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HEPATOLOGY

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Welcome

Future perspectives, new technology and treatments, innovation, best practice, and improving outcomes for patients are all features of this edition of the *European Medical Journal Hepatology*. Compared to our previous publications, this edition will present to you high-quality peer reviewed articles, while our second edition will feature a review of the International Liver Congress taking place this year in Vienna, Austria.

In this edition, leading experts in the field offer a valuable insight into the recent discoveries made and address future challenges, and what they feel needs to happen in order for both treatments and patient outcomes to improve. This journal offers a platform for professionals to exchange ideas, offer guidance, and enhance one's learning.

Acute-on-chronic liver failure commonly occurs in patients with cirrhosis, and is associated with both rapid multi-organ dysfunction and also short-term mortality. The condition, however, does not have a universally accepted definition which can be confusing for the clinician, explains Drs Roland Amathieu and Ali Al-Khafaji in their paper '*Definitions of acute-on-chronic liver failure: the past, the present, and the future*'. In this article the authors assess the strengths and weaknesses of each definition as well as outlining the potential role of 'omic' approaches for its diagnosis. Past, current, and future definitions are all explored and analysed and areas for improvement are highlighted.

To continue with this theme, metabolic syndrome (MetS) is another disease which can cause confusion, as to its definition, among clinicians. Corte et al., in their paper '*Metabolic syndrome in paediatric population: is it time to think back on diagnosis criteria?*' re-evaluate the diagnostic criteria for MetS as well as considering the inclusion of other clinical features, and emphasise just how important it is to uniformly define this condition, especially in the paediatric population. As the number of young people who suffer from obesity grows, it is important to ensure that the conditions associated with it are understood. In doing this, it could help clinicians to identify those at risk of MetS earlier and could slow the progression of other associated conditions.

I would like to thank our esteemed Editorial Board for all of their contributions which have made the publication of this journal possible, along with our authors who have contributed their valuable work, and our peer reviewers who have ensured that the highest quality of information was published. All of the articles presented in this edition have aimed to disseminate practical and relevant knowledge in clear take-home messages.

Spencer Gore

Director, European Medical Journal

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Foreword

Dr Kenneth J. Simpson

Scottish Liver Transplant Unit,
Royal Infirmary of Edinburgh, Edinburgh, UK.

Dear Colleagues,

I would like to sincerely welcome you to another issue of the *European Medical Journal Hepatology.* While preparing this foreword the horrific events in the great European city of Paris were unfolding; at a time when we, as hepatologists, are looking forward with great anticipation to the next International Liver Congress (ILC), the official Annual Meeting of the European Association for the Study of the Liver (EASL). This meeting is to be held in another great European city, Vienna, Austria; let us meet in our thousands in the spirit of friendliness and collaboration, showing our solidarity with our French colleagues that such awful events will not limit our multicultural outlook that is so essential in dealing with the huge worldwide burden of liver disease.

> “Although HCV continues to develop apace, other areas of hepatology also develop and provide exciting advances in understanding pathogenesis or new and novel therapies.”

There is much to look forward to indeed at the next ILC and within this edition of the *EMJ Hepatology.* There is a revolution in the therapy available for our patients with hepatitis C (HCV) infection. At the last ILC, we marvelled at the data suggesting that we could cure this infection, but what of genotype 3 infection and difficult-to-treat groups such as patients with cirrhosis, post-liver transplant, and those with renal failure; we can anticipate much more information regarding these issues. Perhaps most important and exciting is the potential for patients with decompensated liver disease to recompensate their liver failure after effective oral anti-HCV regimes.

Although HCV continues to develop apace, other areas of hepatology also develop and provide exciting advances in understanding pathogenesis or new and novel therapies. Areas such as alcoholic liver disease, non-alcoholic (metabolic syndrome associated) liver disease, and autoimmune and other viral liver diseases have provided interesting and challenging developments.

So let us converge in Vienna for the next ILC with excitement and anticipation, to renew old friendships and develop new international friends and collaborators, to hear the amazing advances in our specialty, and honour the memory of those who lost their lives earlier this month in Paris.

Yours sincerely,

Kenneth J. Simpson

Senior Lecturer in Hepatology, University of Edinburgh, and Honorary Consultant Physician, Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh, Edinburgh, UK.

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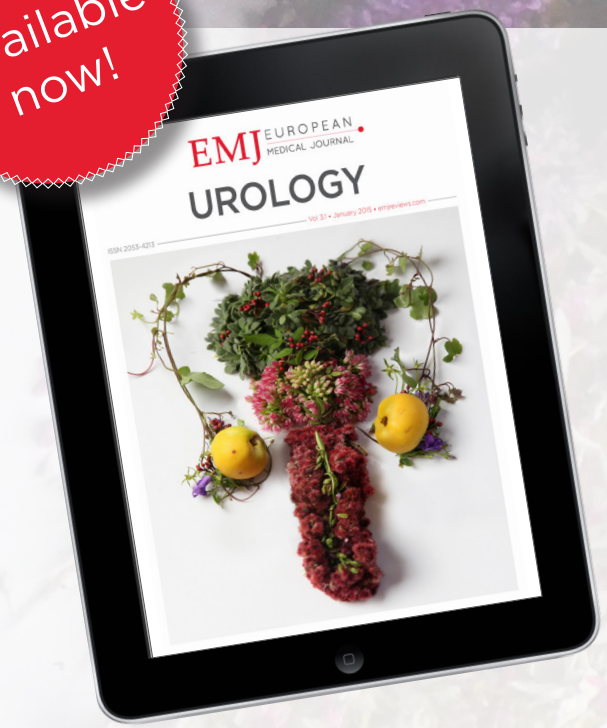

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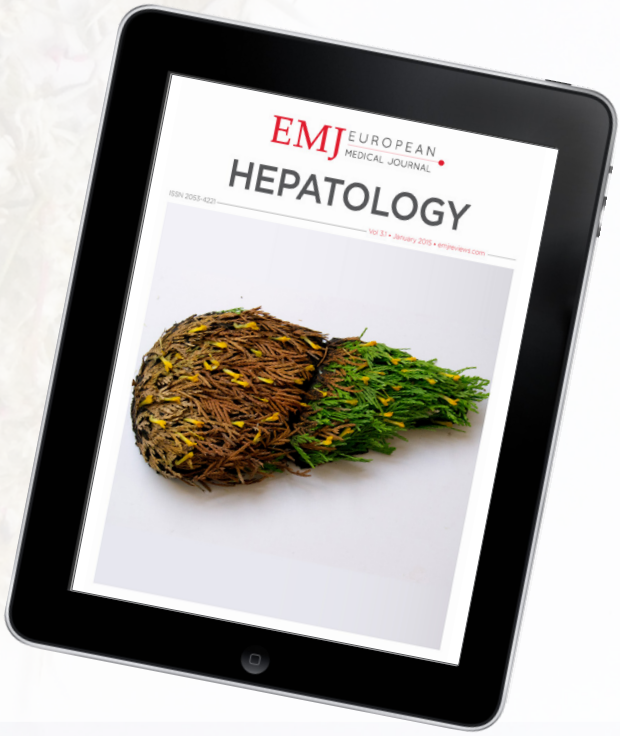

EDITOR'S PICK

With the introduction of effective curative treatment for hepatitis C, the incidence of the hepatitis C virus (HCV)-associated hepatocellular carcinoma (HCC) will be gradually declining within the next 20 years, at least in Western countries which will have wider access to these drugs within the next few years. Since chronic hepatitis B is also fairly well controlled in patients with known infection, alcoholic and non-alcoholic fatty liver disease are the two remaining big aetiologies of chronic liver disease in need of better treatments. Amongst the two, it is non-alcoholic fatty liver disease (NAFLD) that has shown the biggest increase in prevalence globally, mostly in developed countries but also increasingly in developing countries. Despite the fact that severe liver damage occurs only in a small fraction of patients with NAFLD, the proinflammatory environment constitutes an important precancerous condition, making liver cancer the most important obesity-related malignancy, particularly in men. Understanding this means putting a major effort into preventing NAFLD, into identifying what drives the inflammation in the liver, and ultimately into finding a cure for it.

Prof Dr Markus Peck-Radosavljevic

NON-ALCOHOLIC FATTY LIVER DISEASE - CHANGING THE PREVALENCE OF LIVER CANCER?

Benedetta Campana,[1,2] David Semela,[3] Markus Heim,[1,2] *Christine Bernsmeier[1,3,4]

1. Department of Biomedicine, University of Basel, Basel, Switzerland
2. Division of Gastroenterology and Hepatology, University Hospital Basel, Basel, Switzerland
3. Division of Gastroenterology and Hepatology, Cantonal Hospital St. Gallen, St. Gallen, Switzerland
4. Institute of Liver Sciences, King's College London, London, UK
**Correspondence to c.bernsmeier@unibas.ch*

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ABSTRACT

Due to its increasing prevalence, exceeding 25% of the Western population, non-alcoholic fatty liver disease (NAFLD) merits recognition as one of the most frequent chronic liver diseases (CLD) and requires consideration of the associated disease-related complications and their consequences for the surveillance and treatment of patients and the socio-economy worldwide. Along with the increasing incidence of NAFLD-related cirrhosis and end-stage liver disease, the frequency of NAFLD-related hepatocellular carcinoma (HCC) is rising and expected to surpass HCC related to chronic hepatitis C in the upcoming future. These epidemiologic changes will impact on the overall mortality of CLD and the requirement of organs for transplantation. Although the risk of HCC in NAFLD, similar to other CLD, is related to fibrosis (advanced fibrosis increases the risk of HCC 25-fold), there are reports suggesting a considerable rate of HCC also developing in simple hepatic steatosis. Moreover, HCC is nowadays the leading cause of obesity-related cancer mortality; cancers of other origin such as colorectal cancer are more prevalent in patients with NAFLD and obesity. The pathophysiology of HCC has mainly been studied in models of viral hepatitis. Given the expected raise in NAFLD-related HCC, a better understanding of the pathophysiology of carcinogenesis in NAFLD and obesity is desired in order to better define chemopreventive strategies. Here we review the epidemiology, aetiology, and pathogenesis of HCC on the background of NAFLD and deduce potential consequences for the management of patients in respect to the NAFLD epidemic.

Keywords: Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, hepatocellular carcinoma, steatosis, inflammation, cirrhosis, cancer.

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

NAFLD is one of the rising epidemics of the 21st century in developed countries given its close association with obesity and the metabolic syndrome (MetS). The disorder was first described and named 'non-alcoholic steatohepatitis (NASH)' in 1980 by Ludwig et al.[1] It is defined as triglyceride accumulation of >5% in hepatocytes.[2,3] Histological features of NASH are indistinguishable from those that hallmark alcoholic steatohepatitis (fatty vacuoles, lobular hepatitis, mixed inflammatory infiltrate, presence of Mallory bodies, and fibrosis in some cases) but patients present without a personal history of alcohol consumption. For the diagnosis of NAFLD a maximal alcohol intake of 20 g/day of alcohol for women and 40 g/day for men is tolerated. Nowadays NAFL (non-alcoholic fatty liver) and NASH are classified using the NAFLD activity score (NAS) composed of 14 histological features.[2] Recently, the group of Bedossa[4,5] developed and validated the SAF-Score (S: steatosis; A: activity; F: fibrosis for the histopathological classification of liver lesions in patients with morbid obesity. The SAF score allows a better distinction between NAFL and NASH, and high interobserver agreement. To date, the prevalence of NAFLD exceeds 25% in Western countries[6] and requires clear guidelines as to how patients need to be stratified and surveyed.

Epidemiology

Clinical studies estimate the prevalence of NAFLD and NASH in Western countries at 20-30% and 3-5%, respectively.[7] The studies are based on different diagnostic criteria including ultrasound (US),[8] liver biopsies performed before planned liver transplantation, and autopsies. Numbers are even higher in autopsy-based studies including obese patients.[9] The prevalence of NAFLD in normal weight individuals (body mass index [BMI] ≤25 kg/m^2) in the NHANES III study was 4-times lower than in obese patients (7% versus 28%).[7]

Aetiology

NAFLD is associated with obesity, impaired glucose tolerance, Type 2 diabetes mellitus (T2DM) and arterial hypertension – and regarded as the hepatic manifestation of the MetS. Pathophysiologically, there is evidence that the accumulation of intracellular lipids in hepatocytes reduces insulin clearance and promotes insulin resistance (IR), respectively.[10] The presence of IR is regarded as the main predisposing factor for metabolic diseases, independently of the BMI and the visceral fat deposit.[11] However, whether NAFLD is the cause or consequence of metabolic disorders, needs to be clarified. Fortunately NAFL, otherwise termed 'simple steatosis', is supposed to follow a benign course, generally without the risk of complications or life-threatening consequences, although reports of cirrhosis in 4% of patients with simple steatosis have been published.[12]

About 25% of all patients with fatty liver develop inflammation and fibrosis termed NASH.[13] NASH is considered to be the more severe form of NAFLD, bearing the risk of hepatic and extrahepatic complications. In about one-third of patients, NASH leads to cirrhosis, which in turn may lead to liver related death, including the development of hepatocellular carcinoma (HCC).[14] To investigate the natural history, NAFLD patients were re-biopsied at least 3 years after their first histologic assessment in a non-interventional study. Indeed, progression to cirrhosis differs between NAFL and NASH patients: none of the patients diagnosed with NAFL at the time of the first histology showed fibrosis in the repeated biopsy, but one-third of patients with initial diagnosis of NASH had a progression of the degree of fibrosis.[15] NAFLD/NASH is expected to represent the leading indication for liver transplantation within the next few years.[16]

Besides this, the cardiovascular risk is markedly increased in patients with MetS and NAFLD. Patients with NAFLD have increased intima thickness of carotid artery, and also higher incidence of coronary, periphery, and cerebrovascular diseases.[17] While a cohort study showed no impact on survival after a follow-up of 13 years in patients with NAFL, survival was significantly reduced in patients with NASH.[18] A different cohort revealed a higher liver-related mortality in patients with NASH. Overall, mortality was higher if patients were older at diagnosis, had T2DM, or a low level of albumin.[19]

Pathophysiology

The sequence of progression from simple steatosis (NAFL) to inflammation and fibrosis (NASH) remains poorly understood. The mechanisms contributing to the accumulation of fat in hepatocytes and successive induction of a local inflammatory response in the liver need further elucidation. There is a link between hepatic steatosis and increased hepatic activity of the nuclear factor κB (NFκB). The upregulation of NFκB related pro-inflammatory cytokines (interleukin 6 [IL-6], tumour necrosis factor alpha [TNF-α], interleukin 1 beta [IL-1β]) activates Kupffer cells, contributing to inflammation and cell injury.[20]

There is substantial evidence for a genetic component in the development of NAFLD. Observational studies detected lower prevalence in African Americans than in Hispanic Americans.[21] A number of genes have subsequently been associated with the phenotype of NAFLD. One documented genetic association is a missense variant in *PNPLA3*. *PNPLA3* (Patatin like phospholipase domain-containing protein 3) is encoding a lipase protein. A single nucleotide polymorphism (rs738409, encoding I148M) was associated with hepatic fat accumulation.[22] Patients that are homozygous for this allele accumulated twice as much intrahepatic fat, compared to those who didn't carry the mutation. Moreover, differences in the composition of the gut microbiota of obese and NAFLD patients have been observed. Modifications of the microbiota can promote gut permeability, allowing the translocation of endotoxins and other bacterial products and of luminal antigens, which may enhance hepatic inflammation.[23]

HCC IN NAFLD

The epidemic of NAFLD elicits a rising number of NAFLD associated advanced fibrosis and increases the burden of HCC. The overall 5-year survival is as low as 15%, given that only 13% of patients diagnosed with HCC are eligible for therapeutic interventions.[24] HCC screening strategies for patients with NAFLD need to be defined in order to detect HCC early and enable treatment within the means of healthcare resources. Effective chemopreventive strategies for patients at increased risk for HCC are desirable.[16] Numbers for the incidence of HCC in NAFLD vary upon the population studied. A single-centre cohort study recently reported development of HCC in >12% of patients with NASH cirrhosis over a median observation period of 3.2 years, consistent with a yearly HCC incidence of 2.6% after the diagnosis of cirrhosis.[25] Another Danish cohort had reported a yearly incidence of 4.6%.[26] As additional risk factors for the development of HCC, older age, higher BMI, and tobacco and alcohol consumption were detected.[27,28]

The incidence of HCC increased over the last decades, representing 90% of all liver cancers and the third cause of cancer-related mortality worldwide.[29] Known risk factors for developing HCC are chronic hepatitis B (HBV) and chronic hepatitis C virus (HCV) infection, but also chronic alcohol consumption. The incidence of HCC in patients with chronic hepatitis C and advanced fibrosis is higher compared to the incidence in patients with NASH (7% versus 2%, respectively).[25] Because of the high prevalence, cases of NAFLD-associated disease have become one of the leading causes of HCC in the United States. The reported proportion of HCC attributed to the diagnosis of NAFLD ranges between 4-22%.[30] Marrero et al.[28] found underlying 'cryptogenic' cirrhosis in 29% of the investigated cases with HCC; among these, 50% displayed clinical and histological features of NASH.

Although advanced fibrosis increased the risk of HCC in NAFLD about 25-fold,[31,32] there is evidence suggesting that HCC can also develop in a simple steatotic liver or in NASH without the presence of cirrhosis.[33,34] In a retrospective histopathological study, including 128 cases of HCC that had undergone resection, Paradis et al.[34] identified that 65% of HCC, in the group of patients with underlying MetS-related liver disease, developed in livers free of significant fibrosis (F0-F2), versus only 26% in the group of patients with other underlying chronic liver diseases (CLD). A similar study in >800 patients diagnosed with HCC, revealed that 42.6% of tumours developed in a non-cirrhotic liver regardless of the diagnosis.[35] A Japanese study reported a rate of complete cirrhosis in liver tissue surrounding the HCC in only 51% of patients with histologically proven NASH. Moreover, the prevalence of cirrhosis was lower in male compared to female individuals (39% versus 70%).[36] Another cohort found HCC in NAFL without features of inflammation, fibrosis or cirrhosis in 3 out of 50 patients (see Table 1).[37]

A recent meta-analysis compared the risk of developing HCC over time in patients with NAFLD

with and without cirrhosis. The association between NAFLD and risk of HCC seemed to be limited to individuals showing features of cirrhosis.[38] In summary, cirrhosis is apparently not required for the development of HCC in chronic NAFLD-related liver disease. However, the relatively low risk of developing HCC in a non-cirrhotic NAFLD liver does not justify HCC screening of the entire NAFLD population. This is an evolving topic. The differential morphological features and pathophysiological mechanisms of hepatocarcinogenesis, in respect to the underlying liver diseases, i.e. NAFLD versus other CLD, and the individual contributions of obesity, T2DM, steatosis, NASH, and degree of fibrosis need detailed elucidation.

Pathophysiology

As detailed for other malignancies, HCC emerges mostly in patients with CLD and develops over years following a dysplasia-carcinoma sequence.[39] The pathophysiological mechanism of HCC development has mainly been studied in models of chronic viral infection with HBV and HCV. In HCC developing in the livers of patients with NAFLD, hepatic oncogenic drivers such as steatosis and inflammation as well as systemic factors, i.e. obesity and T2DM, have been identified. Hepatic steatosis can cause inflammation with subsequent up-regulation of pro-inflammatory cytokines, e.g. IL-1β, IL-6, and TNFα.[40] In accordance, activation of hepatic Kupffer cells has been described in the liver of obese patients, as well as induced fibrosis.[41] Vice-versa, oxidative stress and inflammatory responses are reduced in obese persons after fasting for 48 hours[42] or during a low calorie (1,000 kcal/day) diet over 4 days.[43] In a murine model, NASH and HCC have been induced after (6-) 12 months of American Lifestyle-Induced Obesity Syndrome (ALIOS) diet and sedentary lifestyle. Mice developed typical metabolic changes of NAFLD, such as higher transaminases and higher triglyceride. These histological alterations reflected the characteristics found in the liver of patients with NAFLD: steatosis, ballooned hepatocytes, Mallory bodies, lobular inflammation, peri-sinusoidal fibrosis, and activation of stellate cells. Conclusively, a higher NAS score was observed after 12 months in mice on the ALIOS diet and sedentary lifestyle, compared to mice on a control diet. Surprisingly, 6 out of 10 mice developed hepatocellular neoplasms after 12 months.[44]

Another study published by the same group explored the effect of 5-alpha-reductase (5αR).[45] 5αR is needed to convert cortisol to inactive metabolites, and testosterone to dihydrotestosterone. Both steroids are involved in the regulation of lipid metabolism. The expression level of 5αR-isoforms (Type 1 and Type 2) was similar in NAFLD and healthy control livers. The authors suggest a potential protective role for reduced expression of 5αR Type 1, since $5\alpha R^{-/-}$ knock-out mice (Type 1, only present in mice) displayed enhanced hepatic steatosis, but developed fewer hepatocellular lesions in response to the ALIOS diet. This mechanism needs to be verified in humans.

Table 1: Epidemiologic studies evaluating the development of hepatocellular carcinoma (HCC) in non-alcoholic fatty liver disease (NAFLD).

