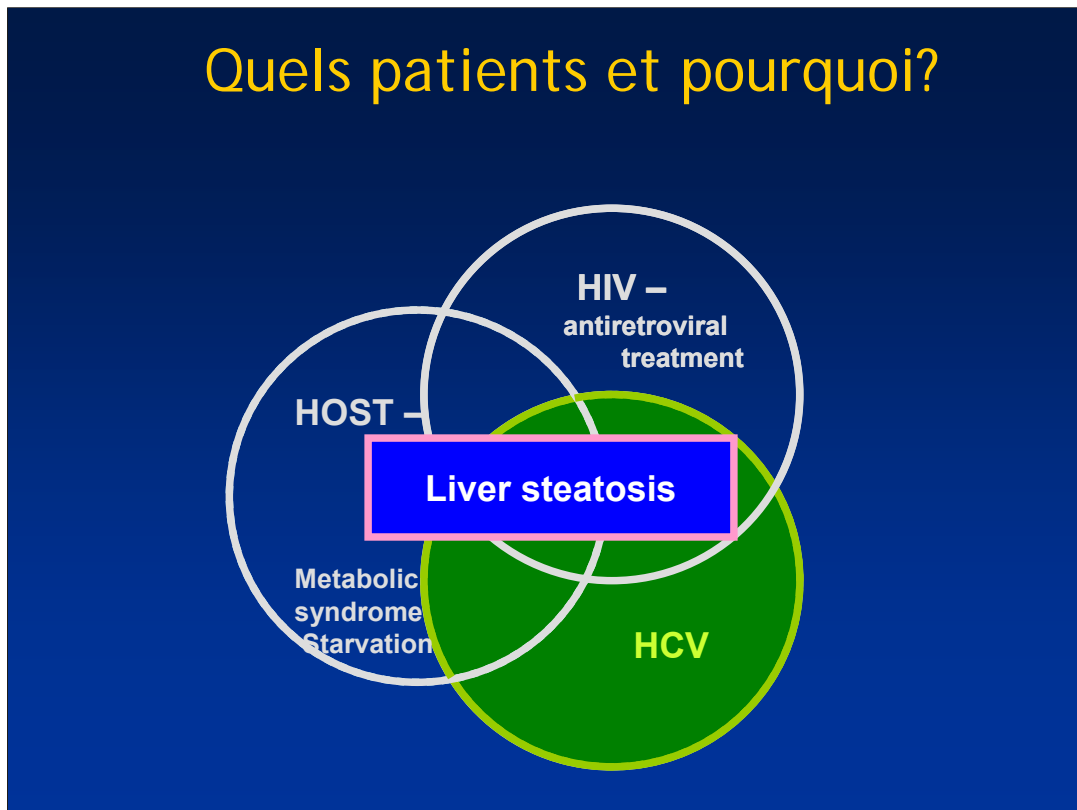
Mitochondrial toxicity seems to be related to the inhibition or the alteration of the human DNA polymerase gamma. This leads to liver mitochondrial DNA depletion and reduced respiratory chain enzyme activity. It occurs more frequently with D drugs (ddC, ddl, d4t) than with non-D drugs, as recently shown in eighty HIV-HCV coinfecting 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, dyslipidemia 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]

Quels patients et pourquoi?



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 coinfecting with HCV.

Quels patients et pourquoi?

VHC

- Steatosis > 30%: 8 – 24 % of the patients
- Factors associated with steatosis

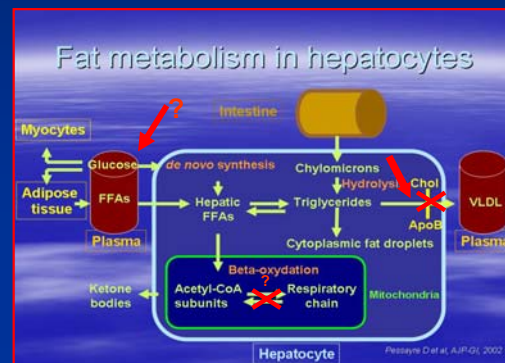
– Causes?

- Obesity, insulin resistance (HCV?), dyslipidemia
- Mitochondrial impairment (genotype 1?)
- Direct cytopathogenic effect (genotype 3)

Castera et al, Gut, 2003
Wyatt J et al, J Clin Pathol, 2004
Patton et al, J Hepatol, 2004

– Consequences?

- Higher risk of advanced fibrosis?



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.

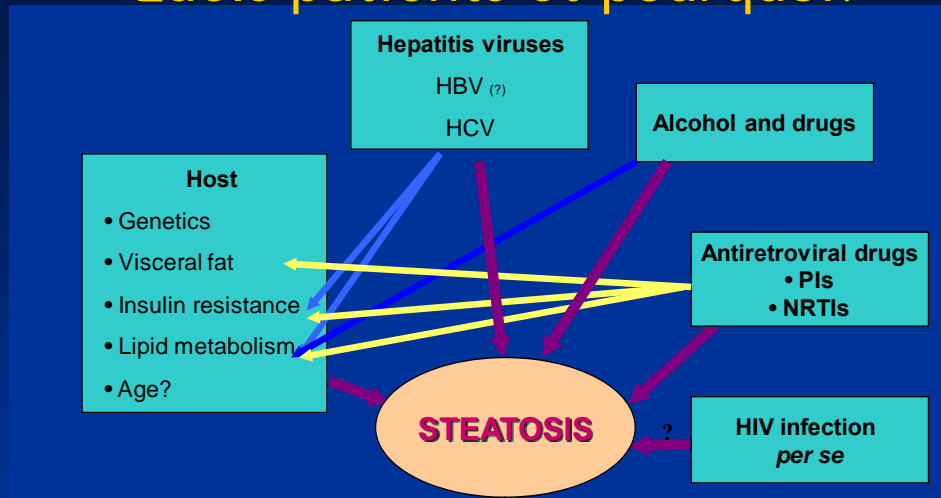
Of interest, HCV has been associated with a higher risk of diabetes and could enhance the risk of steatosis by this way.

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.

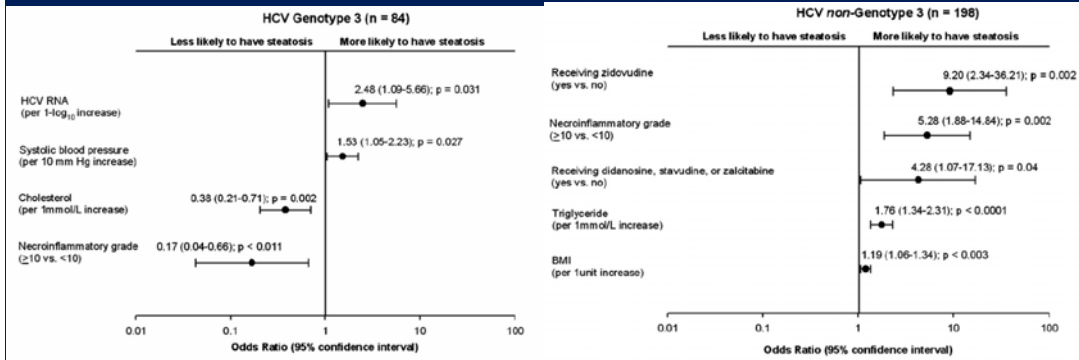
Quels patients et pourquoi?



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

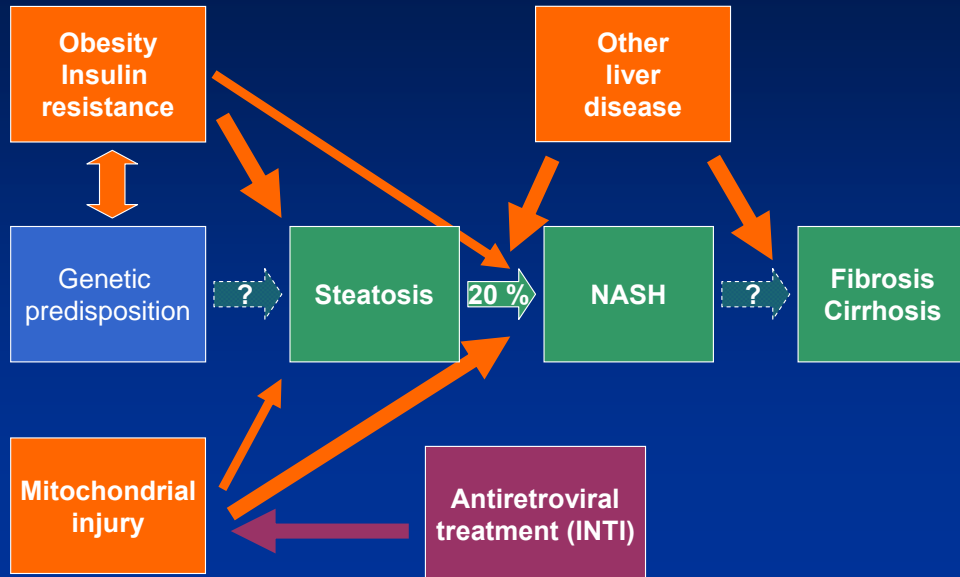
Mohammed S, *J Acquir Immune Defic Syndr* 2007;45:432-438