Study	Design	Number of patients with HCC	HCC in NAFLD	HCC in non-cirrhotic NAFLD
Paradis et al.[34]	Retrospective	128	31	20
Ascha et al.[25]	Retrospective	89	25	0*
Kawamura et al.[31]	Retrospective	16	16	10
Alexander et al.[33]	Retrospective	157	24	24°
Yasui et al.[36]	Retrospective	87	87	43
Guzman et al.[37]	Retrospective	50	5	3
Marrero et al.[28]	Prospective	105	14	0

* only patients with cirrhosis selected
° only non-cirrhotic patients selected

As discussed above, a genome-wide association study identified a variant in the *PNPLA3* gene was associated with susceptibility to NAFLD. The same gene was independently associated with a higher risk of progressive liver fibrosis and HCC. This homozygous mutation is often found in HCC with lower differentiation, multiple foci at diagnosis, and reduced survival for these patients.[46,47] Various studies suggested a higher incidence of neoplastic lesions other than HCC, such as colorectal adenoma and high-grade dysplastic neoplasms of the colon in patients with NAFLD.[48,49] On the other hand, in a meta-analysis of 26 studies, El-Serag et al.[50] found a higher incidence of HCC in patients with diabetes mellitus, regardless of the presence of NAFLD. Also obesity alone has been linked to a higher incidence of malignancies of diverse organs (i.e. colon, endometrium, breast, intestine, liver).[51]

Vaccine and antiviral strategies against HBV and therapy for chronic HCV are effective in lowering the incidence of HCC in these conditions. A recent review by Singh at al.[16] systematically discussed chemopreventive strategies against HCC. The use of statins seemed to reduce the risk of HCC in epidemiological studies, but has not been demonstrated in double-blind placebo-controlled trials. These trials had mostly been designed to evaluate cardiovascular end points and addressed the incidence of HCC as a post-hoc analysis only. Other observational studies in diabetic patients detected a reduced risk of HCC in those treated with metformin. The effect may be promoted through inhibition of cell growth and downregulation of c-Myc. Aspirin, bearing an anti-inflammatory effect, had some antineoplastic properties. However, epidemiological studies might be biased and overestimate the actual effect. Dietary changes have also been evaluated: coffee, known to reduce the risk of fibrosis and cirrhosis, seemed to be effective in reduction of HCC regardless of the chronic underlying liver disease.[16]

RECOMMENDATIONS FOR DIAGNOSTICS, TREATMENT, AND FOLLOW-UP OF PATIENTS WITH NAFLD

In medical practice, patients with persistently elevated transaminases should be referred to a specialised hepatology centre for work up of the underlying condition. Hepatic steatosis can be detected using non-invasive imaging techniques, such as US and magnetic resonance imaging (MRI), but these do not allow for the assessment of hepatic fibrosis. Non-invasive methods to identify fibrosis in NAFLD include NAFLD Fibrosis Score, levels of cytokeratin 18 (CK18), and measuring transient elastography of the liver.[52] The NAFLD Fibrosis score is a calculated score based on age, BMI, albumin, aspartate aminotransferase/alanine transaminase ratio, hyperglycaemia, and platelet count.[53] CK18 is significantly increased in patients with NASH compared to NAFL or absence of steatosis.[54] The assessment of liver stiffness by transient elastography (Fibroscan) in individuals with biopsy proven NAFLD showed a high negative predictive value to exclude advanced fibrosis, but often failed in patients with high BMI.[55]

Although an invasive procedure bearing the risk of complications, liver biopsy is still the diagnostic gold standard and the only method allowing the detection and quantification of fibrosis and inflammation in the steatotic liver. Also, it is important to screen for other CLD since steatosis can be detected in hepatitis C, Wilson's and coeliac disease, or as a consequence of the toxic effects of alcohol or prescription drugs. Since, depending on the population studied, >25% of the population in Western countries are predicted to carry the diagnosis of NAFLD, a general follow-up of each and every patient in a specialised centre cannot be recommended. However, patients with biopsy proven NASH need counselling regarding the risk of disease progression towards cirrhosis and possible life-threatening complications, such as acute-on-chronic liver failure or HCC. Furthermore, potential extrahepatic complications such as malignancies other than HCC and the risk of cardiovascular disease need to be evaluated and treated. Regular follow-up of patients with NASH is recommended in order to monitor disease progression. The recommended treatment for NAFLD is lifestyle modification with the aim to reduce weight but also IR, the major target of current therapeutic approaches.[56] Until now, no effective pharmaceutical therapy has been approved.[52]

A number of clinical trials evaluating novel targets are ongoing. Promising effects were shown in the FLINT trial[57] evaluating obeticholic acid (OCA) versus placebo in patients with NASH. Treatment with OCA for 72 weeks improved histological findings. However pruritus occurring in 23% of patients on OCA requires further clarification of drug-safety.[57] Another multicentre randomised clinical trial evaluated 11β-hydroxysteroid

dehydrogenase Type 1 (11β-HSD1) versus placebo in patients with in MRI-detected NAFLD. 11β-HSD1 reduced hepatic fat content by 2% while there was no difference in the placebo group.[58] In our NAFLD/NASH cohort, we documented a significantly lower secretion of glucose induced glucagon-like peptide-1 (GLP-1) along with a reduced glucose-lowering effect in NAFLD and NASH patients, endorsing ongoing clinical studies evaluating the use of GLP-1-analogs for the treatment of NAFLD.[59]

Gastric bypass or sleeve gastrectomy decrease the BMI and improve extrahepatic diseases related to MetS.[60] However, the beneficial effect on hepatic fibrosis or cirrhosis in patients with NASH could not be proven in cohort studies.[61] Comorbidities of MetS and a multidisciplinary team evaluation, involving endocrinologists, surgeons, and gastroenterologists/hepatologists, clearly need to justify the indication for and the risks of this incisive surgical procedure. A recent study highlights the significantly increased peri and postoperative morbidity and mortality in cirrhotic patients showing 10% of postoperative mortality, 32% of major complications, and 43% deterioration of liver function after major surgery.[62] All patients with NAFLD-related cirrhosis should undergo HCC screening in 6-monthly intervals. HCC in non-cirrhotic patients with NAFLD/NASH has been reported at considerable frequencies, but the true incidence is still regarded too low to support a general HCC screening for all NAFLD patients. Chemopreventive strategies might be beneficial but cannot be recommended yet.

REFERENCES

1. Ludwig J et al. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc. 1980;55(7):434-8.

2. Kleiner DE et al; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313-21.

3. Brunt EM. Nonalcoholic fatty liver disease: what the pathologist can tell the clinician. Dig Dis. 2012;30 Suppl 1:61-8.

4. Bedossa P et al. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. Hepatology. 2012;56(5):1751-9.

5. Bedossa P; FLIP Pathology Consortium. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. Hepatology. 2014;60(2):565-75.

6. Lazo M et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. Am J Epidemiol. 2013;178(1):38-45.

7. Armstrong MJ et al. Presence and severity of non-alcoholic fatty liver disease in a large prospective primary care cohort. J Hepatol. 2012;56(1):234-40.

8. Musso G et al. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med. 2011;43(8):617-49.

9. Zois CD et al. Steatosis and steatohepatitis in postmortem material from Northwestern Greece. World J Gastroenterol. 2010;16(31):3944-9.

10. Tamura Y et al. Effects of diet and exercise on muscle and liver intracellular lipid contents and insulin sensitivity in type 2 diabetic patients. J Clin Endocrinol Metab. 2005;90(6):3191-6.

11. Fabbrini E et al. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology. 2010;51(2):679-89.

12. Matteoni CA et al. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology. 1999;116(6):1413-9.

13. McCullough AJ. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. Clin Liver Dis. 2004;8(3):521-33, viii.

14. Powell EE et al. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. Hepatology. 1990;11(1):74-80.

15. Fassio E et al. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. Hepatology. 2004;40(4):820-6.

16. Singh S et al. Chemopreventive strategies in hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2014;11(1):45-54.

17. Targher G et al. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med. 2010;363(14):1341-50.

18. Ekstedt M et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology. 2006;44(4):865-73.

19. Rafiq N et al. Long-term follow-up of patients with nonalcoholic fatty liver. Clin Gastroenterol Hepatol. 2009;7(2):234-8.

20. Day CP. Natural history of NAFLD: remarkably benign in the absence of cirrhosis. Gastroenterology. 2005;129(1):375-8.

21. Schwimmer JB et al. Heritability of nonalcoholic fatty liver disease. Gastroenterology. 2009;136(5):1585-92.

22. Romeo S et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008;40(12):1461-5.

23. Miura K, Ohnishi H. Role of gut microbiota and Toll-like receptors in nonalcoholic fatty liver disease. World J Gastroenterol. 2014;20(23):7381-91.

24. El-Serag HB et al. Treatment and outcomes of treating of hepatocellular carcinoma among Medicare recipients in the United States: a population-based study. J Hepatol. 2006;44(1):158-66.

25. Ascha MS et al. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. Hepatology. 2010;51(6):1972-8.

26. Sørensen HT et al. Risk of cancer in patients hospitalized with fatty liver: a Danish cohort study. J Clin Gastroenterol. 2003;36(4):356-9.

27. Dyson J et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. J Hepatol. 2014;60(1):110-7.

28. Marrero JA et al. Alcohol, tobacco and obesity are synergistic risk factors for hepatocellular carcinoma. J Hepatol.

2005;42(2):218-24.

29. European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56(4):908-43.

30. Michelotti GA et al. NAFLD, NASH and liver cancer. Nat Rev Gastroenterol Hepatol. 2013;10(11):656-65.

31. Kawamura Y et al. Large-scale long-term follow-up study of Japanese patients with non-alcoholic fatty liver disease for the onset of hepatocellular carcinoma. Am J Gastroenterol. 2012;107(2):253-61.

32. Hashimoto E, Farrell GC. Will non-invasive markers replace liver biopsy for diagnosing and staging fibrosis in non-alcoholic steatohepatitis? J Gastroenterol Hepatol. 2009;24(4):501-3.

33. Alexander J et al. Non-alcoholic fatty liver disease contributes to hepatocarcinogenesis in non-cirrhotic liver: a clinical and pathological study. J Gastroenterol Hepatol. 2013;28(5): 848-54.

34. Paradis V et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. Hepatology. 2009;49(3):851-9.

35. Nzeako UC et al. Hepatocellular carcinoma in cirrhotic and noncirrhotic livers. A clinico-histopathologic study of 804 North American patients. Am J Clin Pathol. 1996;105(1):65-75.

36. Yasui K et al; Japan NASH Study Group, Ministry of Health, Labour, and Welfare of Japan. Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. Clin Gastroenterol Hepatol. 2011;9(5):428-33; quiz e50.

37. Guzman G et al. Does nonalcoholic fatty liver disease predispose patients to hepatocellular carcinoma in the absence of cirrhosis? Arch Pathol Lab Med. 2008;132(11):1761-6.

38. White DL et al. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. Clin Gastroenterol Hepatol. 2012;10(12):1342-1359.e2.

39. Kojiro M, Nakashima O. Histopathologic evaluation of hepatocellular carcinoma with special reference to small early stage tumors. Semin Liver Dis. 1999;19(3): 287-96.

40. Shoelson SE et al. Obesity, inflammation, and insulin resistance. Gastroenterology. 2007;132(6):2169-80.

41. Farrell GC et al. NASH is an inflammatory disorder: pathogenic, prognostic and therapeutic implications. Gut Liver. 2012;6(2):149-71.

42. Dandona P et al. The suppressive effect of dietary restriction and weight loss in the obese on the generation of reactive oxygen species by leukocytes, lipid peroxidation, and protein carbonylation. J Clin Endocrinol Metab. 2001;86(1):355-62.

43. Dandona P et al. Inhibitory effect of a two day fast on reactive oxygen species (ROS) generation by leucocytes and plasma ortho-tyrosine and meta-tyrosine concentrations. J Clin Endocrinol Metab. 2001;86(6):2899-902.

44. Dowman JK et al. Development of hepatocellular carcinoma in a murine model of nonalcoholic steatohepatitis induced by use of a high-fat/fructose diet and sedentary lifestyle. Am J Pathol. 2014;184(5):1550-61.

45. Dowman JK et al. Loss of 5α-reductase type 1 accelerates the development of hepatic steatosis but protects against hepatocellular carcinoma in male mice. Endocrinology. 2013;154(12):4536-47.

46. Valenti L et al. PNPLA3 I148M polymorphism, clinical presentation, and survival in patients with hepatocellular carcinoma. PLoS One. 2013;8(10):e75982.

47. Hassan MM et al. Genetic variation in the PNPLA3 gene and hepatocellular carcinoma in USA: risk and prognosis prediction. Mol Carcinog. 2013;52 Suppl 1:E139-47.

48. Huang KW et al. Patients with nonalcoholic fatty liver disease have higher risk of colorectal adenoma after negative baseline colonoscopy. Colorectal Dis. 2013;15(7):830-5.

49. Wong VW et al. High prevalence of colorectal neoplasm in patients with non-alcoholic steatohepatitis. Gut. 2011;60(6):829-36.

50. El-Serag HB et al. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. Clin Gastroenterol Hepatol. 2006;4(3):369-80.

51. Wolk A et al. A prospective study of obesity and cancer risk (Sweden). Cancer Causes Control. 2001;12(1):13-21.

52. Chalasani N et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55(6):2005-23.

53. Angulo P et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45(4):846-54.

54. Wieckowska A et al. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. Hepatology. 2006;44(1):27-33.

55. Kwok R et al. Systematic review with meta-analysis: non-invasive assessment of non-alcoholic fatty liver disease--the role of transient elastography and plasma cytokeratin-18 fragments. Aliment Pharmacol Ther. 2014;39(3):254-69.

56. Thoma C et al. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. J Hepatol. 2012;56(1):255-66.

57. Neuschwander-Tetri BA et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet. 2014;doi:10.1016/S0140-6736(14)61933-4. [Epub ahead of print].

58. Stefan N et al. Inhibition of 11β-HSD1 with RO5093151 for non-alcoholic fatty liver disease: a multicentre, randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2014;2(5):406-16.

59. Bernsmeier C et al. Glucose-induced glucagon-like Peptide 1 secretion is deficient in patients with non-alcoholic fatty liver disease. PLoS One. 2014;9(1):e87488.

60. Chavez-Tapia NC et al. Bariatric surgery for non-alcoholic steatohepatitis in obese patients. Cochrane Database Syst Rev. 2010;(1):CD007340.

61. Cotrim HP, Daltro C. Liver: does bariatric surgery reduce the severity of NAFLD? Nat Rev Gastroenterol Hepatol. 2010;7(1):11-3.

62. Kim MK et al. Effects of bariatric surgery on metabolic and nutritional parameters in severely obese Korean patients with type 2 diabetes: a prospective 2-year follow up. J Diabetes Investig. 2014;5(2):221-7.

ALCOHOL DEPENDENCE AND ALCOHOLIC LIVER DISEASE

Summary of Presentations from the H. Lundbeck A/S-Supported Symposium, held at the 49th Annual International Liver Congress, London, United Kingdom, on 10th April 2014

***Karl Mann,[1] Sebastian Mueller[2]**

1. Department of Addictive Behaviour and Addiction Medicine, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Heidelberg, Germany
2. Department of Internal Medicine, Salem Medical Center and Center for Alcohol Research, University of Heidelberg, Heidelberg, Germany
**Correspondence to Karl.Mann@zi-mannheim.de*

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MEETING SUMMARY

Alcohol dependence is a disabling condition that has a high prevalence, but in Europe only a small fraction of the people diagnosed with alcohol abuse and dependence are treated, representing the widest treatment gap, as compared with other mental disorders. Early diagnosis and monitoring of alcoholic liver disease (ALD) is still insufficiently solved. Although ALD is the most common cause for liver disease in the Western world, it largely remains underestimated and underdiagnosed for many reasons. The recent introduction of non-invasive elastographic techniques such as transient elastography (TE) has significantly improved the early diagnosis of alcoholic liver cirrhosis (ALC). As demonstrated in the literature, inflammation-associated liver stiffness (LS) rapidly decreases during alcohol detoxification, and is also directly correlated to change in LS in both abstinent and relapsing patients. Newly published data show that LS could be used to monitor and validate hepatoprotective effects during nalmefene usage.

Nalmefene is an opioid system modulator that diminishes the reinforcing effects of alcohol, helping the patient to reduce drinking. Three randomised, multicentre, double-blind, placebo-controlled, parallel-group Phase III studies were designed to assess the efficacy and safety of nalmefene in reducing alcohol consumption. Patients with a high or very high drinking risk level (DRL) at baseline and randomisation show a clinically significant effect from nalmefene treatment, which is generally well tolerated. Moreover, reduced alcohol consumption supported by nalmefene in combination with psychosocial support may indeed help to reduce the alcohol-related burden and the large treatment gap.

Nalmefene – A New Treatment Option in Alcohol Dependence

Professor Karl Mann

Alcohol consumption demographics and management of alcohol dependence

Alcohol dependence is a disabling condition that has a high prevalence, with Europe having the highest per capita (10 to over 12.50 litres) pure alcohol consumption of all world regions.[1] Alcohol consumption and dependence can have multiple negative social consequences, such as disrupted relationships with family and friends, violence, crime and accidents, and lack of productivity in the workplace, often leading to unemployment.[1-4] In Europe, only a small fraction (8.3%) of the people diagnosed with alcohol abuse and dependence are treated, representing the widest treatment gap, as compared with other

mental disorders.[5] In a survey conducted from 2009 to 2012, the main reasons given for not receiving alcohol treatment in the past year by American individuals aged 12 and older (n=67,500) who needed treatment and who perceived a need for it were that they were not ready to stop alcohol use (49.5%) and that they had no health coverage and could not afford the costs related to alcohol treatment (30.3%).[6]

While treatment for alcohol use disorder comprises total abstinence using psychotherapeutic and pharmacological treatment modalities, these results show that many individuals are not able or willing to achieve abstinence, resulting in a medical condition that is under-treated.

This is reflected in the latest guidelines from the European Medicines Agency (EMA, 2010), the US National Institute on Alcohol Abuse and Alcoholism (NIAAA, 2007), the Canadian Centre for Addiction and Mental Health (2012), and the British National Institute for Health and Clinical Excellence (NICE, 2011), that highlight that alcohol consumption reduction is an appropriate treatment goal for certain patients.[7-11] Therefore, novel pharmaceutical agents such as nalmefene, which aim to provide support in the reduction of alcohol consumption, represent a significant improvement in the therapeutic armamentarium.

Nalmefene

Adaptation of the brain to alcohol through brain chemistry and neuroadaptive changes leading to dependence has already been established. One of the affected areas of the brain is the mesolimbic dopamine system, a network of interconnected brain regions that includes the ventral tegmental area, the prefrontal cortex, and the nucleus accumbens.[12] Nalmefene (Selincro®, H. Lundbeck A/S) is an opioid system modulator, with antagonist activity at the μ and δ opioid receptors and partial agonist activity at the κ opioid receptor. It diminishes the reinforcing effects of alcohol, helping the patient to reduce drinking.[13,14] The pharmacological properties of nalmefene enable an 'as needed' dosing: it is rapidly absorbed with peak plasma level at 1 hour, with a half-life of approximately 13 hours (longer than that of naltrexone) and high receptor occupancy (87–100%) within 3 hours and also after 26 hours (83–100%).[15] Nalmefene was approved in February 2013 by the EMA for the reduction of alcohol consumption in adult patients with alcohol dependence who have a high DRL, without physical withdrawal symptoms and who do not require immediate detoxification. Nalmefene should only be prescribed in conjunction with continuous psychosocial support focused on treatment adherence and reducing alcohol consumption.

Phase III studies on nalmefene

Three randomised, multicentre, double-blind, placebo-controlled, parallel-group Phase III studies were designed to assess the efficacy and safety of nalmefene in reducing alcohol consumption. This Phase III programme enrolled about 2,000 patients with alcohol dependence (Table 1). Two studies comprised a 24-week treatment period followed by a 4-week run-out phase (ESENSE 1 & 2) while the third one (SENSE) was a 52-week study.

Table 1: Main characteristics and results for ESENSE 1, ESENSE 2, and SENSE Phase III studies.

Study name	ESENSE 1 (12014A)	ESENSE 2 (12023A)	SENSE (12013A)
Study duration	24 weeks plus 4-week run-out	24 weeks plus 4-week run-out	52 weeks
Patients enrolled	604 (306 NMF+298 PBO)	718 (358 NMF+360 PBO)	675 (509 NMF+166 PBO)
Difference to placebo at 6 months			
HDDs per month	-2.3 (95% CI, -3.8 to -0.8; p=0.0021)	-1.7 (95% CI, -3.1 to -0.4; p=0.012)	-0.9 (95% CI, -2.1 to 0.4; p=0.160)
TAC (g/day)	-11.0 (95% CI, -16.8 to -5.1; p=0.0003)	-5.0 (95% CI, -10.6 to 0.7; p=0.088)	-3.5 (95% CI, -9.2 to -2.2; p=0.232)

CI: confidence interval; HDD: heavy drinking day; NMF: nalmefene; PBO: placebo; TAC: total alcohol consumption.

Objectives, design, and main endpoints

The ESENSE 1 and 2 studies aimed to evaluate the effect of nalmefene on alcohol consumption at study end.[16,17] The SENSE study[18] aimed to evaluate the safety and tolerability of nalmefene at study end, as well as the effect of nalmefene on alcohol consumption at 6 months. In all three studies, eligible patients were aged 18 or older and were diagnosed with alcohol dependence according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV).[19] Exclusion criteria related to alcohol consumption comprised DRLs (EMA/World Health Organization [WHO] criteria) below medium (total alcohol consumption [TAC] <40 g/day in men, <20 g/day in women) at baseline,[20,21] 5 or fewer heavy drinking days (HDD; 60 g/day or more of pure alcohol in men, 40 g/day or more in women) in the 4 weeks prior to screening. Other exclusion criteria were aspartate aminotransferase (S-ASAT) and/or alanine transaminase (S-ALAT) levels >3 times upper the normal limits, psychiatric comorbidities, and a Revised Clinical Institute Withdrawal Assessment for Alcohol score of 10 or higher.

Nalmefene 20 mg nalmefene hydrochloride (~18 mg base) tablets were investigated in an 'as-needed' regimen against placebo tablets. One tablet was to be taken on days where patients anticipated a risk of drinking (preferably 1–2 hours prior to anticipated time of drinking), or if the patient had started drinking, as soon as possible thereafter. Treatment could be used daily when patients felt a risk of drinking every day, but for no more than one tablet per day.

In the ESENSE studies, the first visit (V1) was the starting point of an assessment period of 1-2 weeks, leading to 1:1 randomisation at the second visit (V2) to active treatment (nalmefene) or to placebo for 24 weeks, in combination with motivational and compliance intervention.[16,17] Visits to conduct assessments of efficacy and safety were scheduled at weeks 1, 2, and 4, then on a monthly basis (up to V10). In the subsequent 4-week run-out phase, following re-randomisation patients from the active treatment arm either remained on nalmefene therapy or switched to placebo, while patients from the placebo arm remained on placebo. Following completion of this phase, patients where followed up during a 4-week safety follow-up period.

In the SENSE study, the first visit (V1) was followed by an assessment period of 1-2 weeks, then by 3:1 randomisation (at V2) to a 52-week treatment phase with either 'as-needed' nalmefene 18 mg (base) or placebo.[18,22] Visits to conduct assessments of efficacy and safety were scheduled at weeks 1, 2, and 4, then on a monthly basis. Main endpoints in all three studies were the numbers and change from baseline with respect to monthly number of HDDs, monthly TAC, and WHO DRLs.[20,21]

Main clinical findings

Differences to placebo at 6 months in HDDs were of -2.3, -1.7, and -0.9 HDDs per month in the ESENSE 1 ($p<0.05$), ESENSE 2 ($p<0.05$), and SENSE studies, respectively (Table 1).[16-18,22] Differences to placebo at 6 months in terms of TAC were of -11.0, -5.0, and -3.5 g per day in the ESENSE 1, ESENSE 2, and SENSE studies, respectively. A reduction in alcohol consumption during the assessment period prior to randomisation is a known phenomenon.[23,24] In the nalmefene studies 18% (ESENSE 1), 33% (ESENSE 2), and 39% (SENSE) of patients reduced their alcohol consumption in the period between screening and randomisation.[16-18] Comparable patterns were seen for both HDDs and TAC across all three studies.

The benefit of nalmefene was further studied in a pooled 6-month sample from both ESENSE studies.[22] Subgroup analyses showed that patients with high or very high DRL at baseline and randomisation, with no reduction in alcohol consumption prior to randomisation, were associated with the most pronounced clinical benefits of treatment than the general population of the studies (HDD −3.2/months versus −2.0/months, respectively; reduction in TAC, -14.3 g/day versus −7.6 g/day, respectively). Responder analyses in this subgroup showed a 2-category downward shift in WHO DRL (very high-risk to medium-risk consumption; high-risk to low-risk consumption; overall ratio [OR] for 2-category downward shift, 1.87, 95% confidence interval, 1.35-2.59).[25] Similarly, patients with at least high DRL at baseline and at randomisation in the SENSE study (placebo n=42; nalmefene n=141) identified as most likely to benefit ('target population'), the net treatment effect over placebo in terms of reduction of alcohol consumption was more pronounced at 13 months as compared with the total population (HDD −3.6/month versus −1.6/month, respectively; reduction in TAC, −17.3 g/day versus −6.5g/day, respectively).[18] In the pooled 6-month high DRL sample from the ESENSE studies, adjusted mean change from baseline in Impression-Severity of Illness

and Improvement scales (CGI-S and CGI-I) in the nalmefene group were significant versus placebo at 24 weeks.[26]

These results were consistent with liver function test outcomes for the same sample of patients with high DRLs, as glutamyltransferase (GGT) and alanine aminotransferase (ALT) levels at 24 weeks were significantly reduced versus placebo in almost all outcomes (adjusted geometric means, $p<0.05$ for GGT/ALT in ESENSE 1, $p<0.05$ for ALT in ESENSE 2, $p=0.244$ for GGT in ESENSE 2). Safety results from a pooled analysis of all three Phase III studies showed that the most frequent (>10% of patients) adverse events (AEs) in the nalmefene arm (n=1,144) were nausea, dizziness, insomnia, and headache. Overall AEs were of mild or moderate intensity.[16,17,25,27,28] As previously demonstrated by Rehm et al.,[29,30] alcohol consumption reduction of 36 g/day from a baseline of 96 g/day corresponds to a reduced lifetime mortality risk of 119 per 10,000, while a reduction of 36 g/day (3 drinks) from a baseline of 60 g/day corresponds to a reduced lifetime mortality risk of 38 per 10,000.