Quels patients et pourquoi?



M. Rodríguez-Torres et al. / Journal of Hepatology 48 (2008) 756–764

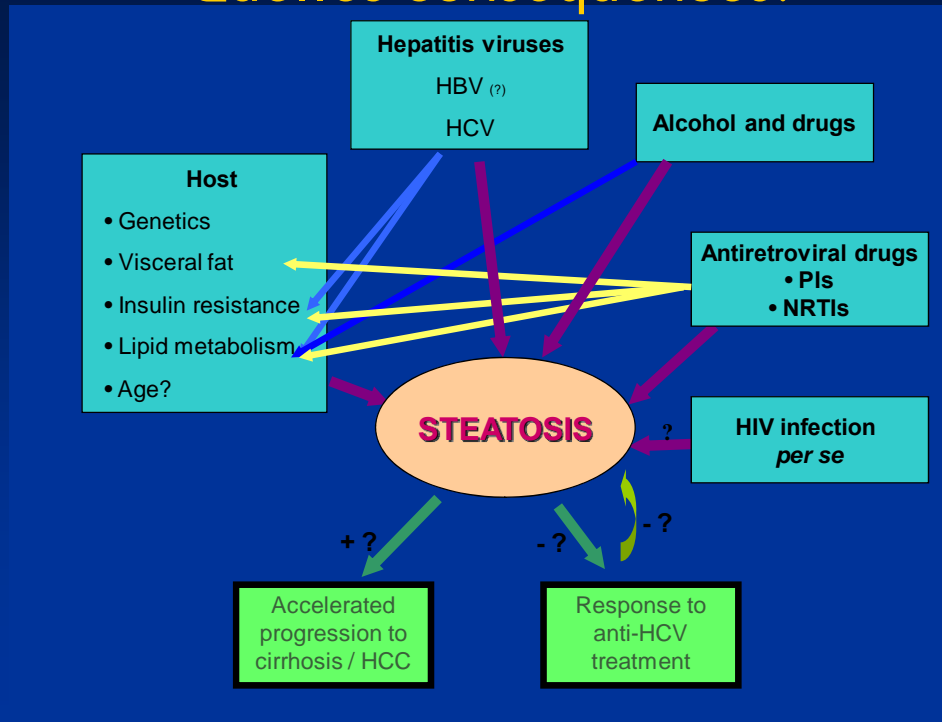
Pathogenesis of non alcoholic liver steatosis: the "two hits hypothesis"



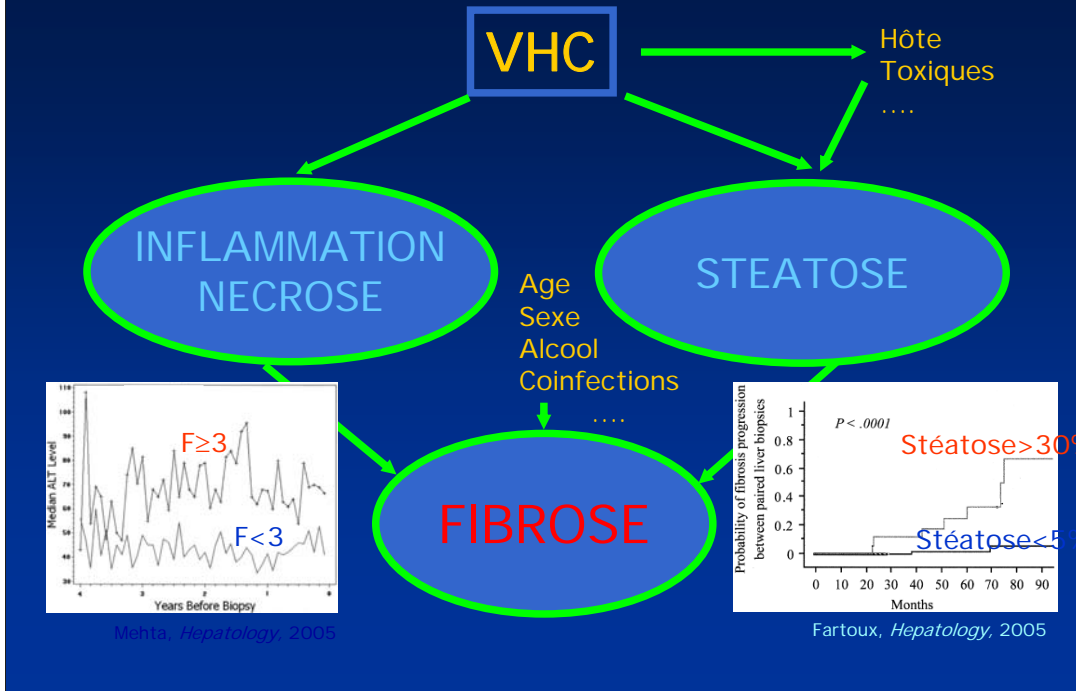
adapted from Ristig et al, AIDS Pat Care, 2005

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?



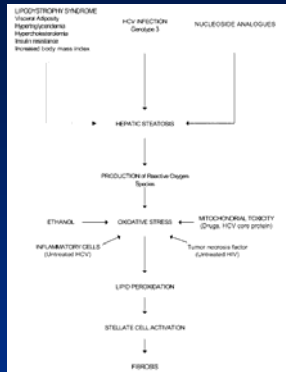
Quelles conséquences?



Mehta, *Hepatology*, 2005

Fartoux, *Hepatology*, 2005

Quelles conséquences?



Mc Govern, CID, 2006

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

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

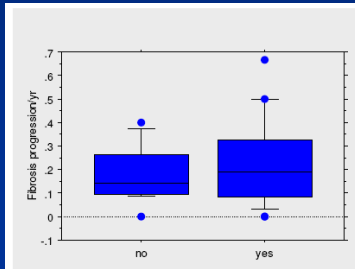
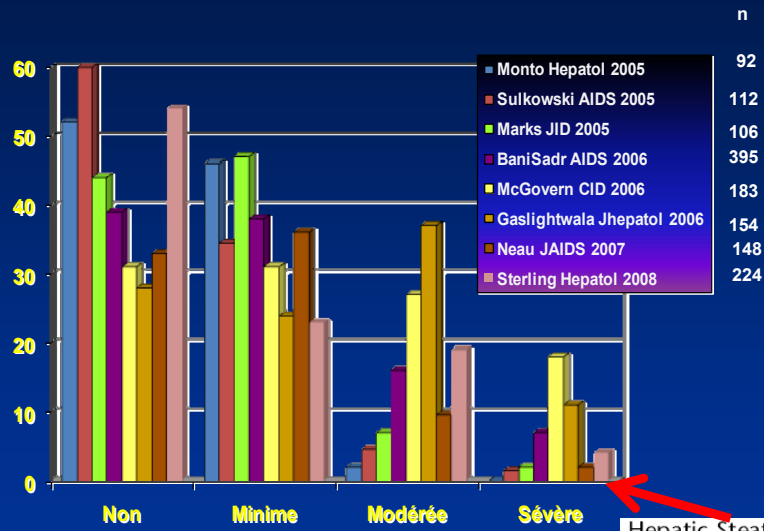


Figure 3
 Box plot showing fibrosis progression rates/year in those with (yes) and without (no) hepatic steatosis.

Verma, BMCresnotes 2008

Combien de patients concernés?