In conclusion, there remains a large treatment gap for alcohol dependence, but the reduced risks associated with reduced alcohol consumption supported by nalmefene, in combination with psychosocial support are meaningful. Patients with a high or very high DRL at baseline and randomisation showed a greater benefit from nalmefene treatment, which was generally well tolerated.

Non-Invasive Assessments for Early Diagnosis of ALD

Professor Sebastian Mueller

As stated above, nalmefene lowers alcohol consumption in patients addicted to alcohol. But nalmefene also significantly reduces transaminase levels. Indeed, in a pooled analysis from the ESENSE 1 and 2 studies, adjusted geometric means at 24 months were significantly lower in the nalmefene arms (n=187) than in the placebo arms (n=220 for GGT; n=218 for ALT), with respect to γ-GGT (43.5 IU/l versus 53.0 IU/l; $p=0.0005$) and ALT (26.0 IU/l versus 30.7 IU/l; $p=0.0001$) levels.[22,25,26] Whether these effects are related to decreased alcohol consumption or additional pharmacological mechanisms, and whether they can prevent disease progression towards cirrhosis remains largely unknown. For many practical and technical reasons, it is difficult to objectify the hepatoprotective effects of nalmefene treatment in patients with ALD. In addition, early diagnosis and monitoring of ALD is still insufficiently solved. Although ALD is the most common liver disease in the Western world, it largely remains underestimated and underdiagnosed for many reasons: it is under-reported by patients, and underestimated by physicians and by healthcare statistics.[31,32]

Establishing a definite diagnosis is crucial to the subsequent management of the disease, particularly the prevention of complications such as bleeding, ascites, peritonitis, and encephalopathy. If left untreated, ALD naturally progresses to alcoholic steatohepatitis (ASH), either leading to liver cirrhosis and hepatocellular carcinoma (HCC), or to alcoholic hepatitis. As these conditions are associated to a significant risk of mortality, early diagnosis and management of ALD need to be upheld. The diagnosis of ALD and alcoholic cirrhosis usually relies on a combination of clinical, laboratory (GGT, glutamate oxaloacetate transaminase [GOT], ferritin, bilirubin, platelets etc.) and imaging findings (ultrasound, computed tomography, and magnetic resonance imaging).[33,34] However, standard screening tools for ALD can overlook as much as 40% of manifest ALC.[32] Liver biopsy can add useful information in patients with ALD especially the exclusion of comorbidities. However, it is an invasive procedure that is associated with mild and severe complications and shows a rather high sampling error of up to 30%. Liver biopsy is therefore not suitable to follow-up patients with ALD. LS has emerged in the last decade as an important non-invasive parameter to assess the degree of fibrosis, thus monitoring and screening ALD patients at high risk to rapidly progress to cirrhosis. The recent introduction of non-invasive elastographic techniques such as TE (Fibroscan), acoustic radiation force impulse imaging, magnetic resonance elastography, or shear wave elastography has significantly improved the early diagnosis of alcoholic cirrhosis.[35]

LS below 6 kPa is considered as normal, while METAVIR F3 and F4 stage of fibrosis (cirrhosis) have established cut-offs for LS of 8 and 12.5 kPa. Several publications revealed that the diagnostic stiffness cut-offs for cirrhosis stage F4 in ALD patients was higher than that of hepatitis C virus patients. Applying the cut-offs of HCV to ALD patients would yield a very high sensitivity but a lower specificity for ALD.[36-38] These techniques

are of particular importance as they contrast significantly with the level of sample error seen with histological assessments (approximately 30%).[39-43] Indeed, the sample error for LS assessment is of about 3%.[44] Moreover, LS as measured by Fibroscan showed an excellent correlation with histological fibrosis stages in alcoholic patients, and exhibited good diagnostic performance warranting systematic use.[36-38,45-47] However, LS can be influenced by multiple factors: fibrosis, inflammation, cholestasis, liver congestion, or venous pressure.[48-51] Inflammation-increased LS could hinder the detection of fibrosis in ALD patients.[52] Moreover, there remains a 'grey area' between 6 and 8 kPa in which diagnosis is difficult to establish. As demonstrated in the literature, inflammation-associated LS rapidly decreases during alcohol detoxification,[38,53] and is also directly correlated to change in LS in both abstinent and relapsing patients.[54]

In 2013, Mueller et al.[55] proposed an algorithm (Figure 1[33,55]) to either exclude or determine fibrosis stage via LS, recommending that all patients with >6 kPa in LS be assessed according to GOT levels. If the latter are of >100 IU/l, then accurate determination of fibrosis stage is not possible and alcohol detoxification is required before a proper evaluation can be conducted. In patients with GOT levels >100 IU/l, F1-2 fibrosis is established for stiffness ranging from 6-8 kPa, then F3 (>8kPa) and F4 (>12.5 kPa) cut-offs as stated above. Recently, a large multicentre study on >2,000 patients with ALD and chronic hepatitis C was conducted to establish a correlation between GOT levels and LS and to establish optimised, GOT-adapted cut-off values.[56] Among the parameters for liver damage, GOT levels were identified to show the most significant association with LS. Consequently, GOT-adapted cut-off values have been proposed for immediate fibrosis stage assessment or for those patients who will not undergo alcohol withdrawal. In conclusion, it appears that novel non-invasive parameter could be used to monitor hepatoprotective effects during nalmefene usage. In patients with ALD, LS reflects both the degree of inflammation, liver damage, and fibrosis. Novel technologies such as TE show a small sampling error and could allow a better validation of hepatoprotective effects of drugs such as nalmefene.

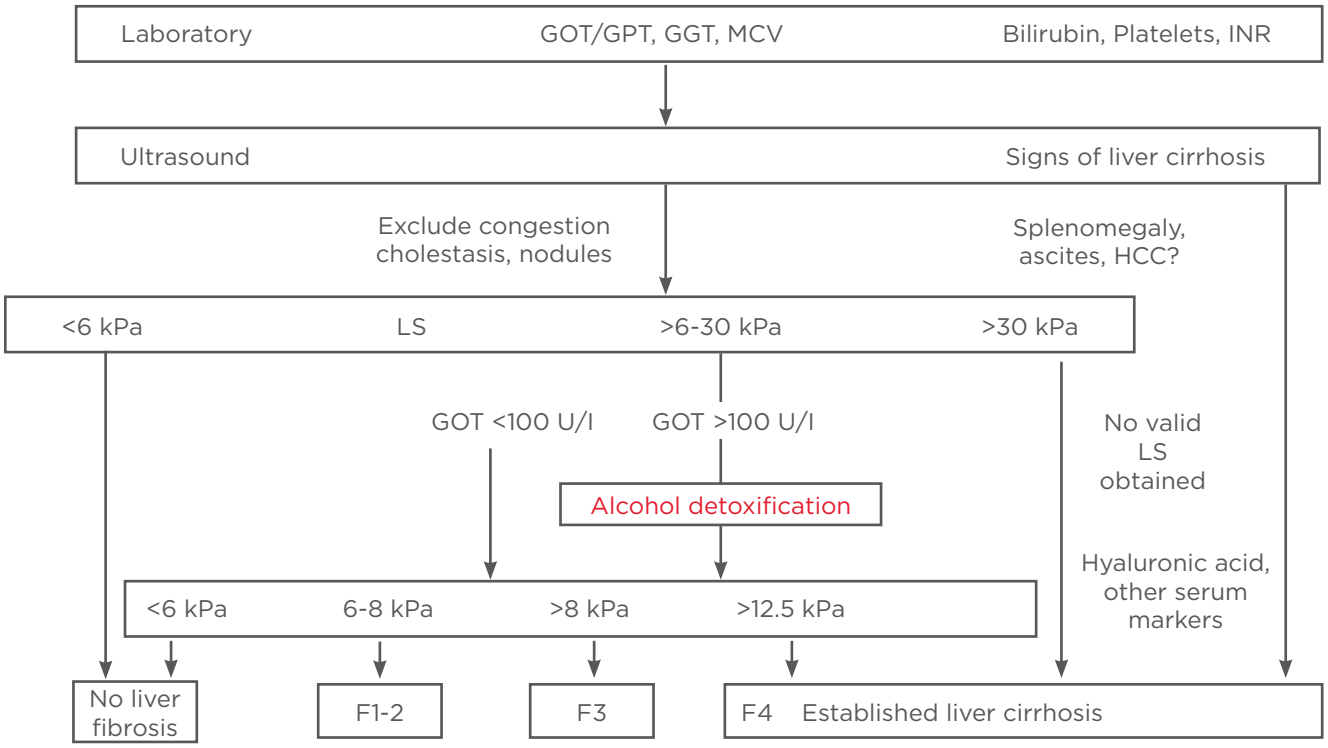

Figure 1: Decision algorithm for fibrosis assessment in alcoholic liver disease.[33,55]
GOT: glutamate oxalacetate transaminase; GPT: glutamate pyruvate transaminase; GGT: glutamyltransferase; MCV: mean corpuscular volume; INR: international normalised ratio; HCC: hepatocellular carcinoma; LS: liver stiffness.

REFERENCES

1. Fleischmann A et al. Global status report on alcohol and health. World Health Organization. 2011.

2. Anderson P, Baumberg, B. Alcohol in Europe. A public health perspective. London: Institute of Alcohol Studies. 2006.

3. Rossow I, Hauge R. Who pays for the drinking? Characteristics of the extent and distribution of social harms from others' drinking. Addiction. 2004;99(9): 1094-102.

4. Caetano R, Cunradi C. Alcohol dependence: a public health perspective. Addiction. 2002;97(6):633-45.

5. Kohn R et al. The treatment gap in mental health care. Bull World Health Organ. 2004;82(11):858-66.

6. Substance Abuse and Mental Health Services Administration, Results from the 2012 National Survey on Drug Use and Health: Summary of National Findings, NSDUH Series H-46, HHS Publication No. (SMA) 13-4795. Rockville, MD: Substance Abuse and Mental Health Services Administration, 2013.

7. EMA. Guideline on the development of medicinal products for the treatment of alcohol dependence. 2010.

8. National Institute on Alcohol Abuse and Alcoholism. Helping patients who drink too much. A Clinician's Guide. Updated 2005 edition.

9. National Institute on Alcohol Abuse and Alcoholism. Rethinking drinking: alcohol and your health. 2010.

10. National Institute for Health and Care Excellence (NICE). Clinical Guideline 115. 2011.

11. Rehm J et al. Alcohol consumption, alcohol dependence and attributable burden of disease in Europe. Potential gains from effective interventions for alcohol dependence. Centre for Addiction and Mental Health. 2012.

12. Clapp P et al. How adaptation of the brain to alcohol leads to dependence: a pharmacological perspective. Alcohol Res Health. 2008;31(4):310-39.

13. Lundbeck. Selincro (Nalmefene). Summary of Product Characteristics. 2013. Available: https://www.medicines.org.uk/emc/medicine/27609/SPC/Selincro+18mg+film-coated+tablets. 18 December 2014.

14. EMA. Nalmefene European Public Assessment Report. 2012.

15. Ingman K et al. Prolonged central mu-opioid receptor occupancy after single and repeated nalmefene dosing. Neuropsychopharmacology. 2005;30(12):2245-53.

16. Mann K et al. Extending the treatment options in alcohol dependence: a randomized controlled study of as-needed nalmefene. Biol Psychiatry. 2013;73(8):706-13.

17. Gual A et al; ESENSE 2 Study Group. A randomised, double-blind, placebo-controlled, efficacy study of nalmefene, as-needed use, in patients with alcohol dependence. Eur Neuropsychopharmacol. 2013;23(11):1432-42.

18. van den Brink W et al; for the SENSE Study Group. Long-term efficacy, tolerability and safety of nalmefene as-needed in patients with alcohol dependence: a 1-year, randomised controlled study. J Psychopharmacol. 2014;28(8):733-44.

19. American Psychiatric Association (APA) (ed.), Diagnostic and statistical manual of mental disorders: Text Revision (DSM-IV) (1994) 4th edition, American Psychiatric Association: Washington, DC.

20. Rehm J et al. Steps towards constructing a global comparative risk analysis for alcohol consumption: determining indicators and empirical weights for patterns of drinking, deciding about theoretical minimum, and dealing with different consequences. Eur Addict Res. 2001;7(3):138-47.

21. World Health Organization. International guide for monitoring alcohol consumption and related harm. 2010.

22. van den Brink W et al. Efficacy of as-needed nalmefene in alcohol-dependent patients with at least a high drinking risk level: results from a subgroup analysis of two randomized controlled 6-month studies. Alcohol Alcohol. 2013;48(5): 570-8.

23. Epstein EE et al. Is alcohol assessment therapeutic? Pretreatment change in drinking among alcohol-dependent women. J Stud Alcohol. 2005;66(3): 369-78.

24. Litten RZ et al; NCIG 001 Study Group. A double-blind, placebo-controlled trial to assess the efficacy of quetiapine fumarate XR in very heavy-drinking alcohol-dependent patients. Alcohol Clin Exp Res. 2012;36(3):406-16.

25. Gual A et al. Efficacy of nalmefene as-needed in alcohol dependent patients with high drinking risk level: subgroup analysis of two randomised controlled studies. Poster presentation P346. WONCA Family Medicine Conference, Prague, Czech Republic, 25–29 June, 2013.

26. Aubin H et al. Clinical relevance of as-needed treatment with nalmefene in alcohol dependent patients. Abstract-0255. Presented at EPA Congress, Munich, Germany, 1-4 March, 2014.

27. van den Brink W et al. Long-term efficacy, tolerability and safety of nalmefene as-needed in alcohol dependence: a randomised, double-blind, placebo controlled study. Poster 302-T-945. Presented at the 35th Annual RSA Scientific Meeting, San Francisco, California, USA, 23-27 June, 2012.

28. van den Brink W et al. Tolerability and safety of as-needed nalmefene in the treatment of alcohol dependence: results from the phase 3 programme. Abstract 0405. Presented at EPA 2014, Munich, Germany, 1-4 March, 2014.

29. Rehm J et al. Epidemiology and alcohol policy in Europe. Addiction. 2011;106 Suppl 1:11-9.

30. Rehm J, Roerecke M. Reduction of drinking in problem drinkers and all-cause mortality. Alcohol Alcohol. 2013;48(4):509-13.

31. European Association for the Study of Liver. EASL clinical practical guidelines: management of alcoholic liver disease. J Hepatology. 2012;57(2):399-420.

32. Mueller S et al. Non-invasive diagnosis of alcoholic liver disease. World J Gastroenterol. 2014;20(40):14626-41.

33. Mueller S. Noninvasive assessment of patients with alcoholic liver disease. Clinical Liver Disease. 2013;2(2):68-71.

34. Torruellas C et al. Diagnosis of alcoholic liver disease. World J Gastroenterol. 2014;20(33):11684-99.

35. Sandrin L et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol. 2003;29(12):1705-13.

36. Nguyen-Khac E et al. Assessment of asymptomatic liver fibrosis in alcoholic patients using fibroscan: prospective comparison with seven non-invasive laboratory tests. Aliment Pharmacol Ther. 2008;28(10):1188-98.

37. Kim SG et al. [The usefulness of transient elastography to diagnose cirrhosis in patients with alcoholic liver disease]. Korean J Hepatol. 2009;15(1): 42-51.

38. Mueller S et al. Increased liver stiffness in alcoholic liver disease: differentiating fibrosis from steatohepatitis. World J Gastroenterol. 2010;16(8):966-72.

39. Abdi W et al. Sampling variability on percutaneous liver biopsy. Arch Intern Med. 1979;139(6):667-9.

40. Maharaj B et al. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the

liver. Lancet. 1986;1(8480):523-5.

41. Cadranel JF et al. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). Hepatology. 2000;32(3):477-81.

42. Regev A et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol. 2002;97(10):2614-8.

43. Bedossa P et al. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology. 2003;38(6):1449-57.

44. Boursier J et al. Reproducibility of liver stiffness measurement by ultrasonographic elastometry. Clin Gastroenterol Hepatol. 2008;6(11):1263-9.

45. Nahon P et al. Assessment of liver fibrosis using transient elastography in patients with alcoholic liver disease. J Hepatol. 2008;49(6):1062-8.

46. Nguyen-Khac E et al. Assessment of asymptomatic liver fibrosis in alcoholic patients using fibroscan: prospective comparison with seven non-invasive laboratory tests. Aliment Pharmacol Ther. 2008;28(10):1188-98.

47. Janssens F et al. Can transient elastography replace liver histology for determination of advanced fibrosis in alcoholic patients: a real-life study. J Clin Gastroenterol. 2010;44(8):575-82.

48. Sagir A et al. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. Hepatology. 2008;47(2):592-5.

49. Arena U et al. Acute viral hepatitis increases liver stiffness values measured by transient elastography. Hepatology. 2008;47(2):380-4.

50. Millonig G et al. Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis. Hepatology. 2008;48(5):1718-23.

51. Millonig G et al. Liver stiffness is directly influenced by central venous pressure. J Hepatol. 2010;52(2):206-10.

52. Mueller S, Sandrin L. Liver stiffness: a novel parameter for the diagnosis of liver disease. Hepat Med. 2010;2:49-67.

53. Trabut JB et al. Rapid decline of liver stiffness following alcohol withdrawal in heavy drinkers. Alcohol Clin Exp Res. 2012;36(8):1407-11.

54. Gelsi E et al. Effect of detoxification on liver stiffness assessed by Fibroscan® in alcoholic patients. Alcohol Clin Exp Res. 2011;35(3):566-70.

55. Mueller S et al. Non-invasive diagnosis of alcoholic liver disease. World J Gastroenterol. 2014;20:14626-41.

56. Mueller S et al. Liver stiffness in HCV and ALD: fibrosis-related cut-off values depend on degree and location of inflammation. Poster P1010. The International Liver Congress, London, UK, 9-13 April, 2014.

THE LIVER MEETING 2014:

Summary of the treatment of genotype 3 HCV infection from the 65th Annual Liver Meeting of the American Association for the Study of Liver Diseases (AASLD), held in Boston, MA, USA, on 7th-11th November 2014

Markus Peck,[1] *Stanislas Pol[2]

1. Department of Gastroenterology and Hepatology, Medizinische Universität Wien (MedUniWien), Vienna, Austria
2. Department of Hepatology, Groupe Hospitalier Cochin-Saint Vincent De Paul, Université Paris Descartes, Inserm UMS20, Institut Pasteur, Paris, France
**Correspondence to stanislas.pol@cch.aphp.fr*

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ABSTRACT

After genotype 1 (GT1), genotype 3 (GT3) is the second most common hepatitis C virus (HCV) genotype worldwide, with an estimated worldwide prevalence of 54.3 million. As its high prevalence already represents a significant burden, this genotype appears to be one of the most difficult to treat using all oral therapies, which is of importance as it is associated with more rapid fibrosis progression and disproportionately increased risks of steatosis (fatty liver disease) and hepatocellular carcinoma. Outstanding developments in clinical research in the last few years have led to the development of direct-acting antivirals, but GT3 still represents a challenge yet to be addressed, and is a clear unmet medical need, especially with associated comorbidities (advanced, liver disease, pre and post-transplant patients, HIV/HCV co-infected patients). The 65th American Association for the Study of Liver Diseases Annual Meeting, The Liver Meeting® 2014, took place in Boston, Massachusetts, USA, on 7th–11th November 2014. This review will summarise the highlights of this meeting on the future of GT3 HCV treatment within pivotal clinical trials.

Keywords: American Association for the Study of Liver Diseases, AASLD, chronic hepatitis C, genotype 3, direct-acting antivirals, meeting highlights.

INTRODUCTION

After genotype 1 (GT1), genotype 3 (GT3) is the second most common hepatitis C virus (HCV) genotype worldwide, with an estimated worldwide prevalence of 54.3 million.[1] As its high prevalence already represents a significant burden, this genotype appears to be one of the most difficult to treat using all oral therapies, which is of importance as it is associated with more rapid fibrosis progression and disproportionately increased risks of steatosis (fatty liver disease) and hepatocellular carcinoma.[2-5] Outstanding developments in clinical research in the last few years have led to the development of direct-acting antivirals (DAAs), which generated a change of paradigm from

interferon-based therapies, as the former require shorter treatment durations and provide high cure rates within acceptable toxicity. Nevertheless, GT3 still represents a challenge yet to be addressed, and is a clear unmet medical need, especially with associated comorbidities (advanced, liver disease, pre and post-transplant patients, HIV/HCV co-infected patients).[2] After decades of treatment with pegylated-interferon (pegIFN) + ribavirin (RBV), the standard of care in GT3 patients is shifting to DAA-based combination therapies, such as sofosbuvir (SOF), a pan-genotypic nucleotide NS5B inhibitor, or daclatasvir (DCV) and ledipasvir (LDV), both potent NS5A inhibitors.[2]

However, due to the diminished antiviral potency in cirrhotics and to the high costs of such therapies associated with longer treatment duration than that for GT1, such clinical situations require additional options to effectively manage the disease and contribute to the disease control. Eradication may then be possible over the course of the coming decades, as anticipated by the medical community.[3] In December 2014, the latest practice guidelines from the American Association for the Study of Liver Diseases (AASLD) proposed an alternative of SOF + RBV combination therapy for 24 weeks (Class I recommendation) or SOF + RBV + pegIFN for 12 weeks (Class IIa recommendation).[4] The latest update of the European Association for the Study of the Liver guidelines[5] published in April 2014 following the International Liver Congress also recommended this triple combination for GT3 patients (level A2 recommendation), over two other options, namely SOF + RBV for 24 weeks (level A2, suboptimal in treatment-experienced cirrhotic patients) and SOF + DCV for 12 (treatment-naïve) or 24 weeks (treatment-experienced; level B1).

Table 1: Key clinical studies presented at the 65th Liver Meeting of the American Association for the Study of Liver Diseases.

Patient characteristics	Previous therapy	Sub-population	Study name/ reference	Type of study	Investigated compounds	Treatment duration	N
Non-cirrhotic patients	TN	GT3	**ELECTRON-2**[27]	Phase II open-label study	SOF + GS-5816 ± RBV	12 weeks	104
		GT3 (n=54) (overall cohort, GT1-6)	[28]	Phase II open-label study	SOF + GS-5816 ± RBV	8 or 12 weeks	154
Cirrhotic or non-cirrhotic patients	TN	GT3 (n=61) (overall cohort, GT1 and GT3)	[10]	Prospective randomised national study	SOF + RBV	16 or 24 weeks	127
	TN (n=42)	GT3 (n=165) (overall cohort, GT1-4)	**PHOTON 1 & 2**[14]	Phase III study	SOF + RBV	12 weeks	497
	TN (n=57)					24 weeks	
	TE (n=66)					24 weeks	
	TE (n=50)	Non-cirrhotic GT3 (n=107)	[29]	Phase II study	SOF + GS-5816 ± RBV	12 weeks	221
		Cirrhotic GT3 (n=103)					
	TN (n=101)	GT3	**ALLY-3**[21]	Phase III study	SOF + DCV	12 weeks	152
	TE (n=51)				SOF + DCV	12 weeks	
	TE (n=50)	GT3	[27]	Phase II multicentre, open-label study	SOF + LDV	12 weeks	75
Cirrhotic patients	TN or TE (n=59)	Interferon-ineligible patients	[15]	Prospective single-centre study	SOF + RBV	8 weeks	59

DCV: daclatasvir; GT: genotype; LDV: ledipasvir; RBV: ribavirin; SOF: sofosbuvir; TE: treatment-experienced; TN: treatment-naïve.

DCV was recently approved in Europe and Japan in combination with other DAAs such as SOF, with or without RBV,[6] and is currently being reviewed in the USA. The 65th AASLD Annual Meeting, The Liver Meeting® 2014, took place in Boston, from 7th–11th November. This review will summarise the highlights of this meeting on the future of GT3 HCV treatment within pivotal clinical trials (Table 1).