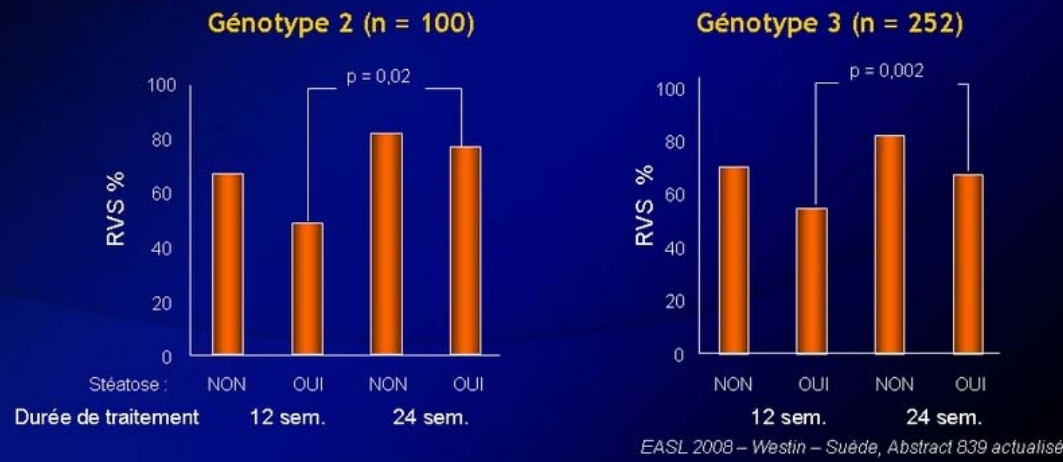


Hepatic Steatosis as an Emerging Cause of Cirrhosis in HIV-Infected Patients

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

Génotypes 2 et 3 : faut-il traiter plus longtemps si une stéatose est présente ? (1)

- **But** : étudier l'impact de la stéatose sur la réponse antivirale chez les sujets infectés par les génotypes 2 ou 3
- Essai NORDynamiC, multicentrique Scandinave incluant 382 pts dont 352 avec biopsie en début de traitement
- Traitement par PEG-IFN α -2a 180 μ g/sem + RBV 800 mg/j pendant 12 ou 24 semaines



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?

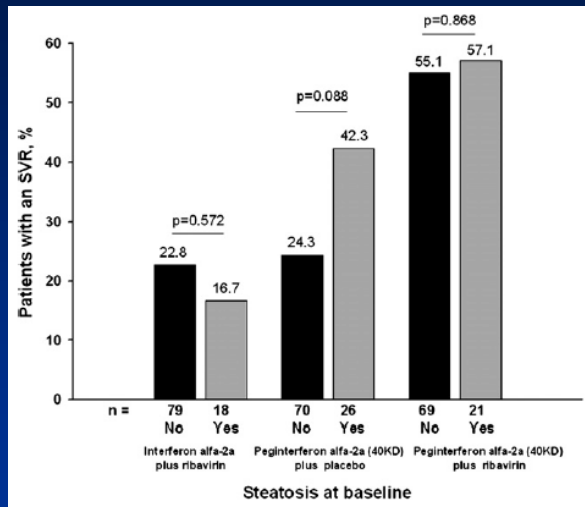


Fig. 3. Sustained virological response rates in patients with and without steatosis at baseline.

M. Rodríguez-Torres et al. / Journal of Hepatology 48 (2008) 756–764

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 (ddl = 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 ddl. 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.

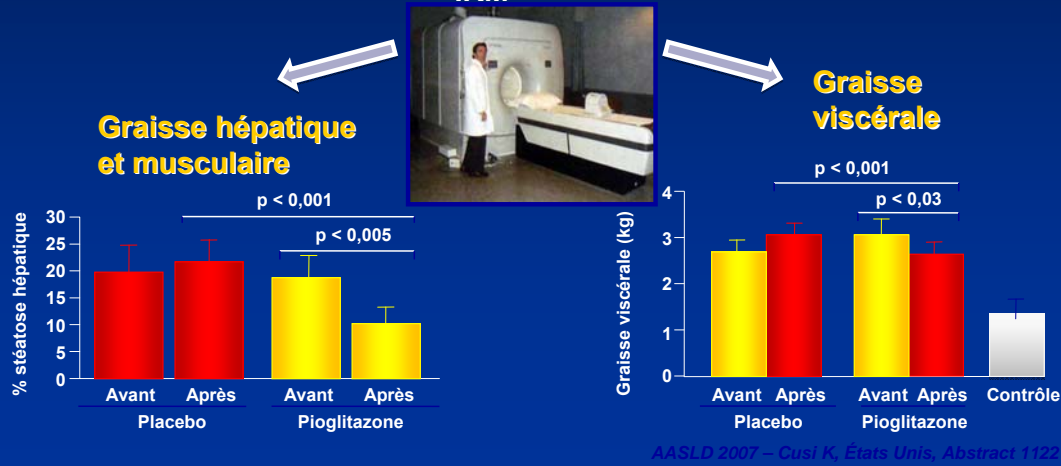
[such as ursodeoxycholic acid, vitamin E, and N acetyl cysteine]