SOF/RBV COMBINATION

SOF is a once-daily (OD) pan-genotypic nucleotide NS5B polymerase inhibitor with demonstrated potent activity against GT1-6 and an acceptable safety profile.[7-9] It was approved in Europe in January 2014 and in the USA in December 2013, in combination with RBV, with or without IFN. Despite being approved, efforts to investigate such compounds within multiple treatment regimens in high unmet-need patients presenting comorbidities or complex clinical settings are still ongoing.

General Population of GT3 Treatment-Naïve Patients

An IFN-free regimen of SOF + RBV (16 or 24 weeks) was evaluated across 16 centres encompassing 127 treatment-naïve patients, of which 61 were GT3 (18% of cirrhotics, 44% of IL28B CC carriers, and 67% with HCV ribonucleic acid (RNA) viral load ≥800,000 IU/ml).[10] After 16 weeks of therapy, sustained virologic response 12 weeks after completion of therapy (SVR12) rates were of 87% (88% in non-cirrhotics, 83% in cirrhotics; Table 2) while after 24 weeks they reached 90% (96% in non-cirrhotics, 60% in cirrhotics), demonstrating high rates for either treatment duration. All virologic failures were due to relapse and the combination was well tolerated with no patients discontinuing treatment due to adverse events (AEs) and a safety profile consistent with that of RBV. The results are consistent with those previously reported on treatment-naïve patients in the FISSION and VALENCE studies, which reported SVR12 rates of 56% at 12 weeks (61% versus 34% in non-cirrhotic/cirrhotic patients) and 94% at 24 weeks (non-cirrhotic versus 92% cirrhotic patients), respectively.[11-13]

Specific Populations

HIV co-infection

The PHOTON-1 and 2 Phase III studies[14] aimed to evaluate the safety (as well as co-administration with antiretrovirals) and efficacy of OD SOF 400 mg + RBV 1,000-1,200 mg/day for 12 or 24 weeks, in 497 GT1-4 HCV patients with concurrent HIV infection. GT3 patients were randomised into three groups: treatment-naïve patients receiving combination therapy for 12 weeks (n=42, Group 1), treatment-naïve patients receiving treatment for 24 weeks (n=57, Group 2), and treatment-experienced patients also treated for 24 weeks (n=66, Group 3). SVR12 (lower limit of quantitation [LLOQ], 25 IU/ml) were of 67% (29% of relapses), 91% (7% of relapses), and 88% (11% of relapses) in Groups 1, 2, and 3, respectively, thus demonstrating superiority of the 24-week modality. SVR12 rates were equal in the first group with respect to cirrhosis, while patients with and without cirrhosis had different results among Groups 2 (100% versus 91%) and 3 (79% versus 95%). Multivariate analyses showed that significant predictors for SVR12 were lower HCV RNA level at baseline and longer treatment duration for GT3 patients. Overall, SOF + RBV was well tolerated and there was no change in CD4 T cell percentage during treatment.

Advanced cirrhosis

Available data to date suggest that SOF regimens are slightly less effective in cirrhotic patients, but little data exist on the value of SOF + RBV in IFN-ineligible patients with advanced liver disease. Therefore, Deterding et al.[15] conducted a study on 59 patients with liver cirrhosis. All patients had transient elastography values higher than 14.5 kPa, while 15 patients had Child B or C cirrhosis. 25 patients had GT3 HCV infection. All patients had HCV RNA values below LLOQ (15 IU/ml) at Week 8 of therapy. HCV RNA was undetectable at Weeks 1, 2, and 4 in 0%, 0%, and 17%, respectively of GT3 patients; 87% of GT3 patients reached LLOQ at Week 4. Fatigue (53%), sleep disorder (25%), and muscle pain (20%) were the most reported AEs.

Real-World Data

HCV-TARGET[16] is a longitudinal observational cohort study (50 centres) evaluating current practices and use of DAAs in Europe and North America, in order to evaluate real-world data and determine its correlation with Phase III study results. Among all patients (n=2,063), 92% of GT3 patients were given SOF + RBV and 9% received triple combination therapy with SOF + pegIFN + RBV. Clinical outcomes on GT3 patients were not available because they received longer treatment and were still undergoing follow-up for sustained

response at the time of the presentation. Interval results from the study were presented with respect to patients in the post-transplant setting.[17] Among all patients (n=227), 95% of GT3 patients were given SOF + RBV and 5% received triple combination therapy with SOF + pegIFN + RBV. Crude sustained virologic response at post-treatment Week 4 (SVR4) in these treatment subpopulations were of 60% (3 patients out of 5) and 100% (1 patient out of 1), respectively.

Table 2: Key results from GT3 studies presented at the 65th Liver Meeting of the American Association for the Study of Liver Diseases.

Study	Treatment arms	TN/TE	Cirrhosis/sub-population	SVR12 (%) or primary endpoint	Most frequently reported (≥5%) AE
SOFOSBUVIR (SOF) + RIBAVIRIN (RBV)					
Russian multicentre study (n=127; only GT3 results presented here)[10]	SOF + RBV, 16 weeks (n=30)	TN	Overall	87	Headache, asthenia, viral respiratory tract infection, fatigue, alopecia, insomnia
	SOF + RBV, 24 weeks (n=31)			**90**	
	SOF + RBV, 16 weeks (n=24)		Non-cirrhotic	88	
	SOF + RBV, 24 weeks (n=26)			**96**	
	SOF + RBV, 16 weeks (n=6)		Cirrhotic	83	
	SOF + RBV, 24 weeks (n=5)			60	
PHOTON 1 & 2 Phase III studies[14]	SOF + RBV, 12 weeks (n=42)	TN		67	Fatigue, insomnia, nausea, headache, upper respiratory tract infection, asthenia, diarrhoea, irritability, cough
	SOF + RBV, 24 weeks (n=57)	TN		**91**	
	SOF + RBV, 24 weeks (n=66)	TE		88	
Prospective, single-centre study[15]	SOF + RBV, 8 weeks + 24 weeks	TN/TE	Advanced cirrhosis	At 8 weeks: 100% of HCV RNA <LLOQ (15 UI/ml)	Fatigue, sleep disorder, muscle pain
SOFOSBUVIR (SOF) + DACLATASVIR (DCV)					
ALLY-3 Phase III trial[21]	SOF + DCV, 12 weeks (n=101)	TN	Overall	**90**	Headache, fatigue, nausea
			Non-cirrhotic	**97**	
			Cirrhotic	58	
	SOF + DCV, 12 weeks (n=51)	TE	Overall	86	
			Non-cirrhotic	**94**	
			Cirrhotic	69	
	SOF + DCV, 12 weeks (whole cohort)	TN/TE	Non-cirrhotic	**96**	
			Cirrhotic	63	
SOFOSBUVIR (SOF) + LEDIPASVIR (LDV)					
Phase II multicentre, open-label study[27]	SOF + LDV, 12 weeks (n=50)	TE	Overall	82	Fatigue, headache, upper respiratory tract infection, insomnia, rash, diarrhoea, nausea
			Non-cirrhotic (56%)	89	
			Cirrhotic (44%)	73	
SOFOSBUVIR (SOF) + GS-5816					
ELECTRON-2 Phase III open-label study[27]	SOF + GS-5816 (25 mg), 12 weeks (n=27)	TN	Non-cirrhotic	**100**	Fatigue, headache, nausea, upper respiratory tract infection, insomnia, rash, diarrhoea, lethargy, vomiting, back pain, pruritus
	SOF + GS-5816 (100 mg), 12 weeks (n=27)			**96**	

Table 2 continued.

Study	Treatment arms	TN/TE	Cirrhosis/sub-population	SVR12 (%) or primary endpoint	Most frequently reported (≥5%) AE
ELECTRON-2 Phase III open-label study[27]	SOF + GS-5816 (25 mg) + RBV, 12 weeks (n=24)	TN	Non-cirrhotic	88	Fatigue, headache, nausea, upper respiratory tract infection, insomnia, rash, diarrhoea, lethargy, vomiting, back pain, pruritus
	SOF + GS-5816 (100 mg) + RBV, 12 weeks (n=26)			**100**	
Phase II open-label study[28]	SOF + GS-5816 (25 mg), 12 weeks (n=27)	TN	Non-cirrhotic	**93**	N/A
	SOF + GS-5816 (100 mg), 12 weeks (n=27)			**93**	
Phase II study[29]	SOF + GS-5816 (25 mg), 12 weeks (n=26)	TE	Non-cirrhotic	85	Headache, fatigue, nausea, insomnia, irritability, diarrhoea, pruritus, rash
	SOF + GS-5816 (25 mg), 12 weeks (n=26)		Cirrhotic	58	
	SOF + GS-5816 (100 mg), 12 weeks (n=27)		Non-cirrhotic	**100**	
	SOF + GS-5816 (100 mg), 12 weeks (n=26)		Cirrhotic	88	
	SOF + GS-5816 (25 mg) + RBV, 12 weeks (n=28)		Non-cirrhotic	**96**	
	SOF + GS-5816 (25 mg) + RBV, 12 weeks (n=25)		Cirrhotic	88	
	SOF + GS-5816 (100 mg) + RBV, 12 weeks (n=26)		Non-cirrhotic	**100**	
	SOF + GS-5816 (100 mg) + RBV, 12 weeks (n=26)		Cirrhotic	**96**	

AE: adverse event; GT: genotype; LLOQ: lower limit of quantification; HCV: hepatitis C virus; N/A: not applicable or data not available; SVR12: sustained virologic response 12 weeks after completion of therapy; TE: treatment-experienced; TN: treatment-naïve.

SOF/DCV COMBINATION

DCV is the first-in-class NS5A replication complex inhibitor and is a potent and pan-genotypic DAA.[18,19] Its pharmacokinetic profile is supportive of OD dosing within a well-tolerated safety profile.[20] As DCV has demonstrated a low potential for drug-drug interactions, it is already approved in combination with other DAAs such as SOF in Europe (HCV GT1-3) and asunaprevir in Japan (HCV GT1).

GT3 Treatment-Naïve/Experienced Patients: ALLY-3 Phase III Study

The landmark ALLY Phase III programme comprises three studies evaluating the efficacy and safety of an all-oral combination of DCV + SOF in patients with high unmet medical needs: ALLY-1, conducted in GT1-6 patients with cirrhosis or post-liver transplant (n=113) receiving the combination with RBV for 12 weeks; ALLY-2, conducted in GT1-6 patients with HIV co-infection and receiving the combination for 8 or 12 weeks (n=203); and ALLY-3, in which GT3 treatment-naïve or experienced patients received the combination for 12 weeks (n=152). While ALLY-1 and ALLY-2 are still ongoing, the results for the ALLY-3 study were first presented in a late-breaking abstract of an oral presentation by Nelson et al.[21] at The Liver Meeting. In the ALLY-3 clinical trial, the first 12-week Phase III study on such combination, GT3 patients, either treatment-naïve (n=101) or treatment-experienced (n=51), were enrolled to receive open-label DCV 60 mg + SOF 400 mg OD for 12 weeks. The treatment phase led to 24 weeks of follow-up. The cohort of treatment-experienced patients

comprised prior treatment-failures, including SOF or alisporivir (prior treatment with NS5A inhibitors was an exclusion criterion). The primary endpoint, evaluated at Week 24, was the rate of sustained virologic response at post-treatment Week 12 (SVR12; HCV RNA less than the lower limit of quantitation [LLOQ, 25 IU/ml] detected or not detected). Other efficacy (SVR4) and safety parameters were reported.

Overall, baseline characteristics were comparable between both cohorts: 21% of patients were cirrhotic (a slightly greater proportion of cirrhotic patients was observed in the treatment-experienced cohort), 40% presented the CC IL28B genotype while 71% had a HCV RNA ≥800,000 IU/ml. 90% and 86% of treatment-naïve patients and treatment-experienced patients achieved SVR12, respectively. Cirrhosis status (determined by liver biopsy, FibroScan or FibroTest) was associated with lower (62.5%) SVR12 rates than in non-cirrhotic patients (96%). This influence of cirrhosis was even more marked in the treatment-naïve cohort (97% versus 58%, respectively) than in the treatment-experienced cohort (94% versus 69%, respectively). Higher SVR12 rates were positively associated with female gender, younger patient age (<65 years), HCV RNA levels <800,000 IU/ml, CC-IL28B genotype, and absence of cirrhosis. 1 patient (1%) experienced on-treatment failure (detectable HCV RNA at the end of treatment) while 16 (11%) patients, mainly cirrhotic, relapsed after study completion. The NS5A-Y93H resistance-associated variant (RAV) emerged in 9 of 16 relapsing patients. No virologic breakthrough was reported.

Overall, the combination was safe and well tolerated. Most frequently reported (≥10%) AEs included headache (20%), fatigue (19%), and nausea (12%). No deaths, treatment-related serious AEs (one patient experienced a serious AE which was not related to the study regimen), or discontinuations due to AEs were reported during the course of the study. DCV + SOF in combination for a shorter 12-week duration achieved high SVR12 rates in GT3 patients (treatment-naïve, 90%; treatment-experienced, 86%). DCV + SOF combination demonstrated high SVR rates in both treatment-naïve and treatment-experienced non-cirrhotic GT3 patients after only 12 weeks. Further options for optimising treatment outcome with DCV + SOF in GT3-infected patients with cirrhosis are currently being evaluated, including addition of RBV to the DCV + SOF combination for 12 or 16 weeks.[22] These results follow those of an open-label randomised study on GT1-3 patients receiving DCV + SOF with or without RBV, and in which 89% of GT3 non-cirrhotic patients achieved an SVR12 but after a treatment duration of 24 weeks,[23] and with no impact of addition of RBV.

SOF/LDV COMBINATION

LDV is a NS5A inhibitor with picomolar potency against HCV GT1a/b that was recently approved in Europe and in the USA in a fixed-dose combination (FDC) with SOF. In a Phase II multicentre, open-label study,[24] 50 treatment-experienced GT3 patients were part of one of two cohorts evaluated to assess the efficacy and safety of a 12-week regimen of LDV + SOF (the other cohort was composed of 25 treatment-naïve/experienced GT6 patients). 44% of GT3 (3a, 98%; 3b, 2%) patients were cirrhotic while 36% of patients carried the IL28B CC genotype. SVR12 rates were of 82%, 89%, and 73% in the whole GT3 cohort, non-cirrhotic GT3 and cirrhotic GT3 patients, respectively. One non-cirrhotic GT3 patient experienced breakthrough at Week 12; all other patients with virologic failure relapsed.

SOF/GS-5816 COMBINATION

GS-5816 is an investigative NS5A inhibitor with pan-genotypic activity, as demonstrated by a previous monotherapy study, and pharmacokinetic properties supporting OD dosing.[25]

Studies on Treatment-Naïve, Non-Cirrhotic Patients

GT3-naïve non-cirrhotic patients

In a previous Phase II trial,[26] the SOF + GS-5816 combination was evaluated across all genotypes in 154 treatment-naïve non-cirrhotic patients, who received a 12-week course of 400 mg SOF with either 25 or 100 mg GS-5816. In GT3 patients, the GS-5816 25 mg and GS-5816 100 mg cohorts achieved SVR12 rates of 93%. At the Liver Meeting, Gane et al.[27] presented the Phase II ELECTRON-2 study which aimed to evaluate the efficacy and safety of the OD FDC of 400 mg SOF + 25/100 mg GS-5816 (+/- RBV) in treatment-naïve non-cirrhotic GT3 patients (n=104). Almost all (96.2%) patients were GT3a.

In this open-label study, patients from the four cohorts received the treatment for 8 weeks. The main clinical endpoint, SVR12 (LLOQ, 15 IU/ml),

was evaluated at Week 20 and reached 100% in the SOF + 25 mg GS-5816 OD without RBV arm and the SOF + 100 mg GS-5816 OD + RBV arm. SVR12 rates in the SOF + 100 mg GS-5816 OD without RBV arm and the SOF + 25 mg GS-5816 OD with RBV arm were of 96% and 88%, respectively. Most patients achieved LLOQ by Week 2, while 10 out of 11 patients with the NS5A-Y93H RAV achieved SVR12. No advantage in efficacy was identified with triple-combination with RBV over FDC with SOF and GS-5816 only. In the SOF + GS-5816 25 mg OD arms, two virologic relapses (at treatment Week 4) and one discontinuation due to AE were reported, while no virologic failures were observed in SOF + GS-5816 100 mg OD arms. One patient from the SOF + 100 mg GS-5816 OD without RBV arm withdrew consent.

Overall, the FDC was well tolerated; most frequently reported (≥10%) AEs included fatigue, headache, and nausea. No deaths were reported during the course of the study. In another Phase II open-label study,[28] efficacy and safety parameters of the OD FDC of 400 mg SOF + 25/100 mg GS-5816±RBV were evaluated. 154 treatment-naïve, non-cirrhotic patients from three cohorts (GT1 [n=55], GT3 [n=54], and GT2/4/5/6 [n=45]) were enrolled, for a study duration of 8 or 12 weeks. In Part A, patients were randomised 1:1 to SOF + GS-5816 25 mg or SOF + GS-5816 100 mg for 12 weeks. In Part B, GT1 and GT2 were randomised 1:1:1:1 to SOF + GS-5816 25 mg, SOF + GS-5816 25 mg + RBV, SOF + GS-5816 100 mg, or SOF + GS-5816 100 mg + RBV for 8 weeks. In GT3 patients, SVR12 (LLOQ 15 IU/ml) rates were of 93% for both SOF + GS-5816 25 mg and SOF + GS-5816 100 mg arms and not clearly different than those achieved in the European VALENCE study in non-cirrhotic patients who were given 24 weeks of SOF + RBV (94%).[13] As in the previous study, the safety profile of this combination for 8 or 12 weeks was acceptable, with low incidences of treatment discontinuation and serious AEs.

GT3 Treatment-Experienced Patients

In a Phase II study,[29] OD FDC of 400 mg SOF + 25/100 mg GS-5816 (+/- RBV) was further evaluated across three cohorts consisting of treatment-experienced GT3 patients, without (cohort 1, n=107) or with cirrhosis (cohort 2, n=103), and GT1 patients with prior failures to protease inhibitor regimens, with or without cirrhosis (cohort 3, n=111). In all three cohorts, patients were randomised 1:1:1:1 to SOF + GS-5816 25 mg, SOF + GS-5816 25 mg + RBV, SOF + GS-5816 100 mg, or SOF + GS-5816 100 mg + RBV for 12 weeks. All non-cirrhotic patients (100%) within the SOF + GS-5816 100 mg and SOF + GS-5816 100 mg + RBV arms (cohort 1) reached SVR12, while 85% (four relapses [all virologic failures]) and 96% (one relapse) of patients who received SOF + GS-5816 25 mg without or with RBV, respectively. In the second cohort of cirrhotic patients, 88% (3 relapses) and 96% (1 relapse) of patients from the SOF + GS-5816 100 mg and SOF + GS-5816 100 mg + RBV arms reached SVR12, respectively, while 58% (11 relapses) and 84% (3 relapses, 1 withdrawal of consent) of patients who received SOF + GS-5816 25 mg without or with RBV, respectively.

Overall, the FDC was well tolerated with low incidences of serious AEs or discontinuation. The most frequently reported AEs (≥10%) in patients were headache, fatigue, nausea, and insomnia. RBV co-administration was positively correlated to increased incidence of pruritus and rash. The conclusion of the authors, and the company, was that RBV co-administration does not yield additional benefits and 100 mg regimens of GS-5816 are superior to 25 mg regimens. Consequently, SOF 400 mg and GS-5816 100 mg have been co-formulated in a FDC for Phase III evaluation.

CONCLUSION

In conclusion, different therapeutic options may be proposed to GT3-infected patients including 24 weeks of SOF + RBV, SOF + DCV, or of SOF + LDV + RBV, or 12 weeks of SOF + pegIFN + RBV in treatment-naïve as well as in treatment-experienced patients. If SVR12 is frequently achieved in >90% of non-cirrhotic patients, the rates remain lower (65-80%) in cirrhotic patients who probably need longer duration of therapies or reinforced therapies (SOF + NS5A inhibitors [DCV, LDV, or GS-5816] + RBV) for 12 or 24 weeks. This lower rate reflects a potential lower slope of the viral decline associated with both GT3 and cirrhosis with PEG-including or PEG-free regimens.

REFERENCES

1. Messina JP et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2014;doi:10.1002/hep.27259. [Epub ahead of print].

2. Pol S et al. Treatment of hepatitis C virus genotype 3-infection. Liver Int. 2014;34 Suppl 1:18-23.

3. Gane E. Hepatitis C beware--the end is nigh. Lancet. 2014;384:1557-60.

4. AASLD/IDSA/IAS–USA. Recommendations for testing, managing, and treating hepatitis C. Available at: http://www.hcvguidelines.org. Accessed: November 2014.

5. Pawlotsky JM et al. European Association for the Study of the Liver (EASL) recommendations on treatment of hepatitis C. EASL. 2014.

6. Bristol-Myers Squibb Pharmaceutical Limited. Daklinza. Summary of Product Characteristics. 2014. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/003768/smops/Positive/human_smop_000703.jsp&mid=WC0b01ac058001d127%5D. Accessed: November 2014.

7. Poordad F et al. Exploratory study of oral combination antiviral therapy for hepatitis C. N Engl J Med. 2013;368(1): 45-53.

8. Gane EJ et al. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. N Engl J Med. 2013;368(1): 34-44.

9. Rodriguez-Torres M et al. Sofosbuvir (GS-7977) plus peginterferon/ribavirin in treatment-naïve patients with HCV genotype 1: a randomized, 28-day, dose-ranging trial. J Hepatol. 2013;58(4):663-8.

10. Chulanov V et al. Sofosbuvir plus ribavirin for the treatment of Russian patients with chronic HCV genotype 1 or 3 infection. Abstract 982. 65th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, USA, 7-11 November, 2014.

11. Zeuzem S et al. Sofosbuvir + ribavirin for 12 or 24 weeks for patients with HCV genotype 2 or 3: the VALENCE trial. Hepatology. 2013;58 (Suppl. 1):733A.

12. Lawitz E et al. Sofosbuvir for previously untreated chronic hepatitis C infection. N Engl J Med. 2013;368(20):1878-87.

13. Zeuzem S et al; VALENCE Investigators. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. N Engl J Med. 2014;370(21): 1993-2001.

14. Rockstroh JK et al. Sofosbuvir and ribavirin therapy for the treatment of HIV/HCV coinfected patients with HCV GT1-4 infection: the PHOTON-1 and -2 trials. Abstract 195. 65th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, USA, 7-11 November, 2014.

15. Deterding K et al. Delayed early HCV RNA response during IFN-free therapy with sofosbuvir in interferon-ineligible patients with advanced cirrhosis. Abstract 969. 65th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, USA, 7-11 November, 2014.

16. Jensen DM et al. Safety and efficacy of sofosbuvir-containing regimens for hepatitis C: real-world experience in a diverse, longitudinal observational cohort. Abstract 45. 65th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, USA, 7-11 November, 2014.

17. Brown Jr RS et al. Safety and efficacy of new DAA-based therapy for hepatitis C post-transplant: interval results from the HCV-TARGET longitudinal, observational study. Late-Breaking abstract LB-4. 65th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, USA, 7-11 November, 2014.

18. Gao M et al. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. Nature. 2010;465(7294):96-100.

19. Pol S et al. Daclatasvir for previously untreated chronic hepatitis C genotype-1 infection: a randomised, parallel-group, double-blind, placebo-controlled, dose-finding, phase 2a trial. Lancet Infect Dis. 2012;12(9):671-7.

20. Nettles RE et al. Multiple ascending dose study of BMS-790052, a nonstructural protein 5A replication complex inhibitor, in patients infected with hepatitis C virus genotype 1. Hepatology. 2011;54(6):1956-65.

21. Nelson DR et al. All-oral 12-week combination treatment with daclatasvir and sofosbuvir in patients infected with HCV genotype 3: ALLY-3 Phase 3 study. Late-Breaking abstract LB-8. 65th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, USA, 7-11 November, 2014. In press.

22. Bristol-Myers Squibb. Safety and efficacy study of daclatasvir 60mg, sofosbuvir 400mg, and ribavirin (dosed based upon weight) in subjects with chronic genotype 3 hepatitis C infection with or without prior treatment experience and compensated advanced cirrhosis for 12 or 16 weeks. NCT02319031. https://www.clinicaltrials.gov/ct2/show/NCT02319031.

23. Sulkowski MS et al. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med. 2014;370(3):211-21.

24. Gane EJ et al. High efficacy of LDV/SOF regimens for 12 weeks for patients with HCV genotype 3 or 6 infection. Late-Breaking abstract LB-11. 65th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, USA, 7-11 November, 2014.

25. Lawitz E et al. GS-5816, a once-daily NS5A inhibitor, demonstrates potent antiviral activity in patients with genotype 1-4 HCV infection in a 3 day monotherapy study. Abstract 1082. 64th Annual Meeting of the American Association for the Study of Liver Diseases, Washington, DC, USA, 1-5 November, 2013.

26. Everson GT et al. Safety and efficacy of treatment with the interferon-free, ribavirin-free combination of sofosbuvir + GS-5816 for 12 weeks in treatment naïve patients with genotype 1–6 HCV infection. Abstract O111. The International Liver Congress 2014, London, United Kingdom, 9-13 April.

27. Gane EJ et al. Once-daily sofosbuvir with GS-5816 for 8 weeks with or without ribavirin in patients with HCV genotype 3 without cirrhosis result in high rates of SVR12: the ELECTRON-2 study. Abstract 79. 65th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, USA, 7-11 November, 2014.

28. Tran TT et al. Safety and efficacy of treatment with sofosbuvir + GS-5816 ± ribavirin for 8 or 12 weeks in treatment-naïve patients with genotype 1-6 HCV infection. Abstract 80. 65th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, USA, 7-11 November, 2014.

29. Pianko S et al. High efficacy of treatment with sofosbuvir + GS-5816 ± ribavirin for 12 weeks in treatment-experienced patients with genotype 1 or 3 HCV infection. Abstract 197. 65th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, Massachusetts, USA, 7-11 November, 2014.

DEFINITIONS OF ACUTE-ON-CHRONIC LIVER FAILURE: THE PAST, THE PRESENT, AND THE FUTURE

***Roland Amathieu,[1] Ali Al-Khafaji[2]**

1. Department of Critical Care Medicine and Anesthesiology, AP-HP, Henri Mondor Hospital, School of Medicine, Université Paris Est Créteil, Créteil, France
2. Department of Critical Care Medicine, University of Pittsburgh School of Medicine, and University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA
**Correspondence to roland.amathieu@hmn.aphp.fr*

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ABSTRACT

Acute-on-chronic liver failure (ACLF) is an entity used to define patients with liver cirrhosis presenting with acute decompensation. For over 20 years, ACLF has taken multiple definitions and/or classifications. Unfortunately, to date, there has not been a universally accepted definition/classification of this entity. In this short review, we discuss the definition evolution of ACLF, the strengths and weaknesses of the existing definitions and classifications, and finally the potential role of the 'omic' approaches for the diagnosis of this complex syndrome.

Keywords: Acute-on-chronic liver disease, definition, classification, cirrhosis, chronic liver disease, metabolomics.

INTRODUCTION

Chronic liver diseases (CLD) are defined by the following triad: 1) prolonged course of a hepatic disease >6 months; 2) inflammatory and/or degenerative morphological findings; and 3) uncertain prognosis.[1] CLD consist of several aetiologies and different states of functional and/or morphological liver deterioration. Nevertheless, regardless of the aetiology, CLD could lead to both histological modifications of the liver and chronic liver insufficiency. CLD caused by steatohepatitis (alcohol or obesity) or chronic viral hepatitis leads to morphological changes in the liver. These changes could be attributed to four processes: 1) cell damage and degeneration; 2) cell death and necrosis; 3) liver regeneration; and 4) fibrogenesis. Cirrhosis is the consequence and final stage of various CLD.[2] Associated to this phenomenon, in cirrhotic patients, increased intrahepatic vascular resistances leads to portal hypertension and its complications, namely gastrointestinal (GI) bleeding from varices and/or ascites. Moreover, major functions of the liver are also impaired such as immunological function with increased infection sensibility and several perturbations in anabolism and catabolism liver function. Unfortunately, there are no correlations between morphological changes and the severity of functional impairment. Nevertheless, put together, all these perturbations, often asymptomatic when cirrhosis is 'compensated', become symptomatic when the cirrhosis is 'decompensated'.

Natural history of the disease could be progressive, with a slow decrease of liver function but without the potential for full recovery leading to end-stage of cirrhosis. End-stage of cirrhosis is characterised by chronic decompensation of the liver. At which point, the only definitive treatment is liver transplantation (LTx). Patients with CLD may have acute decompensation (AD) that is usually precipitated by an event that represents a direct or indirect hepatic insult. For example, indirect insult could be infection or extra-hepatic surgery. Direct insult could be new viral hepatitis infection (like virus Delta or E), viral hepatitis reactivation, or hepatotoxic drug misuse. In case of AD, partial or full recovery to the original liver function level is assumed after treatment. In those patients, short-term mortality increases dramatically when extra-

hepatic organ failures are present. Three clinical scenarios are possible regarding the natural history of the CLD: CLD without cirrhosis and AD, CLD with cirrhosis and AD, and CLD with cirrhosis and end-stage liver disease. These three categories of patients are different in terms of mechanism and prognosis (Figure 1).

Acute-on-chronic liver failure (ACLF) is a complex syndrome with an acute deterioration of liver function superimposed on CLD. Both the exact definition and underlying pathogenesis of ACLF remain unclear. Instead of using the term 'acute decompensation', ACLF is used to define and classify all acute events of liver decompensation in patients with CLD or cirrhosis regardless of the presence of other organ failure. In >20 years, this syndrome has taken several different definitions, leading different outcomes according to mortality. From all the available definitions, three common points are emphasised: 1) Presence of CLD; 2) Rapid deterioration but theoretically reversible liver function; and 3) high short-term mortality. ACLF is associated with a short and medium-term mortality of 50-90%.[3,4] A new definition and classification will allow to better stratify patients with ACLF. Nevertheless, proposed definitions by Asian and Americano-European Study of the Liver societies are not clear with the definition of the CLD. On the other hand, new classifications proposed by the European and North American studies focus only on cirrhotic patients and define the patient principally with extra-hepatic failure which could be confusing too. None of those definitions or classifications takes into account the probability of liver function recovery. Unfortunately, despite recent efforts to well define this syndrome, there is no universally accepted definition.

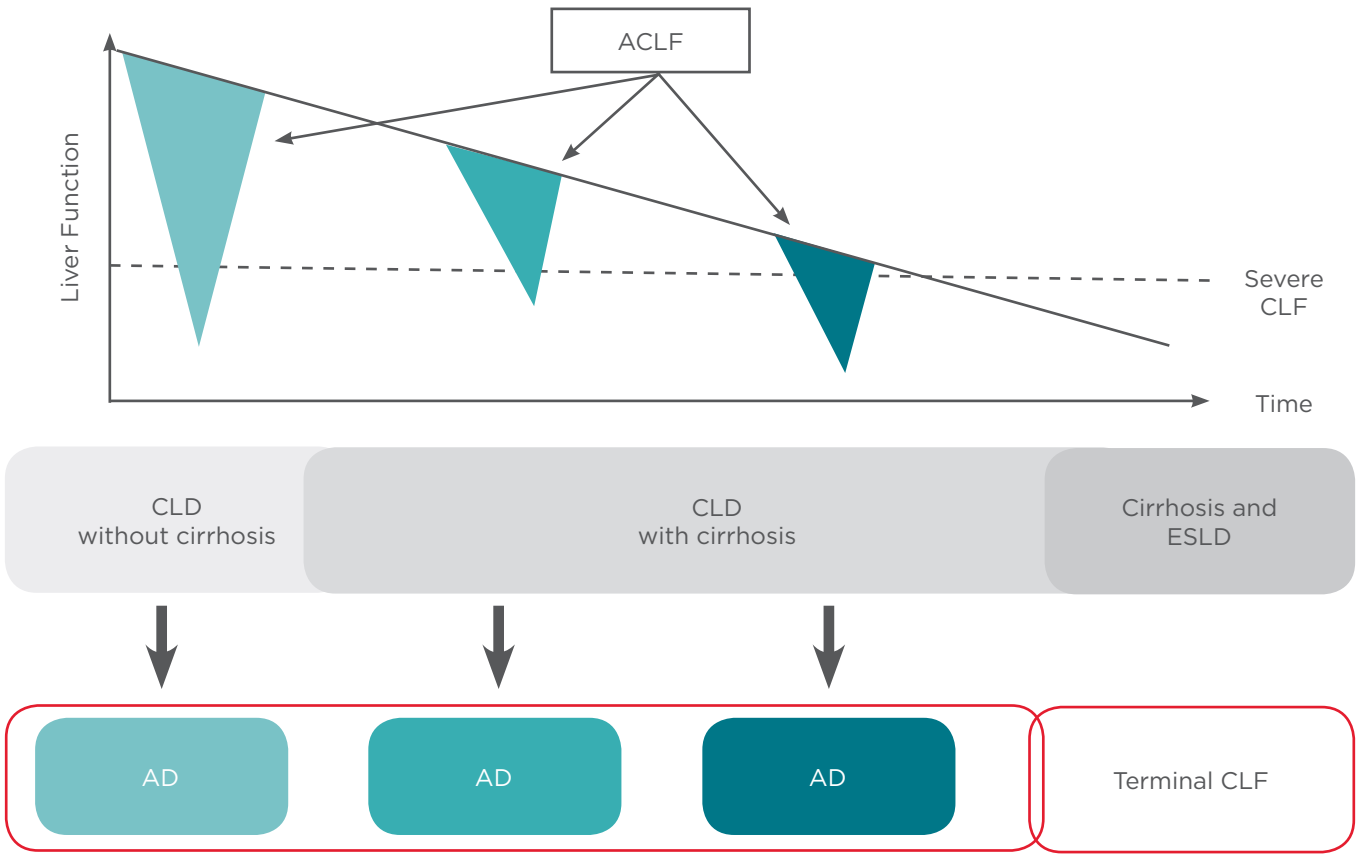

Figure 1: Schematic representation of natural history of chronic liver disease (CLD), acute decompensation (AD), and end-stage liver disease (ESLD).

This figure describes the concept of acute-on-chronic liver failure (ACLF) in CLD patients with or without cirrhosis, chronic liver failure (CLF), and ESLD. It also describes arbitrary evolution of CLD with cirrhosis, at the top of the figure, progressive decreases of the liver function leading to terminal liver failure and, on the bottom of the figure, three categories of patients: CLD without cirrhosis, CLD with cirrhosis, and cirrhosis and ESLD. ACLF (at the top) is characterised by acute liver impairment but with partial or total recovery of the liver function after treatment.

Table 1: Different definitions of acute-on-chronic liver failure (ACLF) found in the literature.

	Definition	Aetiology of CLD	Ref
1	Acute insult manifesting as jaundice (bilirubin ≥10 mg/dl) and coagulopathy (PTA <40%), complicated within 4 weeks with ascites and/or HE with previously diagnosed or undiagnosed chronic hepatitis B (with or without cirrhosis).	Hepatitis B virus	18
2	Acute deterioration of liver function in established and compensated CLD following a life-threatening complication (HE or ascites or bleeding or HRS) in patient with or without cirrhosis.	Hepatitis B virus	19
3	Defined as a rise in MELD score of >5 points within 4 weeks before transplantation.	Various	20
4	Acute decompensation of cirrhosis manifested by increased jaundice.	Various	21
5	ACLF was diagnosed in cirrhotic patients with acute hepatitis A or E presenting with clinical evidence of liver failure (significant ascites and/or HE).	Various	22
6	Defined as acute decompensation of CLD with severe liver dysfunction and high grade of HE (2 or more).	Hepatitis B virus	23
7	Cirrhotic patient with decompensation such as GIB, HE, admitted to ICU required organ support	Various	24

PTA: prothrombin activity; HE: hepatic encephalopathy; CLD: chronic liver disease; HRS: hepatorenal syndrome; MELD: model for end-stage liver disease; GIB: gastrointestinal bleeding; ICU: intensive care unit.

New approaches, more global and biological, of this polymorphic syndrome are needed. 'Omic' approaches, such as metabolomic, are probably interesting biological approaches to help clinicians to best define and classify the patients with this syndrome and predict liver function recovery. In this review, we discuss the evolution and accuracy of the different definitions of the ACLF and propose the need for 'biological' approaches of this syndrome.

ACLF Definitions: the Past

The term 'acute-on-chronic liver failure' appears for the first time in 1995.[5] It gains interest at the end of the last century probably as a consequence of the development of the different kinds of liver support. Initially, it describes a condition with superimposed insult on the liver in patients with CLD. On the other hand, it describes the notion that an organ (the liver in this case) with chronic impairment could have superimposed acute impairment but with possible return to the previous state. Then, patients with chronic liver failure (CLF) and acute liver failure (ALF) should be treated by liver support as a bridge to the recovery of their function or to the LTx. Unfortunately, despite the first meta-analysis, which showed decreased mortality in the ALCF group, no controlled trials have been able to support this hypothesis.[6,7] Subsequently, several definitions were proposed to define this syndrome. At the beginning, all of them focused on the loss of liver function with various clinical and biological signs (Table 1). Few definitions take into account organs other than the liver in the definition. High short-term mortality of this syndrome (between 50-90%) was common in all of them. The presence of a large panel of definitions is a problem for the interpretation of the studies regarding outcomes or therapeutic trials on patients with ACLF. Taking into account this point and the increase of interest for these patients, notably regarding the LTx, more consensual definitions were raised at the beginning of the new century. Typically two definitions, especially due to the difference of CLD aetiology, from the 'Western countries' and 'Eastern countries' (i.e. mainly Asian) were proposed.

ACLF Definitions: the Present

Two definitions of the ACLF are mostly used. One is proposed by the Asian-Pacific Association of the Study of the Liver and the others by the American Association for the Study of the Liver (AASLD) and the European Association for the Study of the Liver (EASL).[8,9] The Asian definition focuses exclusively on liver failure. ACLF is defined asacute hepatic

insult manifesting as jaundice (with bilirubin ≥5 mg/dl), coagulopathy (with international normalised ratio ≥1.5 or prothrombin activity <40%), and complicated within 4 weeks by ascites and/or hepatic encephalopathy with previously diagnosed or undiagnosed CLD. Current definition of ACLF proposed by EASL-AASLD symposium includes the notion of high mortality and extra-hepatic organ failure. ACLF is then defined as an "acute deterioration of pre-existing CLD, usually related to a precipitating event and associated with increased mortality at 3 months due to multi-organ failure." The precipitating event may be an extra-hepatic insult such as sepsis, or GI bleeding. It may also be a direct hepatic mechanism with viral infestation or reactivation, or drug induced liver injury.[3] Two points should be clarified; first, all patients with CLD are included in those definitions and not only patients with cirrhosis. CLD without cirrhosis does not have the same clinical presentation, treatment, or prognosis when compared to CLD with cirrhosis. Consensual definition of CLD is lacking. Future works are needed to establish new criteria (clinical, radiological, biological, and/or histological) to best define it. Moreover, those criteria of CLD will probably be also helpful to best recognise unknown underlying CLD and distinguish patients with ALF from a patient with ALF or CLF. Second, the notion of recovery is lacking in both of them. How so you differentiate between impairment of liver function that leads to end-stage disease or from the ones which will recover?

To address the first issue, Jalan et al.[10] attempted to classify patients with CLD. They proposed a new classification of ACLF in three categories (A, B, or C) according to underlying presence of cirrhosis and for the cirrhotic patient, history of pervious decompensation. Group A includes CLD patients without cirrhosis. Group B includes well-compensated cirrhosis, and group C includes patients with advanced cirrhosis with previous decompensation. Prospective evaluation of this new classification is necessary to determine its accuracy. Recently, two large studies have tried to better classify ACLF patients: one from EASL–Chronic Liver Failure Consortium (EASL-CLIF) Consortium in Europe, called the CLIF Acute On Chronic Liver Failure in Cirrhosis (CANONIC) study, and the other from the North-American Consortium for the Study of End-Stage Liver Disease study.[11,12] The first study included around 1,400 patients hospitalised with cirrhosis for an AD. This study classified ACLF based on mortality (Table 2).

Table 2: Definition of chronic liver failure consortium - acute-on-chronic liver failure (CLIF-ACLF) grades.

Grade	No ACLF	ACLF Grade 1	ACLF Grade 2	ACLF Grade 3
Definition	No organ failure or single organ failure (coagulation, circulation, or respiration) and creatininaemia <1.5 mg/dl and no hepatic encephalopathy.	Single kidney failure or single organ failure (coagulation, circulation, or respiration) and creatininaemia between 1.5 and 1.9 mg/dl or hepatic encephalopathy and creatininaemia between 1.5 and 1.9 mg/dl	Two organ failures	Three organ failures
1 and 3 months mortality	4.7% and 14%	22.1% and 40.7%	32% and 52.3%	76.7 and 79.1%

Coagulation failure is defined by the international normalised ratio >2.5 or platelet count <20 g/l; circulation failure is defined by use of any dose of terlipressin, dopamine, dobutamine, epinephrine, or norepinephrine. Lung failure is defined by PaO_2/FiO_2 ratio <200 or SpO_2/FiO_2 ratio <89. Kidney failure is defined by creatininaemia >2 mg/dl or need to renal replacement therapy. Hepatic encephalopathy Grade >2 defines neurological failure.

In the CLIF classification (CLIF-ACLF Grades), cirrhotic patients were classified according to organ failure, mainly kidney and brain (i.e. hepatic encephalopathy) failure. The North American Study proposes classification of ACLF specific to cirrhotic patients with sepsis (infection-related acute-on-chronic liver failure [I-ACLF]). The goal of this classification is to help the clinician with bedside decision-making to accurately identify potential survivors for cost-effective healthcare resource utilisation. I-ACLF is defined as a cirrhotic patient with suspected or documented infection and at least one organ failure (hepatic encephalopathy Grade 2/3, renal replacement therapy, mechanical ventilation, shock). Approximately 500 patients were included in this multicentre prospective study. As expected, for both studies, mortality was well correlated with the number of organ failures. Major points of the new classifications are: 1) they included only patients with proven or strongly suspected liver cirrhosis; 2) they included all aetiologies of cirrhosis; 3) they included well-documented cirrhotic patients hospitalised for an acute event; 4) for one of them (European study), there is external validation of the accuracy of the classification.[13] The interesting point with these classifications is that they best stratify cirrhotic patients with ACLF according to the risk of death. The major implication is for its use in the inclusion criteria to have a more homogenous population for future randomised clinical trials. However, consensual definition of ACLF is still lacking. The ambiguity and variability in the definition/classification of ACLF does not allow the clinician to make rapid and proper diagnoses of ACLF, to distinguish between patients with ACLF that require transplantation and those that require only intensive medical treatment. Specific biomarkers that confirm the diagnosis, exclude other diseases, and best predict patients with poor outcome should be stated to best define ACLF.

ACLF Definition: the Future

Bioclinical classification as proposed by Moreau et al.[12] is probably a major improvement concerning the characterisation of the ACLF according to its prognosis. Nevertheless, the score used is complex and not readily adaptable to clinical care. The view of the ACLF syndrome as a systemic syndrome with extra-hepatic organ failures responsible of increased mortality is interesting, but it is also counterintuitive to define an 'acute hepatic failure' as 'extra-hepatic failure'. To better define ACLF syndrome, new biomarkers or biological fingerprints could probably be helpful. Nevertheless, it is now widely accepted that the search for a single biomarker that can be used in routine clinical practice to diagnosis patients with ACLF is probably unrealistic. Future definition and characterisation of this systemic syndrome could probably be completed and clarified using the 'omic' concept, and specifically, the metabolomic approach. Metabolomics, which is the study of metabolic changes in an integrated biological system using multiparametric analyses, may help identify biomarkers that characterise the metabolic profiles of a disease, and/or evaluate metabolic modifications after treatment has been initiated.[14] Metabolomics, using proton nuclear magnetic resonance (1H-NMR) spectroscopy, when applied to liver disease, has shown a close relationship between metabolic abnormalities and the severity of the disease in sera and tissues.[15,16] Recently, a serum metabolite fingerprint for ACLF, obtained with 1H-NMR, was identified.[17]

The hypothesis in this study was that cirrhotic patients with acute events have had a specific metabolic response as compared to cirrhotic patients with stable cirrhosis. Metabolomic profiles of the sera of 93 patients with compensated or decompensated cirrhosis (CLF group) but stable liver function, and 30 patients with cirrhosis and hospitalised for the management of an acute event who may be responsible of ACLF (i.e. ACLF group) were analysed. Both groups were well-separated using a multivariable statistic method and the specific metabolomics fingerprint of patients in intensive care unit was identified. Several metabolites were identified and reflected major changes in liver function, such as energy metabolism, urea metabolism, or amino acid metabolism, but also major extra-liver function changes, such as renal impairment, or were related to inflammation/necrosis. This primary results are interesting but should be confirmed by a large multicentric population including various aetiologies.

CONCLUSION

Despite major efforts, recent definitions and classifications proposed by leading organisations or studies are still confusing for the clinician notably to make difference between ACLF and CLD or ACLF in cirrhotic patient and cirrhosis decompensation. Future research should produce an accurate 'universal' definition of this complex syndrome, in-patients with CLD, and including

cirrhotic patients. In the same way, a study of the variations of different biomarkers or biological fingerprints could be interesting in order to best classify and define the prognostics of those patients. An interesting way to find it could be a biological approach using the 'omic' platforms.

REFERENCES

1. Kuntz E, Kuntz HD (eds.), Hepatology: Principles and Practice: History, Morphology, Biochemistry, Diagnostics, Clinic, Therapy (2006) 2nd edition, Springer: Heidelberg.

2. Friedman SL. Liver fibrosis -- from bench to bedside. J Hepatol. 2003;38 Suppl 1:S38-53.

3. Jalan R et al. Acute-on chronic liver failure. J Hepatol. 2012;57(6):1336-48.

4. Katoonizadeh A et al. Early features of acute-on-chronic alcoholic liver failure: a prospective cohort study. Gut. 2010;59(11):1561-9.

5. Ohnishi H et al. [Acute-on-chronic liver failure]. Ryoikibetsu Shokogun Shirizu. 1995;(7):217-9.

6. Bañares R et al; RELIEF study group. Extracorporeal albumin dialysis with the molecular adsorbent recirculating system in acute-on-chronic liver failure: the RELIEF trial. Hepatology. 2013;57(3):1153-62.

7. Kjaergard LL et al. Artificial and bioartificial support systems for acute and acute-on-chronic liver failure: a systematic review. JAMA. 2003;289(2):217-22.

8. Olson JC et al. Intensive care of the patient with cirrhosis. Hepatology. 2011;54(5):1864-72.

9. Sarin SK et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). Hepatol Int. 2009;3(1):269-82.

10. Jalan R et al; World Gastroenterology Organization Working Party. Toward an improved definition of acute-on-chronic liver failure. Gastroenterology. 2014;147(1):4-10.

11. Bajaj JS et al; North American Consortium For The Study Of End-Stage Liver Disease Nacseld. Survival in infection-related acute-on-chronic liver failure is defined by extrahepatic organ failures. Hepatology. 2014;60(1):250-6.