Comment traiter?

Analyse approfondie des mécanismes d'action de la pioglitazone chez des patients atteints de NASH ayant été inclus dans un essai récemment publié (*Belfort R et al, New Engl J Med 2006*)

Évaluation du contenu graisseux des tissus hépatique, musculaire et adipo-cytaire en spectroscopie par résonance magnétique

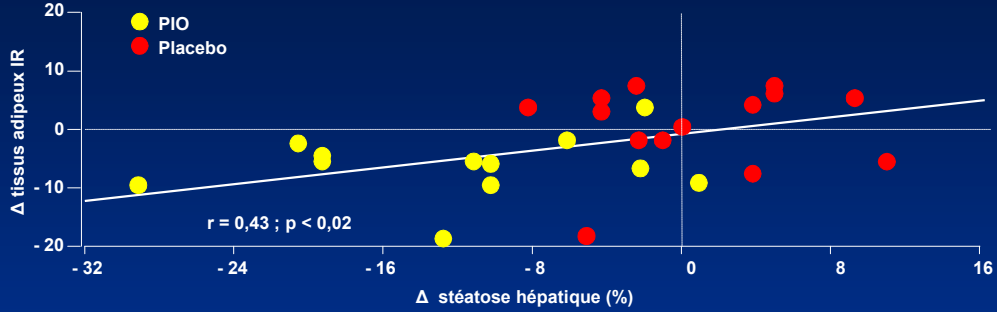
IRM



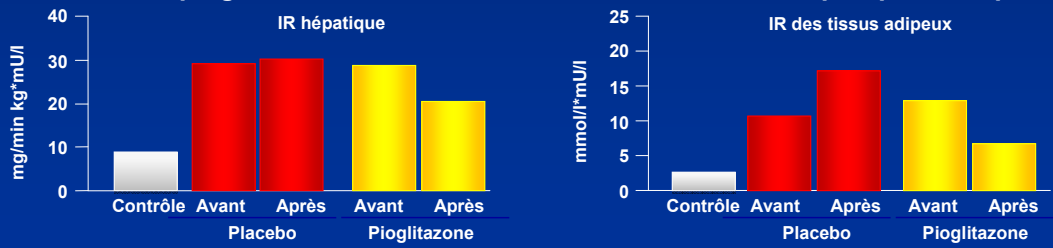
La même équipe a récemment publié (*Belfort R et al, New Engl J Med 2006*) que la pioglitazone améliorait les lésions hépatiques des patients atteints de NASH. L'amélioration des lésions semblait principalement liée à l'amélioration de l'insulinorésistance. Afin de progresser dans la compréhension des mécanismes impliqués dans les effets de la pioglitazone, les auteurs ont analysé les effets de la pioglitazone sur l'insulinorésistance au niveau des tissus adipeux et musculaires.

Comment traiter?

Corrélation entre l'évolution de la stéatose et l'insulino résistance adipeux



Effets de la pioglitazone sur l'insulinorésistance des tissus hépatique et adipeux



AASLD 2007 – Cusi K – États Unis, Abstract 1122

Voir commentaire diapositive précédente.