12. Moreau R et al; CANONIC Study Investigators of the EASL–CLIF Consortium. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. Gastroenterology. 2013;144(7):1426-37, 1437.e1-9.

13. Silva PE et al. Single-centre validation of the EASL-CLIF Consortium definition of acute-on-chronic liver failure and CLIF-SOFA for prediction of mortality in cirrhosis. Liver Int. 2014;doi:10.1111/liv.12597. [Epub ahead of print].

14. Dunn WB et al. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. Chem Soc Rev. 2011;40(1):387-426.

15. Amathieu R et al. Metabolomic approach by 1H NMR spectroscopy of serum for the assessment of chronic liver failure in patients with cirrhosis. J Proteome Res. 2011;10(7):3239-45.

16. Martínez-Granados B et al. Metabolic profile of chronic liver disease by NMR spectroscopy of human biopsies. Int J Mol Med. 2011;27(1):111-7.

17. Amathieu R et al. Serum 1H-NMR metabolomic fingerprints of acute-on-chronic liver failure in intensive care unit patients with alcoholic cirrhosis. PloS One. 2014;9(2):e89230.

18. Huang K et al. Survival and prognostic factors in hepatitis B virus-related acute-on-chronic liver failure. World J Gastroenterol. 2011;17(29):3448-52.

19. Zhai S et al. The ratio of Th-17 to Treg cells is associated with survival of patients with acute-on-chronic hepatitis B liver failure. Viral Immunol. 2011;24(4):303-10.

20. Bahirwani R et al. Acute-on-chronic liver failure before liver transplantation: impact on posttransplant outcomes. Transplantation. 2011;92(8):952-7.

21. Novelli G et al. Predictive parameters after molecular absorbent recirculating system treatment integrated with model for end stage liver disease model in patients with acute-on-chronic liver failure. Transplant Proc. 2010;42(4):1182-7.

22. Radha Krishna Y et al. Clinical features and predictors of outcome in acute hepatitis A and hepatitis E virus hepatitis on cirrhosis. Liver Int. 2009;29(3):392-8.

23. Wang ZX et al. Impact of pretransplant MELD score on posttransplant outcome in orthotopic liver transplantation for patients with acute-on-chronic hepatitis B liver failure. Transplant Proc. 2007;39(5):1501-4.

24. Karvellas CJ et al. Bacteremia, acute physiology and chronic health evaluation II and modified end stage liver disease are independent predictors of mortality in critically ill nontransplanted patients with acute on chronic liver failure. Crit Care Med. 2010;38(1):121-6.

HEPATIC-BASED INBORN ERRORS OF METABOLISM

***Tormod Lund**

Surgical Department, Vestre Viken Hospital Trust, Drammen, Norway
**Correspondence to tolund@vestreviken.no*

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ABSTRACT

Inborn errors of metabolism (IEMs) are a vast, diverse, and heterogeneous set of genetic disorders. Hepatic-based IEMs are a significant cause of morbidity and mortality, and represent a common indication for liver transplantation (LTx) in the paediatric population. This review focuses on four of the most common hepatic-based IEMs where Tx, either as whole organ liver or as isolated hepatocytes, may be an option: familial amyloid polyneuropathy, Wilson's disease, alpha-1 antitrypsin deficiency, and phenylketonuria.

Keywords: Inborn errors of metabolism, inherited metabolic disorders, liver transplantation, hepatocyte transplantation, gene therapy.

INTRODUCTION

Inborn errors of metabolism (IEMs) are a vast, diverse, and heterogeneous set of genetic disorders that are caused by alterations of a specific chemical reaction in metabolism. The term was coined by Sir Archibald Garrod in 1902.[1] Although individually rare, IEMs are collectively common with an estimated incidence >1:1,000.[2] There are hundreds of different IEMs mapped to date, and the number will probably continue to grow until all variants of enzymes and transporters that specify homeostatic mechanisms in humans are identified.[3,4] IEMs are a significant cause of morbidity and mortality, especially in childhood. A vast number of key metabolic reactions occur in the liver. This review focuses on the most common hepatic-based IEMs where transplantation (Tx), either as whole organ or as isolated hepatocytes, may be an option. Based on data from the European Liver Transplant Registry, familial amyloid polyneuropathy (FAP), Wilson's disease (WD), and alpha-1 antitrypsin deficiency (A1ATD) combined accounts for ~55% of all liver transplants for IEMs (Table 1[5-13]). In phenylketonuria (PKU), due to its relative frequency, arduous management, and grave complications, hepatocyte transplantation (HTx) is being investigated.[14]

Table 1: Hepatic-based inborn errors of metabolism (IEMs) and liver transplantation in Europe from 1988-2009.

Disorder	Incidence	No. of transplants performed
Familial amyloid polyneuropathy	1:500 to 1:100,000[5,6]	1280[7]
Wilson's Disease	1:30,000 to 1:100,000[8]	812[7]
Alpha-1 antitrypsin deficiency	1:2,000 to 1:5,000[9]	542[7]
Hereditary haemochromatosis	1:200 to 1:300[10]	468[7]
Primary hyperoxaluria	1:120,000[11]	230[7]
Tyrosinaemia Type 1	1:100,000[12]	98[7]
Homozygous hypercholesterolaemia	1:1,000,000[13]	21[7]

NB: Transplants for non-hepatic-based IEMs are excluded.

FAP

FAP is caused by a mutation in the gene that encodes transthyretin (TTR), and was first described by Mario Corino de Andrade in 1952.[15] There are >100 mutations in the *TTR* gene associated with disease,[16] the most common being V30M. FAP is an autosomal dominant disease found throughout the world, but not all carriers develop the disease. For example, Northern Sweden has a high carrier frequency of the V30M mutation - 1.5% of the population - but only 5% develop symptoms before the age of 40 years. In contrast, endemic areas in Portugal have ten times lower carrier frequency (0.18%), but high penetrance (87% before the age 40).[17] Unexpectedly, homozygote carriers do not have more severe disease than heterozygotes.[17] TTR transports thyroxin and retinol in serum and cerebrospinal fluid, and is secreted by hepatocytes.[18] TTR is a significant plasma protein (approximately 25 mg/dl),[19] and has a tendency to form amyloid in essentially all vascular organs. These amyloid deposits are found, to some degree, in 25% of the population older than 80 years,[20] usually without clinical significance. In TTR V30M, a single amino acid substitution results in a structural change of the protein, causing altered metabolism and enhanced amyloid fibril formation,[21] resulting in neuro and cardiomyocyte-toxicity at a younger age.

FAP is characterised by progressive peripheral and autonomic neuropathy or cardiomyopathy in early adulthood and results in severe disability and death within 10-15 years.[22] It is caused by deposits of mutant TTR displacing normal cellular structures, resulting in impairment of organ function.[16] Clinically, FAP should be considered in patients with a progressive axonal polyneuropathy of unknown origin, especially when associated with autonomic dysfunction or cardiac manifestation. Biopsy of an affected organ may then confirm the diagnosis. Family history is of paramount importance.[23] Medical treatment options for FAP are evolving. The working hypothesis is that if one can stabilise TTR in its tetrameric form, amyloid formation may be prevented. Tafamidis, a meglumine salt, has been shown to slow the neurological deterioration in FAP,[24] and has been approved in Europe and Japan. Another strategy is inhibition of TTR synthesis on the RNA level. Again, mainly two strategies for this exist; degradation of mRNA by antisense oligonucleotides or gene silencing using small interfering RNAs. Phase I studies for both strategies have recently been completed, and Phase II/III studies are ongoing. Both strategies appear safe, and efficiently reduce the amount of circulating TTR.[25-27]

Liver transplantation (LTx) has been performed as treatment for FAP since the early 1990s. Worldwide, over 2,000 liver transplants have been registered (www.fapwtr.org), of which 1,200 have been performed in Europe alone.[5] The overall 5-year patient survival in Europe following Tx is 76%, but 100% 10-year survival has been reported by a Japanese group.[28] TTR is not hepatotoxic, meaning that the explanted liver from a FAP patient can be transplanted into another patient with terminal liver failure. The first such 'domino' procedure was performed in 1995.[29] The V30M liver will indeed continue to produce mutant TTR, but the generally slow progression of the disease may justify the use in older recipients.[30] There is mainly one drawback with LTx for FAP (aside from the necessity of immunosuppression and risks of surgery): the transplanted liver continues to produce normal TTR, which, in some patients, continues to be incorporated into existing fibril deposits, especially in the heart.[31] Combined heart-liver Tx has been performed in patients with FAP,[32] but is not likely to become a widely available treatment option.

WD

WD is caused by mutations in the gene encoding ATP7B, and was first described Samuel Alexander Kinnier Wilson in 1912.[33] There are >500 mutations associated with WD in the *ATP7B* gene (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=ATP7B). WD is an autosomal recessive disorder with a prevalence of about 1:30,000 to 1:100,000,[8] but the prevalence may be considerably higher in some areas, e.g. East Asia.[34] Genotype-phenotype correlations in WD have yet to be established, as the number of homozygotes is exceedingly small, and the prevalence of compound heterozygotes is high.[35]

ATP7B is found exclusively in the hepatocyte and permits the efficient excretion of copper into the bile. Copper is an important cofactor for many proteins, but the average diet provides an abundance and the majority ends up being excreted.[36] The most common effect of mutation in *ATPB7* is protein misfolding. This misfolding causes altered metabolism, decreased stability, and loss of copper-transport activity.[37,38] The resulting copper accumulation is toxic, especially in the

liver and brain.[39] Recently, it has been shown that copper is required for tumour growth and signalling in some cancers, however, the rate of hepatobiliary malignancies in WD is very low.[40,41] WD is characterised by liver disease in the second decade, followed by neuropsychiatric disorders in the third decade, but the clinical presentation is highly heterogeneous and may include Fanconi syndrome, cardiomyopathy, osteomalacia, and anaemia.[39] The median delay in diagnosis is reported to be 2 years.[42] Clinically, a diagnostic scoring system has been developed[43] with a high sensitivity and specificity.[44] It combines clinical findings, lab results, liver biopsy, and mutation analysis. Pragmatically, the presence of low serum caeruloplasmin and higher urine copper is sufficient to conclude the diagnosis of WD in most cases.

Kayser-Fleischer rings are present in about 50% of cases of WD at diagnosis.[8] Liver biopsy is used to define liver status in cases with ambiguous biochemical parameters and to evaluate hepatic copper levels with specific stains.[45] Medical treatment of WD include copper-chelators (penicillamine or trientine) and zinc salts, with the goal to establish and maintain normal copper homeostasis. Medical treatment can generally prevent and even reverse symptoms of copper overload, at least when initiated at an early stage in the disease.[46] However, non-responders or lack of compliance to the drug treatment may result in disease progression and acute liver failure with mortality approaching 100%. Sporadically, therapeutic plasma exchange and various forms of albumin dialysis have been reported as effective techniques for rapidly reducing serum copper levels in WD crisis, delaying or even preventing the need for LTx.[47] HTx has been proven safe, and at least transciently effective, in treating other IEMs, and could conceivably be utilised as support until chelation treatment shows its effect.[48] LTx is the treatment modality of choice in most cases of WD crisis. In Europe, >800 patients with WD have been transplanted. The 5-year overall survival rate is 85%.[5]

A1ATD

A1ATD is an autosomal recessive disease caused by mutations in the *SERPINA1* gene. The disease was discovered in 1963 by Carl-Bertil Laurell.[49] In the *SERPINA1* gene there are >120 mutations identified, but the majority of patients with severe disease are homozygous for the Z mutation.[9] A1ATD has an estimated prevalence of 1:2,000 – 5,000.[9] Not all develop disease: it is estimated that 10-35% of patients with ZZ genotypes do not exhibit any clinical symptoms.[50] In an epidemiological study carried out in Sweden over 40 years, <10% of the 127 infants that were identified had clinically significant liver disease over the first four decades of life.[51,52]

A1AT is mainly synthesised by hepatocytes and to a small degree in the lungs. The physiologic serum concentration of A1AT for adults ranges from 1.0-1.7 g/l, but as an acute phase protein, it is up-regulated during inflammation, infection, cancer, and pregnancy.[53] The most important function of A1AT is inactivation of released proteolytic enzymes in the lungs. In patients with the ZZ variant, A1AT proteins have a single amino acid substitution causing structural change, accumulation in the rough endoplasmic reticulum and decreased secretion.[54] The function of A1AT is also reduced.[55] In this sense, A1ATD is similar to the amyloidoses (e.g. FAP). As a result of decreased secretion of A1AT, overt protease activity ensues in the lungs, resulting in destruction of lung matrix components, alveolar structures, and blood vessels. Injury to liver cells also occurs, but susceptibility to disease is determined by processing abilities for misfolded A1AT. Interindividual differences in this cellular machinery are thought to be responsible for the different susceptibility to chronic liver disease.[56] Higher rates of liver cancer are found in A1ATD due to hepatic inflammation and increased liver cell turnover.[57] A1ATD typically appears with chronic obstructive pulmonary disease (COPD), emphysema, and disseminated bronchiectasis, usually between the forth and the fifth decade, but earlier onset may occur, especially in smokers.[58] In younger patients there is often a long lapse before A1ATD is diagnosed,[59] as the symptoms are attributed to a more likely diagnosis of asthma. The progression to liver cirrhosis in patients with A1ATD is usually slow. However, some patients develop early end-stage disease, with the need for LTx at a young age.

Diagnosis of A1ATD is usually made by measurement of serum A1AT concentration in combination with determination of C-reactive protein (the latter to exclude ongoing inflammation), and established with genotyping. Treatment of A1ATD lung manifestations does not differ from standard treatments of COPD.[60] Substitution therapy with A1AT derived from pooled human plasma is performed in some European countries,

but robust evidence of efficacy is so far limited.[61] Recently, a Phase I/II clinical trial with an adeno-accosiated virus as vector delivering human A1AT complementary DNA (cDNA) has been completed,[62] and may usher in a new era in the treatment of A1ATD. For hepatic manifestations, the anticonvulsive drug carbamazepine is currently being evaluated in a Phase III clinical trial.[63,64] Carbamazepine has been shown to enhance autophagy and perhaps other intracellular mechanisms for degrading deposits of misfolded A1AT. For advanced liver disease, Tx is the treatment of choice. More than 500 patients have been transplanted for A1ATD in Europe, and the 5-year overall patient survival is 85%.[5]

PKU

PKU is an autosomal recessive disease caused by mutations in the phenylalanine hydroxylase (*PAH*) gene. The disease was first described by Asbjorn Folling.[65] In the *PAH* gene >500 mutations have been mapped, and most have effects on PAH activity.[66] Established genotype-phenotype correlations are emerging.[67] PKU has a prevalence of about 1:10,000 in Europe, but for some areas it is higher.[68] PAH converts phenylalanine (Phe) into tyrosine, and is found exclusively in the liver.[69] Loss of PAH activity results in increased concentrations of Phe in the blood. Phe is an essential amino acid, and its entry into the brain is mediated by the large neutral amino acid carrier L-type amino acid transporter 1 (LAT1). Two other amino acids—tyrosine (precursor of dopamine and noradrenaline) and tryptophan (precursor of serotonine), also enter the brain via the LAT1 carrier. Since they compete for the same carrier, high concentrations of Phe in the blood impairs brain uptake of tryptophan and tyrosine.[70] Accordingly, cerebral protein synthesis rate is decreased in PKU patients when concentration of Phe is high.[71] Furthermore, animal studies have shown that high concentration of Phe and its metabolites (principally phenyl lactate and phenylacetate) exert deleterious effects on markers of bioenergetics activity in neural tissue.[72] Together with the deficiency of tyrosine and its downstream products, these factors may explain the neurotoxicity in PKU.

PKU is classified by the severity of hyperphenylalaninaemia. The normal range of blood Phe concentrations is 0.8–1.8 mg/dl; concentrations above 20 mg/dl denote classic PKU.[73] Clinically, untreated PKU leads to disturbed brain development with profound retardation, microcephaly, epilepsy, and other neurologic symptoms. Most countries have a newborn screening programme, and early detection and implementation of a Phe-restricted diet widely prevents neurological symptoms. However, the diet regimen is arduous and even patients with well-controlled PKU exhibit a variety of subtle physical, cognitive, and behavioural symptoms.[67,74-76] Perhaps unsurprisingly, it has been shown that patients with PKU spend considerably more time managing their disease than patients with Type 1 diabetes.[77] Sapropterin dihydrochloride, a pharmaceutical form of the chaperone PAH cofactor tetrahydrobiopterin, lowers plasma Phe concentrations for up to half of patients with PKU.[78] Responders reportedly also experience increases in Phe tolerance[79] and increased quality of life (QoL).[80]

One patient with PKU has received a LTx for reasons unrelated to PKU, and the patient's blood Phe level normalised after transplant.[81] HTx has been performed in one patient who had poor dietary control, with temporary improvement of blood Phe levels.[82] Clinically, HTx involves isolation of hepatocytes from livers rejected for solid organ Tx, and is performed with an infusion of the cells via a portal catheter into the liver, in a manner much resembling that of islet Tx. It is minimally invasive, and generally performed under local anesthesia. In the case of PKU, the cells need only improve a single enzyme deficiency. A clinical trial with hepatocytes for PKU is currently ongoing in the United States.[14]

CONCLUSION

Even though IEMs are regarded as simple mendelian diseases, clear genotype-phenotype correlations are rarely seen.[4] It is increasingly recognised, partly through the advent of next-generation sequencing, that multiple causative alleles, modifier alleles, or both, are common.[83] Hopefully improvements in our understanding of these genetic mechanisms will result in robust methods that can identify patients needing treatment before devastating symptoms occur. Gene therapy, and cellular Tx have the potential of dramatically improving the QoL of patients suffering from IEMs in the near future. For now, improved medical treatments and whole organ LTx may increasingly be considered in hepatic based IEMs.

REFERENCES

1. Garrod AE. The incidence of alkaptonuria: a study in chemical individuality. Lancet. 1902;2:1616-20.

2. Sanderson S et al. The incidence of inherited metabolic disorders in the West Midlands, UK. Arch Dis Child. 2006;91:896-9.

3. Peltonen L, McKusick VA. Genomics and medicine. Dissecting human disease in the postgenomic era. Science. 2001;291: 1224-9.

4. Lanpher B et al. Inborn errors of metabolism: the flux from Mendelian to complex diseases. Nat Rev Genet. 2006;7:449-60.

5. Conceição I, De Carvalho M. Clinical variability in type I familial amyloid polyneuropathy (Val30Met): comparison between late- and early-onset cases in Portugal. Muscle Nerve. 2007;35:116–8.

6. Benson M, "Amyloidosis," Scriver CR et al (eds.), The metabolic and molecular bases of inherited disease (2000), McGraw-Hill: New York City, NY, pp. 5345-78.

7. Adam R et al; All contributing centers (www.eltr.org); European Liver and Intestine Transplant Association (ELITA). Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). J Hepatol. 2012;57:675–88.

8. Ala A et al. Wilson's disease. Lancet. 2007;369:397-408.

9. Silverman EK, Sandhaus RA. Clinical practice. Alpha1-antitrypsin deficiency. N Engl J Med. 2009;360:2749-57.

10. Merryweather-Clarke AT et al. Global prevalence of putative haemochromatosis mutations. J Med Genet. 1997;34:275–8.

11. Cochat P et al. Primary hyperoxaluria type 1: still challenging! Pediatr Nephrol. 2006;21:1075-81.

12. de Laet C et al. Recommendations for the management of tyrosinaemia type 1. Orphanet J Rare Dis. 2013;8:8.

13. Goldstein JL, Brown MS. Familial hypercholesterolemia: identification of a defect in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity associated with overproduction of cholesterol. Proc Natl Acad Sci U S A. 1973;70:2804—8.

14. Mazariegos G et al. Liver transplantation for pediatric metabolic disease. Mol Genet Metabol. 2014;111: 418-27.

15. Andrade C. A peculiar form of peripheral neuropathy; familiar atypical generalized amyloidosis with special involvement of the peripheral nerves. Brain. 1952;75:408-27.

16. Merrill D et al. The molecular biology and clinical features of amyloid neuropathy. Muscle Nerve. 2007;36:411-23.

17. Olsson M et al. A possible role for miRNA silencing in disease phenotype variation in Swedish transthyretin V30M carriers. BMC Med Genet. 2010;11:130.

18. Holmgren G et al. Biochemical effect of liver transplantation in two Swedish patients with familial amyloidotic polyneuropathy (FAP-met30). Clin Genet. 1991;40:242-6.

19. Robbins J. Thyroxine-binding proteins. Prog Clin Biol Res. 1976;5:331-55.

20. Westermark P et al. Fibril in senile systemic amyloidosis is derived from normal transthyretin. Proc Natl Acad Sci U S A. 1990;87:2843-5.

21. Hamilton JA, Benson MD. Transthyretin: a review from a structural perspective. Cell Mol Life Sci. 2001;58:1491-521.

22. Ando Y et al. Transthyretin-related familial amyloidotic polyneuropathy. Arch Neurol. 2005;62:1057-62.

23. Planté-Bordeneuve V, Said G. Familial amyloid polyneuropathy. Lancet Neurol. 2011;10:1086-97.

24. Coelho T et al. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. Neurology. 2012;79:785-92.

25. Ueda M, Ando Y. Recent advances in transthyretin amyloidosis therapy. Transl Neurogener. 2014;3:19.

26. Ackermann EJ et al. Clinical development of an antisense therapy for the treatment of transthyretin-associated polyneuropathy. Amyloid. 2012;19 Suppl 1:43-4.

27. Coelho T et al. Safety and efficacy of RNAi therapy for transthyretin amyloidosis. N Engl J Med. 2013;369: 819-29.

28. Yamashita T et al. Long-term survival after liver transplantation in patients with familial amyloid polyneuropathy. Neurology. 2012;78:637-43.

29. Furtado A et al. Sequential liver transplantation. Transplant Proc. 1997;29:467-8.

30. Merrill D, Benson MD. Liver transplantation and transthyretin amyloidosis. Muscle Nerve. 2013;47: 157-62.

31. Ihse E et al. Variation in amount of wild-type transthyretin in different fibril and tissue types in ATTR amyloidosis. J Mol Med (Berl). 2011;89:171-80.

32. Nardo B et al. Combined heart and liver transplantation in four adults with familial amyloidosis: experience of a single center. Transplant Proc. 2004;36:645-7.

33. Wilson SAK. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain. 1912;34:295-509.

34. Hahn SH et al. Pilot study of mass screening for Wilson's disease in Korea. Mol Genet Metab. 2002;76:133-6.

35. Merle U et al. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. Gut. 2007;56:115-20.

36. Roberts EA, Schilsky ML; American Association for Study of Liver Diseases (AASLD). Diagnosis and treatment of Wilson disease: an update. Hepatology. 2008;47:2089-111.

37. Payne AS et al. Functional expression of the Wilson disease protein reveals mislocalization and impaired copper-dependent trafficking of the common H1069Q mutation. Proc Natl Acad Sci U S A. 1998;95:10854-9.

38. Tsivkovskii R et al. The role of the invariant His-1069 in folding and function of the Wilson's disease protein, the human copper-transporting ATPase ATP7B. J Biol Chem. 2003;278:13302-8.

39. Gitlin JD. Wilson disease. Gastroenterology. 2003;125:1868-77.

40. Brady DC et al. Copper is required for oncogenic BRAF signaling and tumorigenesis. Nature. 2014;509:492–6.

41. Pfeiffenberger J et al. Hepatobiliary malignancies in Wilson disease. Liver Int. 2014;doi:10.1111/liv.12727. [Epub ahead of print].

42. Prashanth LK et al. Wilson's disease: diagnostic errors and clinical implications. J Neurol Neurosurg Psychiatry. 2004;75:907-9.

43. Ferenci P et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int. 2003;23:139-42.

44. Koppikar S, Dhawan A. Evaluation of the scoring system for the diagnosis of Wilson's disease in children. Liver Int. 2005;25:680-1.

45. Ovchinsky N et al. Liver biopsy in modern clinical practice: a pediatric point-of-view. Adv Anat Pathol. 2012;19:250-62.

46. Medici V et al. Wilson disease--a practical approach to diagnosis, treatment and follow-up. Dig Liver Dis. 2007;39:601-9.

47. Reynolds HV et al. Copper removal strategies for Wilson's disease crisis in the ICU. Anaesth Intensive Care. 2014;42: 253-7.

48. Filippi C, Dhawan A. Current status of human hepatocyte transplantation and

its potential for Wilson's disease. Ann N Y Acad Sci. 2014;1315:50-5.

49. Laurell CB, Eriksson S. The electrophoretic alpha 1-globulin pattern of serum in alpha 1-antitrypsin deficiency. Scand J Clin Lab Invest. 1963;15:132-40.

50. Tobin MJ et al. Alpha 1 antitrypsin deficiency: the clinical and physiological features of pulmonary emphysema in subjects homozygous for Pi type Z. A survey by the British Thoracic Association. Br J Dis Chest. 1983;77:14-27.

51. Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. N Engl J Med. 1976;294:1316-21.

52. Piitulainen E et al. Alpha1-antitrypsin deficiency in 26-year-old subjects: lung, liver, and protease/protease inhibitor studies. Chest. 2005;128:2076-81.

53. Köhnlein T, Welte T. Alpha-1 antitrypsin deficiency: pathogenesis, clinical presentation, diagnosis, and treatment. Am J Med. 2008;121:3-9.

54. Purkayastha P et al. Alpha 1-antitrypsin polymerization: a fluorescence correlation spectroscopic study. Biochemistry. 2005;44:2642-9.

55. Ogushi F et al. Z-type alpha 1-antitrypsin is less competent than M1-type alpha 1-antitrypsin as an inhibitor of neutrophil elastase. J Clin Invest. 1987;80:1366-74.

56. Teckman JH et al. The proteasome participates in degradation of mutant alpha 1-antitrypsin Z in the endoplasmic reticulum of hepatoma-derived hepatocytes. J Biol Chem. 2001;276:44865-72.

57. Topic A et al. Alpha-1-antitrypsin in pathogenesis of hepatocellular carcinoma. Hepat Mon. 2012;12:e7042.

58. Brode SK et al. Alpha-1 antitrypsin deficiency: a commonly overlooked cause of lung disease. CMAJ. 2012;184:1365-71.

59. Köhnlein T et al. Diagnostic delay and clinical modifiers in alpha-1 antitrypsin deficiency. Ther Adv Respir Dis. 2010;4:279-87.

60. Fabbri LM et al. Update in chronic obstructive pulmonary disease 2005. Am J Respir Crit Care Med. 2006;173:1056-65.

61. Stockley RA et al; Alpha One International Registry (A.I.R.). Augmentation therapy for alpha-1 antitrypsin deficiency: towards a personalised approach. Orphanet J Rare Dis. 2013;8:149.

62. Chiuchiolo MJ et al. Phase I/II study of intrapleural administration of a serotype rh.10 replication-deficient adeno-associated virus gene transfer vector expressing the human α1-antitrypsin cDNA to individuals with α1-antitrypsin deficiency. Hum Gene Ther Clin Dev. 2014;25:112-33.

63. Hidvegi T et al. An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. Science. 2010;329: 229-32.

64. Wang Y, Perlmutter DH. Targeting intracellular degradation pathways for treatment of liver disease caused by α1-antitrypsin deficiency. Pediatr Res. 2014;75:133-9.

65. Fölling A. Über Ausscheidung von Phenylbrenztraubensäure in den Harn als Stoffwechselanomalie in Verbindung mit Imbezillität. Hoppe-Seyler's Zschr Physiol Chem. 1934;227:169-81.

66. Scriver CR. The PAH gene, phenylketonuria, and a paradigm shift. Hum Mutat. 2007;28:831-45.

67. Camp KM et al. Phenylketonuria Scientific Review Conference: state of the science and future research needs. Mol Genet Metab. 2014;112:87-122.

68. Loeber JG. Neonatal screening in Europe; the situation in 2004. J Inherit Metab Dis. 2007;30:430-8.

69. Hsieh MC, Berry HK. Distribution of phenylalanine hydroxylase (EC 1.14.3.1) in liver and kidney of vertebrates. J Exp Zool. 1979;208:161-7.

70. Pietz J et al. Large neutral amino acids block phenylalanine transport into brain tissue in patients with phenylketonuria. J Clin Invest. 1999;103:1169-78.

71. Hoeksma M et al. Phenylketonuria: high plasma phenylalanine decreases cerebral protein synthesis. Mol Genet Metab. 2009;96:177-82.

72. Duarte JM et al. Metabolic disturbances in diseases with neurological involvement. Aging Dis. 2013;5:238-55.

73. Lindner M, "Treatment Of Phenylketonuria Variants: European Recommendations," Blau N (ed.), PKU and BH4: advances in phenylketonuria and tetrahydrobiopterin (2006), SPS Verlagsgesellschaft mbH: Heilbronn, pp. 180-7.

74. Waisbren SE et al. Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis. Mol Genet Metab. 2007;92:63-70.

75. Waisbren SE, Azen C. Cognitive and behavioral development in maternal phenylketonuria offspring. Pediatrics. 2003;112:1544-7.

76. Christ SE et al. Executive function in early-treated phenylketonuria: profile and underlying mechanisms. Mol Genet Metab. 2010;99 Suppl 1:S22-32.

77. Eijgelshoven I et al. The time consuming nature of phenylketonuria: a cross-sectional study investigating time burden and costs of phenylketonuria in the Netherlands. Mol Genet Metab. 2013;109:237–42.

78. Utz JR et al. START, a double blind, placebo-controlled pharmacogenetic test of responsiveness to sapropterin dihydrochloride in phenylketonuria patients. Mol Genet Metab. 2012;105:193-7.

79. Singh RH, Quirk ME. Using change in plasma phenylalanine concentrations and ability to liberalize diet to classify responsiveness to tetrahydrobiopterin therapy in patients with phenylketonuria. Mol Genet Metab. 2011;104:485-91.

80. Douglas TD et al. Longitudinal quality of life analysis in a phenylketonuria cohort provided sapropterin dihydrochloride. Health Qual Life Outcomes. 2013;11:218.

81. Vajro P et al. Correction of phenylketonuria after liver transplantation in a child with cirrhosis. N Engl J Med. 1993;329:363.

82. Stéphenne X et al. Hepatocyte transplantation using the domino concept in a child with tetrabiopterin nonresponsive phenylketonuria. Cell Transplant. 2012;21:2765-70.

83. Lu JT et al. Genotype-phenotype correlation--promiscuity in the era of next-generation sequencing. N Engl J Med. 2014;371:593-6.


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METABOLIC SYNDROME IN PAEDIATRIC POPULATION: IS IT TIME TO THINK BACK ON DIAGNOSIS CRITERIA?

Claudia Della Corte, Anna Alisi, *Valerio Nobili

Hepatometabolic Department, "Bambino Gesù" Children's Hospital, Rome, Italy
**Correspondence to nobili66@yahoo.it*

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ABSTRACT

Metabolic syndrome (MetS) represents an emerging disease in the paediatric population; it is characterised by a cluster of cardiometabolic abnormalities, including visceral obesity, dyslipidaemia, hypertension, and Type 2 diabetes mellitus, that directly increase the risk of developing cardiovascular disease and diabetes. Currently, several definitions of MetS are available in the paediatric setting, causing confusion and discrepancy in the identification of these patients. Moreover, in recent years, several other comorbidities, besides those traditionally used to define MetS, which are also linked to the disease have been identified, making its definition even more difficult. Among these, mainly non-alcoholic fatty liver disease and obstructive sleep disorders have been strictly linked to MetS. In this review, we discuss the importance to re-evaluate diagnostic criteria for MetS, in order to uniformly define this disease in children, considering also the inclusion of the other emerging clinical features.

Keywords: Metabolic syndrome, non-alcoholic fatty liver disease, obesity, children, cardiovascular risk.

INTRODUCTION

Childhood obesity and its metabolic complications are rapidly emerging as one of the greatest challenges of the 21st century. The epidemic spread of obesity in the last 20 years has in fact led, in a paediatric setting, to the appearance of diseases previously considered a prerogative of adulthood, such as metabolic syndrome (MetS) and Type 2 diabetes mellitus (T2DM). MetS, described for the first time in the 1988 by Reaven, is characterised by a cluster of metabolic abnormalities comprising insulin resistance (IR), dyslipidaemia, visceral obesity, and hypertension, associated with an enhanced cardiovascular risk in adulthood.[1] Although the pathophysiological mechanism underlying the development of MetS is still only partially understood, the most widely accepted hypothesis identifies IR and excessive production of free fatty acids as the key components in the development of this disease.[2] In the paediatric population an important role in the pathogenesis of MetS is played by intrauterine events and factors that emerge during the early years of development. In fact, the presence of maternal gestational diabetes, low birth weight, and infant feeding practices contribute to enhance the future risk of MetS. Other factors are socio-economic or environmental (an obesogenic environment, for example), similar to adults.

In the last decade, many criteria have been proposed by the various scientific societies in an attempt to define MetS in children, changing the diagnostic criteria of the adults and using them to diagnose children and adolescents. The major limitation to the application of these criteria is represented by the fact that many of them (body mass index [BMI], waist circumference, blood pressure, and lipid profile) are continuous age-dependent variables. In fact, although several authors have applied the diagnostic criteria of adults to the paediatric population, inserting specific numerical cut-offs expressed in percentiles, the effects have led to great diversity in the results of various epidemiological studies. More importantly, none of the MetS definitions consider the influences of growth and puberty, for instance, physiological insulin resistance in puberty, changes in fat and fat-free mass, and changes in sex steroid secretion.[3]

Until now more than 40 definitions for childhood MetS have been proposed, most based on adaptations of adult criteria.[4] Several studies have clearly demonstrated that the prevalence of MetS in the paediatric age group may vary widely using different definitions, ranging from 2.2% to 52.1% among different studies.[5] Table 1 displays three of the most commonly used definitions for MetS in the paediatric population.

Despite the diversity of such data, it is clear that there is an increased prevalence of MetS among obese children and that, in this population, the prevalence of MetS increases with the increase of the degree of obesity. Recent data (International Obesity Taskforce) reported that around 150 million school-aged children and 50 million children are overweight and obese, respectively, with consequent early and long-term obesity-related comorbidities, including MetS. The health consequences of childhood obesity and MetS are wide-ranging, as obesity appears to be on the causal pathway of every major chronic disease.[2] Several studies have, in fact, reported that the metabolic alterations related to obesity and MetS are multisystemic and include other organs in addition to the best-known targets of MetS, changing the actual scenario of paediatric MetS (Figure 1). Recently, other abnormalities, such as chronic proinflammatory and prothrombotic states, non-alcoholic fatty liver disease (NAFLD), and sleep apnoea have been added to the entity of the syndrome, making its definition even more complex and raising doubts about the accuracy of the metabolic features considered as criteria for the diagnosis of MetS.[4]

Table 1: Criteria for the diagnosis of metabolic syndrome (MetS) in children.

Parameters	Diagnostic criteria for MetS			
	International Diabetes Federation (IDF)*		National Cholesterol Education Program/ Adult Trial Panel III°	American Heart Association (AHA)^
Age	10-16 years	>16 years	12-19 years	12-19 years
Waist circumference	≥90th percentile (or adult cut-off if lower)	In Caucasian population ≥90 cm	≥90th percentile for age and sex	≥90th percentile for age, sex, and ethnicity
Triglycerides	≥150 mg/dl (≥1.7 mmol/l)	≥150 mg/dl (1.7 mmol/l) or specific treatment for this lipid abnormality	>110 mg/dl (1.24 mmol/l)	≥110 mg/dl (1.24 mmol/l)
HDL cholesterol	<40 mg/dl (≤1.3 mmol/l)	<40 mg/dl (1.03 mmol/l) in males <50 mg/dl (1.29 mmol/l) in females or specific treatment for this lipid abnormality	<40 mg/dl (1.03 mmol/l)	≤10th percentile for race and sex
Fasting glucose	>100 mg/dl (5.6 mmol/l)	>100 mg/dl (5.6 mmol/l) or known T2DM	>110 mg/dl (6.1 mmol/l)	≥100 mg/dl (5.6 mmol/l)
Blood pressure (BP)	Systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg	Systolic BP ≥130 or diastolic BP ≥85 mmHg or treatment of previously diagnosed hypertension	Systolic or diastolic above the 90th percentile (age, gender, and height-specific)	≥90th percentile for age, sex, and height

HDL: high density lipoprotein; T2DM: Type 2 diabetes mellitus.
* For the diagnosis of MetS, three of the five criteria must be present.
° For the diagnosis of MetS the presence of three or more of the criteria is required.
^ For the diagnosis of MetS, central obesity and two of four other components must be present.

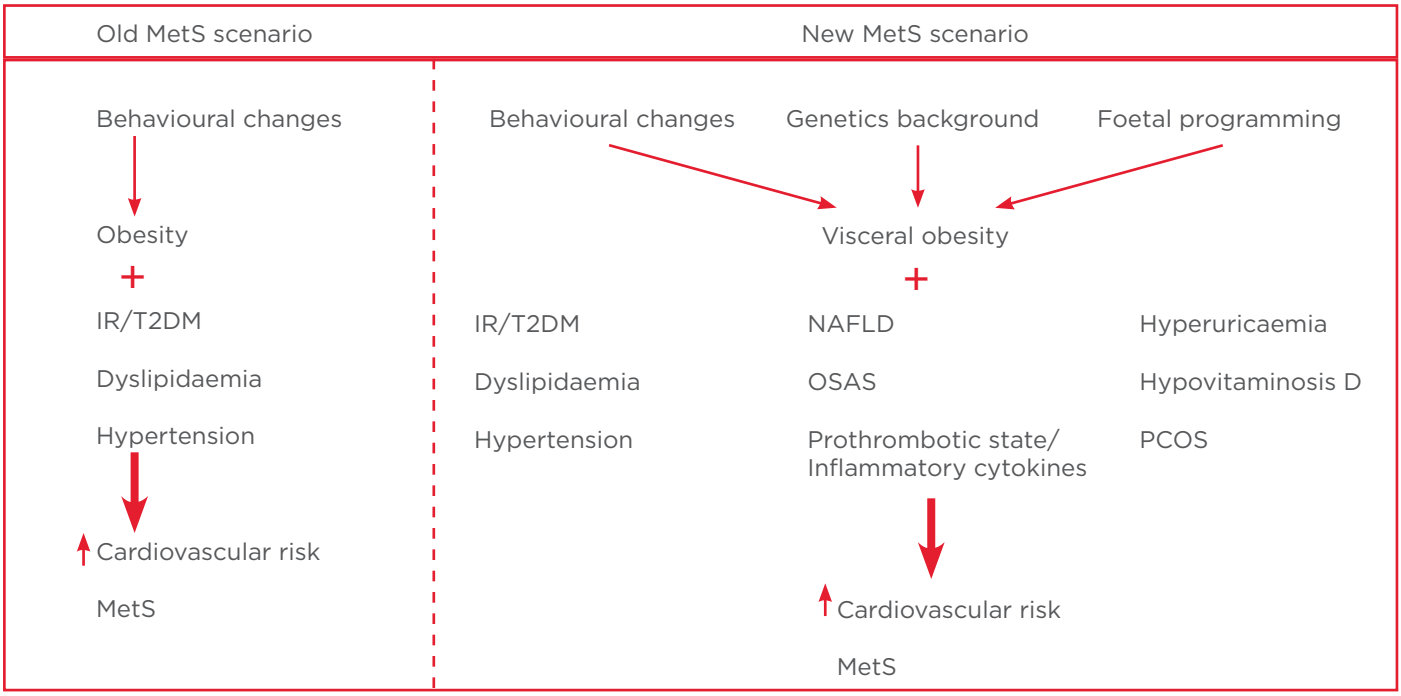

Figure 1: The evolving scenario of metabolic syndrome (MetS) in children.
T2DM: Type 2 diabetes mellitus; PCOS: polycystic ovary syndrome; NAFLD: non-alcoholic fatty liver disease; OSAS: obstructive sleep apnoea syndrome; IR: insulin resistance.

The primary management for MetS is healthy lifestyle promotion, which includes moderate calorie restriction with changes in dietary composition, reducing saturated fat and increasing fibre, which is associated with a moderate intensification in daily physical activity. Unfortunately, because lifestyle changes are difficult to obtain and maintain for children and their families, drug treatments are crucial to prevent the progression of severe organ damage. In the present review, we discuss the importance of establishing clear criteria to define MetS, highlighting the latest research, which extends the metabolic features of MetS to other clinical manifestations, suggesting the need for a re-evaluation of the actual criteria for the diagnosis of paediatric MetS.

NAFLD

The term 'non-alcoholic fatty liver disease' includes a spectrum of liver disease, ranging from simple fat accumulation in the hepatocytes, called simple steatosis, to various degrees of liver inflammation and fibrosis, and even cirrhosis (non-alcoholic steatohepatitis [NASH]).[6] In the last decade, many advances have been made in the understanding of pathogenesis, the clinical implications, and the treatment of this liver disease in children. Unfortunately, because of a scarcity of data regarding long-term follow up, the prognosis of paediatric NAFLD is still doubtful.[7] NAFLD in children displays the same basic morphological lesions observed in their adult counterparts, but the pattern of distribution of these lesions is frequently different. Three distinct histological types were traditionally identified: Type 1 NASH, characterised by steatosis with ballooning degeneration and/or perisinusoidal fibrosis, without portal involvement; Type 2 NASH, characterised by steatosis with portal inflammation and/or fibrosis, in the absence of ballooning degeneration or perisinusoidal involvement; and a NASH overlap type, in which characteristics from both types were present. Most paediatric subjects have Type 2 NASH, which is more likely to be associated with advanced fibrosis.

With regards to the pathogenetic mechanisms that lead to the development of NAFLD in children, multiple metabolic factors, mainly IR, visceral obesity, and dyslipidaemia, interact with each other, creating a network of metabolic derangements involved in both the development and progression of liver damage. It is easy to note the close overlap between the principal pathogenetic factors of MetS and NAFLD, for which fatty liver is now

widely considered as the hepatic manifestation of MetS. In recent years, genome-wide association studies have identified some single-nucleotide polymorphisms, associated with the feature of MetS[8] and NAFLD/NASH, in children.[9] More recently, in addition to well-known environmental factors, emerging studies have reported a possible common genetic susceptibility pattern for NAFLD and MetS.[10] 1 year ago, Xu et al.[11] reported that the rs1800849 variant of the *UCP3* gene is associated with MetS components and increased risk of NAFLD in obese Chinese. Moreover, recent evidence strongly suggests not only a relationship between NAFLD and MetS in obese children, adolescents, and adults, but also the key role exerted by liver fat deposition in the pathogenesis of MetS.[12] Clinically, several studies reported a strict association between these two entities[13] and a case-control study of 300 overweight/obese children (150 with biopsy-proven NAFLD and 150 without) reported that the presence of MetS traits increased 5-times the odds of having NAFLD, compared to age-matched obese children without MetS.[14] Further cross-sectional studies conducted on different ethnic groups have supported the connection between NAFLD and MetS in the paediatric population. Kelishadi et al.[15] have, in fact, reported that in 1,107 Iranian subjects (aged 6–18 years), central obesity may be used as a predictor of NAFLD, assessed by ultrasound and levels of alanine aminotransferase (ALT). It has also been reported that in obese Chinese children, the overlap between NAFLD and MetS may be found in 84.61% of cases.[16]

All these data reinforced the idea that NAFLD represents the hepatic involvement of MetS, and suggested the possible role of NAFLD in the development of metabolic complications during life. In this regard, several studies in the last few years have demonstrated an association between NAFLD and structural or functional cardiovascular abnormalities, including myocardial IR, alterated cardiac energy metabolism, and abnormal left ventricular (LV) structure, independently of cardiovascular risk and metabolic risk factors.[17] Interestingly, Pacifico et al.[18] have recently demonstrated that obese children with NAFLD exhibit features of early LV diastolic and systolic dysfunction compared to obese patients without NAFLD, and that children with more severe liver histology (NASH) have worse cardiac dysfunction. Considering the emerging long-term effects of MetS and NAFLD on cancer and cardiovascular disease (CVD) development, the understanding of the interplay between these two entities may help to identify and promptly treat this high-risk population. Moreover, a prompt intervention on these subclinical abnormalities may be important because treatment to reverse the process is most likely to be effective earlier in the disease.

Lifestyle modification remains the first-line therapy for paediatric NAFLD, but it is usually difficult to achieve. Therefore, in the last decade, based on new knowledge in terms of risk factors and pathogenesis of NAFLD, several studies have evaluated the effects of different molecules, such as insulin-sensitisers, antioxidants, and cytoprotective agents in the treatment of paediatric fatty liver. Metformin, among insulin-sensitiser agents, and vitamin E, such as antioxidants, are the principal drugs evaluated in children with NAFLD. The results of studies conducted in children reported divergent results; recently, the American TONIC trial[19] reported that metformin and vitamin E, either alone or in association, have little effect in reducing serum ALT levels, with partial effects, mainly for vitamin E, on liver histology (hepatocyte ballooning). Furthermore, among the new pharmacological studies for NAFLD, the effect of treatment with docosahexaenoic acid (DHA) requires particular attention. It has been demonstrated that in children with NAFLD, DHA supplementation ameliorates liver fat content, insulin sensitivity index, serum ALT, and triglycerides levels.[7] Other interesting approaches are actually ongoing in NAFLD animal models or in adults. Among these, nuclear receptors are very interesting agents which act to regulate the expression of specific genes controlling a broad range of cellular and metabolic functions. The most studied are the peroxisome proliferator-activated receptors, involved in the activation of hepatic stellate cells, and the farnesoid X receptor, also implicated in the pathogenesis of NAFLD.[7]

OBSTRUCTIVE SLEEP DISORDERS

Obstructive sleep apnoea syndrome (OSAS) is characterised by episodes of chronic intermittent hypoxia and sleep fragmentation and, in obese adults, it has been considered as a respiratory manifestation of the MetS.[20] In fact, according to the International Diabetes Federation Recommendations,[21] adults with OSAS should also be evaluated for cardiovascular disorders and, conversely, the possibility of OSAS should be considered in all patients with diabetes and MetS. Concomitantly with the emerging epidemic of

obesity in childhood, studies evaluating the prevalence of OSAS in children have shown a substantial increase with obesity, such that, for each increase of 1 kg/m^2 of BMI above the mean in children, the risk of OSAS increases by 12%.[22]

OSAS, by exposing children to recurrent intermittent hypoxaemia or oxidative stress, may amplify the adverse effects of adiposity on systemic inflammation and metabolic features associated with vascular disease and diabetes.[23] In fact, emerging data have demonstrated that OSAS leads to a pattern of cardiovascular and metabolic alterations, similar to obesity, due to the production of reactive oxygen species secondary to chronic intermittent hypoxaemia and oxidative stress. These observations suggest that OSAS may exacerbate the deleterious metabolic effects of overweight in adults and children. In fact, children with OSAS have higher levels of blood pressure, C-reactive protein and increased IR, as well as LV hypertrophy,[24] suggesting that childhood OSAS may also increase the risk of developing severe chronic cardiovascular and metabolic conditions.[25,26] Moreover, recently, Nobili et al.[27] have demonstrated a strict association between presence and severity of NASH and OSAS in children with fatty liver, independent of IR and visceral obesity. A growing number of experimental studies have reported an interesting interplay between OSAS and NASH pathogenesis, based on the induction, by hypoxia, of hepatic triglyceride accumulation, inflammation, and fibrosis. On the basis of recent advances concerning the relationship between OSAS and NASH, it has been suggested that children with NAFLD should be routinely screened for OSAS. Further studies are needed to better define the real impact of hypoxaemia correction by OSAS treatment on laboratory, ultrasonography, and histological features of NAFLD, and on metabolic impairments.[27]

HYPERURICAEMIA

Although the serum uric acid (UA) level is not included in any definition of MetS, several studies have shown strong associations between UA levels and MetS or its components.[28,29] In particular, increased serum UA levels are associated with a risk of CVD or renal disease in adulthood.[30,31] The pathogenesis of the association between hyperuricaemia and MetS is not fully understood, but IR is thought to play a pivotal role. In fact, the excess insulin concentrations increase sodium and UA reabsorption by the kidney partially explained this association. Hyperuricaemia, in turn, reduces nitric oxide bioavailability, which is essential for insulin action. Therefore, UA seems to be involved itself in IR pathogenesis, inducing a vicious cycle that is associated with the onset of some components of the MetS.[32]

To date, there is little information regarding this relationship in the paediatric population, both due to the difficult definition of MetS in children and the age-dependent reference values for UA.[33] Recently, in a cohort of 148 Hispanic children with overweight/obesity a prevalence of hyperuricaemia of 53% has been reported, and in the group of patients with hyperuricaemia worse metabolic parameters have been reported, such as higher waist circumference, blood pressure, and Homeostasis Model Assessment index. In this study, the level of UA-associated with less favourable metabolic features in obese children was 5.4 mg/dl.[34] According to these results, subsequent studies reported a strict association between high UA levels and MetS.[35] Pacifico et al.[36] demonstrated an independent association between UA concentrations and the presence of MetS in 120 obese children, and in this study increased UA levels were also associated with carotid atherosclerosis. Recent evidence suggests, in fact, an important pro-atherogenic effect of UA since many studies have associated serum UA concentrations with increased oxidative stress, endothelial dysfunction, inflammation, and hyperinsulinaemia. Therefore, hyperuricaemia should be considered in the scenario of MetS as an independent risk factor causing an increased cardiovascular risk in the paediatric setting also.