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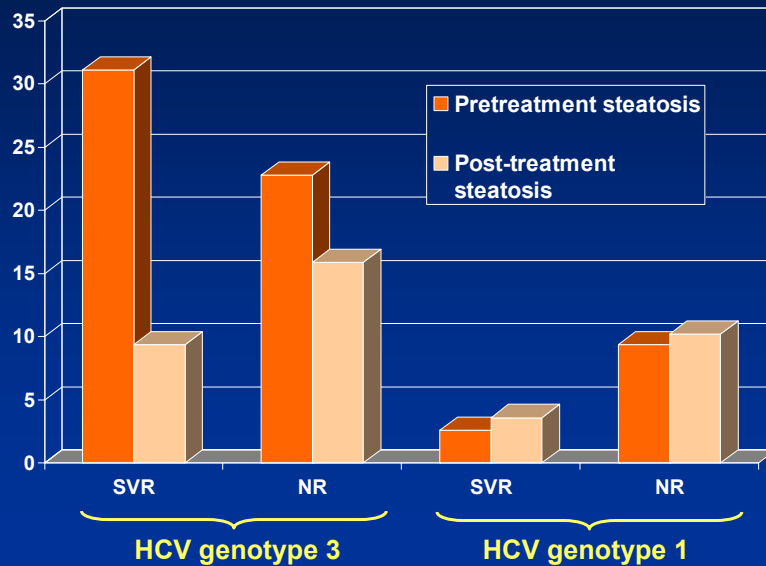
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Last, different drugs have been used for the specific treatment of non alcoholic fatty liver disease, but their interest is still to be established.

[such as ursodeoxycholic acid, vitamin E, and N acetyl cysteine]

Comment traiter?



Patton et al, J Hepatol, 2004

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 coinfecting 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]

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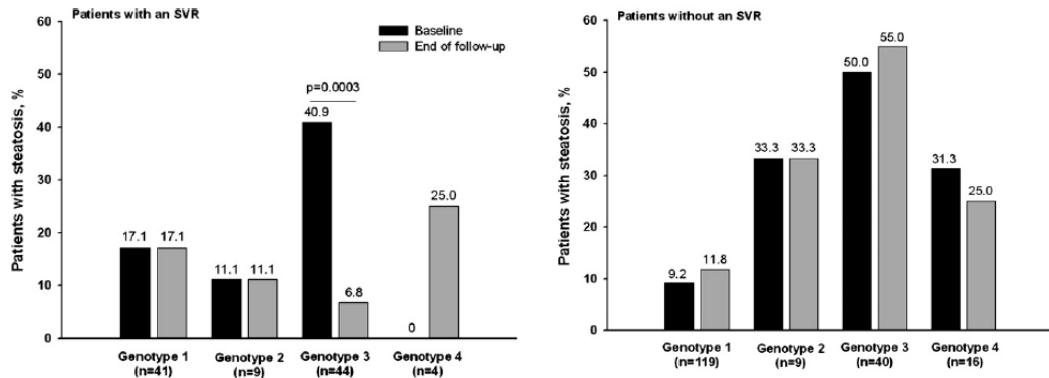


Fig. 4. Prevalence of steatosis at baseline and end of follow-up in patients with (top) and without (bottom) a sustained virological response.

M. Rodríguez-Torres et al. / Journal of Hepatology 48 (2008) 756–764

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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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Uridine

- *In vivo*: essai randomisé double aveugle sur 20 patients lipotrophiques sous HAART non modifié
- Uridine 36 g 3x/j pendant 10 jours/mois/3mois vs placebo
- Augmentation significative masse grasse
- Pas de modification stéatose

(Sutinen J, antivir ther, 2007)

Uridine

- Efficacité dose dépendante *in vitro* sur hépatocytes exposés à ddC, d4T, AZT+3TC (sur fonction cellulaire et taux d'ADNmt), mais pas à la ddl (*Walker, antivir ther, 2003*)
- Efficacité *in vitro* sur adipocytes (*Walker, antivir ther, 2006*)
- Efficacité *in vivo* plus importante et plus rapide que autres mesures?

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[such as ursodeoxycholic acid, vitamin E, and N acetyl cysteine]

Des questions?

- Quelle est la prévalence des différents types de stéatose (en particulier la stéatose microvésiculaire)?

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- Y a t il un terrain génétique? Lequel?
- Quelle est l'influence de la stéatose sur l'histoire naturelle hépatique?
 - dans des études prospectives
 - selon le type de stéatose
- Est ce que l'évaluation histologique est nécessaire à la fois à visée diagnostique et pronostique? Quelle place pour les techniques non invasives?
- Quel peut être l'impact des modifications du traitement antirétroviral sur l'évolution de la stéatose?

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 coinfecting 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.]