HYPOVITAMINOSIS D

Over the last decade, a growing body of observational data from several lines of scientific inquiry indicating a relationship of serum 1,25-dihydroxyvitamin D to chronic metabolic, cardiovascular, and neoplastic diseases has emerged.[37] Several evidences have demonstrated that vitamin D levels are important for optimal functioning of many organs and tissues throughout the body not related to calcium homeostasis.[37] Many evidences also reported an inverse association between fat accumulation and low vitamin D concentrations, ascribed not only to the sequestration of this fat-soluble vitamin in adipose tissue, but also to the negative effect of adipokines produced by adipocytes (i.e. leptin) on synthesis of the active form of vitamin D.[38,39]

In fact, low vitamin D levels have been linked with higher rates of MetS, hypertension, diabetes, myocardial infarction, peripheral arterial disease, and CVD.[37] As previously reported, although CVD events occur most frequently during or after the fifth decade of life, pathologic evidence suggests that precursors of CVD originate in childhood.[40] A significant association between vitamin D and cardiovascular risk factors in youth would suggest that the successful repletion of vitamin D has the potential to improve the cardiovascular risk profile during childhood and adolescence, and to lower the risk of developing CVD in adulthood. From these perspectives, in the last few years, several clinical paediatric trials using different dosage of vitamin D supplementation have been made in order to evaluate the metabolic effects of vitamin D repletion.[41] Moreover, a paediatric trial on the effect of vitamin not only on metabolic characteristics but also on histological features of NASH is now ongoing in the paediatric setting of Italy.[42] The now available results seem to demonstrate positive effects of vitamin D supplementation on metabolic impairments in children, but further studies are needed to identify the optimal dosage and timing of treatment.

CONCLUSION

In the last decade, following the epidemic trail of childhood obesity, an important increase in the prevalence of MetS has been observed in children and adolescents. Given the relatively recent occurrence of MetS in childhood, long-term follow-up studies are limited. However, it is conceivable that the metabolic derangement observed in obese children will have dramatic repercussions on their health earlier than that observed in adults, with a consequent worsening of the prognosis in terms of morbidity and mortality when they are still in youth.[43] Moreover, recent data reported that MetS and its consequences now represent the most part of healthcare expenditure in the United States.[44]

Despite the challenges and difficulties of clearly defining paediatric MetS, it is clear that the prevalence of the single component of MetS is increasing in paediatric settings and that these aspects identify youths at higher risk of developing metabolic impairments, and therefore CVD. In the recent years, several studies reported that MetS is actually associated with many clinical conditions besides CVD and T2DM, including chronic low-grade inflammation, oxidative stress, hyperuricemia, hypertension, dyslipidaemia, hyperandrogenism and polycystic ovary syndrome, NAFLD, OSAS, and certain forms of cancer.[45] Moreover, it has been reported that some of these, such as NAFLD and OSAS for their strict pathogenetic interplay with MetS, should be considered as the hepatic and respiratory manifestations of MetS, respectively. In fact, recent guidelines suggest screening patients with these conditions for MetS and vice versa. In our opinion, considering the close relationship between pathogenetic mechanisms of MetS, NAFLD, and OSAS, and the important and independent effect of these conditions on CVD and metabolic impairments, all actual definitions proposed for MetS in children are unsatisfactory. A careful revision of actual criteria for diagnosis of MetS, also including these emerging features of MetS, is needed. This revision could, in the near future, facilitate the development of specific screening programs for children. There is, in fact, a need for new and sensitive early screening methods that are able to provide a large amount of information about subjects at risk or who are presenting early signs of metabolic injuries. An early identification of these patients at higher risk could permit a prompt intervention in order to prevent or slow the progression of developing metabolic disorders in the initial stages of disease.

REFERENCES

1. Biro FM, Wien M. Childhood obesity and adult morbidities. Am J Clin Nutr. 2010;91(5):1499S-1505S.

2. Katz DL. Childhood obesity trends in 2013: mind, matter, and message. Child Obes. 2013;9(1):1-2.

3. Pilia S et al. The effect of puberty on insulin resistance in obese children. J Endocrinol Invest. 2009;32(5):401-5.

4. Kassi E et al. Metabolic syndrome: definitions and controversies. BMC Med. 2011;9:48.

5. Tavares Giannini D et al. Metabolic syndrome in overweight and obese adolescents: a comparison of two different diagnostic criteria. Ann Nutr Metab. 2014;64(1):71–9.

6. Brunt EM. Nonalcoholic fatty liver disease: what the pathologist can tell the clinician. Dig Dis. 2012;30 Suppl 1:61-8.

7. Alisi A et al. Paediatric nonalcoholic fatty liver disease. Curr Opin Gastroenterol. 2013;29(3):279-84.

8. Pollex RL, Hegele RA. Genetic determinants of the metabolic syndrome. Nat Clin Pract Cardiovasc Med. 2006;3(9):482-9.

9. Nobili V et al. A 4-polymorphism risk score predicts steatohepatitis in children with nonalcoholic fatty liver disease. J Pediatr Gastroenterol Nutr. 2014;58(5):632-6.

10. Alisi A et al. Non-alcoholic fatty

liver disease and metabolic syndrome in adolescents: pathogenetic role of genetic background and intrauterine environment. Ann Med. 2012;44(1):29-40.

11. Xu YP et al. Association between UCP3 gene polymorphisms and nonalcoholic fatty liver disease in Chinese children. World J Gastroenterol. 2013;19(35):5897-903.

12. Nobili V et al. The potential role of fatty liver in paediatric metabolic syndrome: a distinct phenotype with high metabolic risk? Pediatr Obes. 2012;7(6):e75-80.

13. Manco M et al. Waist circumference correlates with liver fibrosis in children with non-alcoholic steatohepatitis. Gut. 2008;57(9):1283-7.

14. Schwimmer JB et al. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. Circulation. 2008;118(3):277-83.

15. Kelishadi R et al. Association of the components of the metabolic syndrome with non-alcoholic fatty liver disease among normal-weight, overweight and obese children and adolescents. Diabetol Metab Syndr. 2009;1:29.

16. Fu JF et al. Non-alcoholic fatty liver disease: an early mediator predicting metabolic syndrome in obese children? World J Gastroenterol. 2011;17(6):735–42.

17. Pacifico L et al. Nonalcoholic fatty liver disease and the heart in children and adolescents. World J Gastroenterol. 2014;20(27):9055-71.

18. Pacifico L et al. Left ventricular dysfunction in obese children and adolescents with nonalcoholic fatty liver disease. Hepatology. 2014;59(2):461-70.

19. Lavine JE et al; Nonalcoholic Steatohepatitis Clinical Research Network. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. JAMA. 2011;305(16):1659-68.

20. Bonsignore MR et al. Adipose tissue in obesity and obstructive sleep apnoea. Eur Respir J. 2012;39(3):746–67.

21. Shaw JE et al; International Diabetes Federation Taskforce on Epidemiology and Prevention. Sleep-disordered breathing and type 2 diabetes: a report from the International Diabetes Federation Taskforce on Epidemiology and Prevention. Diabetes Res Clin Pract. 2008;81(1):2-12.

22. Redline S et al. Risk factors for sleep-disordered breathing in children. Associations with obesity, race, and respiratory problems. Am J Respir Crit Care Med. 1999;159:1527–32.

23. Gozal D. Sleep, sleep disorders and inflammation in children. Sleep Med. 2009;10 Suppl 1:S12–6.

24. Villa MP et al. Early cardiac abnormalities and increased C-reactive protein levels in a cohort of children with sleep disordered breathing. Sleep Breath. 2012;16(1):101-10.

25. Leung LC et al. Twenty-four-hour ambulatory BP in snoring children with obstructive sleep apnea syndrome. Chest. 2006;130(4):1009–17.

26. Gozal D, Kheirandish-Gozal L. Cardiovascular morbidity in obstructive sleep apnea: oxidative stress, inflammation, and much more. Am J Respir Crit Care Med. 2008;177(4): 369–75.

27. Nobili V et al. Obstructive sleep apnea syndrome affects liver histology and inflammatory cell activation in pediatric nonalcoholic fatty liver disease, regardless of obesity/insulin resistance. Am J Respir Crit Care Med. 2014;189(1):66-76.

28. Tsouli SG et al. Elevated serum uric acid levels in metabolic syndrome: an active component or an innocent bystander? Metabolism. 2006;55(10):1293-301.

29. Sui X et al. Uric acid and the development of metabolic syndrome in women and men. Metabolism. 2008;57(6):845-52.

30. Baker JF et al. Serum uric acid and cardiovascular disease: recent developments, and where do they leave us? Am J Med. 2005;118(8):816-26.

31. Cirillo P et al. Uric acid, the metabolic syndrome, and renal disease. J Am Soc Nephrol. 2006;17(12 Suppl 3):S165-8.

32. Santos RD. Elevated uric acid, the metabolic syndrome and cardiovascular disease: cause, consequence, or just a not so innocent bystander? Endocrine. 2012;41(3):350-2.

33. Tang L et al. Hyperuricemia in obese children and adolescents: the relationship with metabolic syndrome. Pediatr Rep. 2010;2(1):e12.

34. Civantos Modino S et al. Hyperuricemia and metabolic syndrome in children with overweight and obesity. Endocrinol Nutr. 2012;59(9):533-8.

35. Mangge H et al. Uric acid best predicts metabolically unhealthy obesity with increased cardiovascular risk in youth and adults. Obesity (Silver Spring). 2013;21(1):E71-7.

36. Pacifico L et al. Serum uric acid and its association with metabolic syndrome and carotid atherosclerosis in obese children. Eur J Endocrinol. 2009;160(1):45-52.

37. Wang C. Role of vitamin d in cardiometabolic diseases. J Diabetes Res. 2013;2013:243934.

38. Wortsman J et al. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72(3):690-3.

39. Tsuji K et al. Leptin stimulates fibroblast growth factor 23 expression in bone and suppresses renal 1alpha,25-dihydroxyvitamin D3 synthesis in leptin-deficient mice. J Bone Miner Res. 2010;25(8):1711-23.

40. Salo A, Logomarsino JV. Relationship of vitamin D status and cardiometabolic risk factors in children and adolescents. Pediatr Endocrinol Rev. 2011;9(1):456–62.

41. Kelishadi R et al. Effects of vitamin D supplementation on insulin resistance and cardiometabolic risk factors in children with metabolic syndrome: a triple-masked controlled trial. J Pediatr (Rio J). 2014;90(1):28-34.

42. Bambino Gesù Hospital and Research Institute. DHA and vitamin D in children with biopsy-proven NAFLD (VitD_DHA). NCT02098317. Available at: http://clinicaltrials.gov/show/NCT02098317.

43. D'Adamo E et al. Metabolic syndrome in pediatrics: old concepts revised, new concepts discussed. Pediatr Clin North Am. 2011;58(5):1241-55.

44. Finkelstein EA et al. Obesity and severe obesity forecasts through 2030. Am J Prev Med. 2012;42(6):563-70.

45. Weiss R et al. What is metabolic syndrome, and why are children getting it? Ann. N Y Acad Sci. 2013;1281:123-40.

PAEDIATRIC METABOLIC CONDITIONS OF THE LIVER

***Elroy P. Weledji**

Faculty of Health Sciences, University of Buea, Buea, Cameroon
**Correspondence to elroypat@yahoo.co.uk*

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ABSTRACT

Paediatric metabolic disorders with the most clinical manifestations of deranged hepatic metabolism are discussed. The conditions which will be stressed are those for which effective treatment is available and early diagnosis is essential. Accurate diagnosis of other disorders for which no treatment is, as yet, available is also important as a guide to prognosis and for accurate genetic counselling. With the advancement in amniocentesis techniques there is a growing role for gene therapy. For selected metabolic disorders, paediatric liver transplantations have been successful.

Keywords: Metabolic disorders, liver, amniocentesis, paediatric, transplantation.

INTRODUCTION

Many inborn errors of metabolism (IEM) are recessive diseases. Recessive conditions are rare, as these disorders are only present when an individual is homozygous for a gene from unaffected heterozygous 'carrier' parents (1 in 4 chance). The overall incidence of autosomal recessive (AS) disorders is about 2.5 per 1,000 live births in the United Kingdom. Worldwide, diseases such as thalassaemia and sickle cell disease are very common and the frequency may be as high as 20 per 1,000 live births.[1] The clinical features of AS disorders are usually severe; patients often present in the first few years of life and have a high mortality. This is unlike dominant inheritance where some traits are 'non-penetrant'. Prenatal diagnosis is becoming possible in suspected (family history) IEM by obtaining specimens of amniotic cells for cytogenetics or enzyme studies. Carrier states can also be identified, so that sensible genetic counselling can be given.[2,3] Liver transplantation (LTx) may not only replace the diseased organ but can also potentially correct the metabolic defect. Both graft and patient survival for adults transplanted for metabolic liver disease is similar to that of other indications for liver transplant, as seen in the 80-90% 1-year survival after paediatric LTx for chronic liver disease (Tables 1 and 2). The controversy is whether LTx should be considered when the disorder is associated with severe impairment of other organ(s).[4-6]

ABNORMALITIES OF METAL METABOLISM

Wilson's Disease (WD)

WD is a very rare IEM associated with the accumulation of toxic amounts of copper in the liver, brain, kidneys, and cornea. It is inherited as an AS gene and occurs worldwide, particularly in countries where consanguinity is common. WD is caused by mutations in the *ATP7B* gene which leads to abnormal functioning copper transporting ATPase, decreased hepatocellular excretion of copper into bile, and failure to incorporate copper into caeruloplasmin.[7] Stains for copper usually show a periportal distribution associated with lipofuscin deposits. In childhood, the principal presentation is a hepatic disorder which may mimic all forms of liver disease. Neurological signs of basal ganglia involvement such as clumsiness, slurring of the speech, deteriorating school performance, or changes in personality are common in young adults.[8] There is growing evidence that genetic variation in *PRNP* encoding the prion glycoprotein may modify the neurological cause of WD.[9] Although it may be absent in young children and not specific to WD, the Kayser-Fleischer ring, which is due to copper

deposition in Descemet's membrane in the cornea, is identified frequently by slit-lamp examination.[7] Haemolytic anaemia, vitamin D resistant rickets, renal rickets, or the Fanconi syndrome (generalised renal tubular reabsorptive defects) may also be the first indications of disease. The diagnosis depends on the measurement of the amount of copper in the liver, although high levels of copper are found in other chronic cholestatic disorders such as sclerosing cholangitis. Measurement of ^{64}Cu incorporation may be helpful as the liver copper is elevated to 25-times the upper limit of normal, except in the presence of advanced cirrhosis when it may fall within the normal range.[7,10] The serum copper and caeruloplasmin are usually reduced, due to stability of apoceruloplasmin from the high liver copper level.[11] Untreated, WD is invariably fatal. Chelation therapy with D-penicillamine (20 mg/kg/day) and trientine (20 mg/kg/day) induces cupruria leading to clinical and biochemical improvement. Treatment given when fulminant hepatic failure or decompensated cirrhosis is established is rarely successful. Toxic side-effects of the drugs are unusual and include skin rashes, leukopenia, and renal damage. Inducing metallothionein and blocking intestinal absorption of copper elemental zinc (50 mg, three times per day) is useful in the initial stages. A diet low in copper should also be given. Tetrathiomolybdate is a chelator and blocker of copper absorption but still experimental.[7] Early diagnosis and effective treatment have improved the prognosis. Neurological damage is, however, permanent and death is from liver failure, bleeding varices, or intercurrent infection.[2] All siblings and children of patients should be biochemically or genetically screened. LTx is considered for fulminant WD with acute liver failure, decompensated cirrhosis, and disease progression despite medical therapy. Transplantation for neurological symptoms without severe liver disease is controversial.[4-6]

Indian Childhood Cirrhosis

This condition of children is seen in the Indian subcontinent and it is still not clear whether this condition has a major genetic component in which copper metabolism is abnormal in these children, or whether the copper loading of the liver results from increased dietary intake. The liver pathology in this condition is characterised by the presence of a fine, micronodular cirrhosis associated with typical hyaline bodies (Mallory's bodies) identical to those which are seen in alcoholic hepatitis.

Table 1: Paediatric liver metabolic diseases: indications for liver transplantation.

Wilson's disease	• **Fulminant Wilson's disease with acute liver failure** • **Decompensated cirrhosis** • **Disease progression despite medical therapy** • **Neurological symptoms without severe liver disease (controversial).**
Hereditary haemochromatosis	• Transplant if decompensated cirrhosis • Risks of transplantation are increased due to associated cardiomyopathy and post-transplant sepsis.
Alpha-1-antitrypsin deficiency	• 15% develop liver failure by adolescence • Transplant if decompensated cirrhosis or liver failure • 5-year post-transplant survival 80%.
Cystic fibrosis	• 15% develop cirrhosis • Risks of transplantation increased by associated cardiopulmonary disease, malnutrition, and chronic infection.
Hereditary tyrosinaemia (Type 1)	• Transplantation considered early in childhood to prevent hepatocellular carcinoma (develops in a third by 2 years).
Primary hyperoxaluria	• Characterised by nephrocalcinosis and renal failure • Treated by combined liver and renal transplantation.
Urea cycle defects	
Glycogen storage diseases Types 1 and 4	

Table 2: Prerequisites for successful paediatric liver transplantation.

• Donor and recipient should be ABO blood group compatible, but preferably ABO matched
• Donor age <50 years
• Satisfactory donor liver function
• Negative donor virology for hepatitis A, B, C, and HIV
• Appropriate size-match or surgically size reduced graft
• 80-90% 1-year survival after liver transplantation for chronic liver disease

The hepatic copper level is markedly increased but without the periorbital distribution seen in WD. Furthermore, there is insufficient evidence to determine whether removal of copper has any effect on the progression of liver damage.[1,12]

Haematochromatosis

Idiopathic haemochromatosis (IHC) is a relatively rare inherited disease characterised by excess iron deposition in various organs, leading to eventual fibrosis and functional organ failure. Iron loading of the liver cells leads to hepatocellular damage and fibrosis. It is inherited as an AS with only homozygotes manifesting the clinical features of the disease. It is associated with HLA-A3 and B14 and may be used for screening relatives of patients for the disease.[1,13] IHC is best diagnosed by liver biopsy as it defines the extent of tissue damage, assesses tissue iron, and measures the hepatic iron concentration. The pathology is similar for idiopathic or from secondary iron overloading states such as sideroblastic anaemias, thalassaemia with multiple transfusions, and alcoholic cirrhosis. Cirrhosis with nodularity is a late feature. It is becoming increasingly apparent that the liver iron content (>180 µmol/g dry weight) is the most sensitive marker of the disease as the amount of iron in the biopsy correlates well with the total body iron load.[14] It is possible to monitor therapy with repeated biopsies and quantitative iron techniques using magnetic resonance imaging.[15] The serum ferritin (SF) is elevated (usually >500 µg/l) and is evidence of excessive parenchymal deposition. Liver function tests are often normal, even with established cirrhosis.[3,14] Although most affected individuals present clinically in the fifth decade, it is important to screen all first-degree relatives with the SF test to detect early and asymptomatic disease in all cases of IHC. 30% of patients with cirrhosis will develop primary hepatocellular carcinoma (HCC) and may be the mode of presentation. There is excessive excretion of iron following administration of chelating agents and, along with long-term venesection and monitoring, cirrhosis may be prevented.[13,14] LTx is indicated for decompensated cirrhosis, but the risks are increased due to associated cardiomyopathy and post-transplant sepsis.[6]

DISORDERS OF CARBOHYDRATE METABOLISM

Paediatric Non-Alcoholic Fatty Liver Disease (NAFLD)

NAFLD is the leading cause of chronic liver disease in children.[16,17] NAFLD has emerged to be extremely prevalent, and predicted by obesity and male gender. Both genetic and environmental factors seem to be involved in the development and progression of the disease, but its pathophysiology is not entirely clear. It is defined by hepatic fat infiltration >5% hepatocytes, in the absence of other causes of liver pathology.[16] It includes a spectrum of disease ranging from intrahepatic fat accumulation (steatosis) to various degrees of necrotic inflammation and fibrosis (non-alcoholic steatohepatatis). NAFLD is associated, in children as in adults, with severe metabolic impairments, determining an increased risk of developing the metabolic syndrome. It can evolve to cirrhosis and HCC, with the consequent need for LTx.[17]

Glycogen Storage Disease (GSD)

All mammalian cells can manufacture glycogen, but the main sites of its production are the liver and muscle. Glycogen is a high molecular weight glucose polymer, and in GSD there is either an abnormality in the molecular structure or an increase in glycogen concentration due to a specific enzyme defect. The majority are inherited

as ASs except for subtype 4 (Hers disease) which is sex-linked.[1,18] They present in infancy except for Type 5 (McArdle disease) disease affecting muscle only which presents in adults. Table 3 shows the classification and clinical features. 16 novel pathogenic mutations in the phosphorylase system (*PHKA2*, *PHKB*, *PHKG2*, and *PYGL*) genes have given rise to GSD Types 6 and 9 and close monitoring for the long-term liver and cardiac complications are important.[19] All these conditions are associated with inefficient glycogen utilisation which leads to hypoglycaemia and glycogen deposition in various tissues. Hepatocellular adenoma is associated with GSD Type 1, 3, and 4 and is a common reason for consulting the paediatric hepatologist.[20] Mortality and morbidity in the early years is high unless the risk of acute episodes of hypoglycaemia and lactic acidosis is recognised and an efficient regimen to maintain normoglycaemia throughout the whole 24-hour period is instituted. This has usually been done by 2-hourly feeds of glucose and starch, the latter as a slow release source of glucose. Nasogastric feeding with glucose at night is a major advance. As the other biochemical parameters also improve, there is catch-up growth and normal somatic development. However, it should be emphasised that the successfully treated patients lose their ability to withstand hypoglycaemia. The crisis associated with intercurrent infections and other stressful situations require intravenous glucose and sodium bicarbonate with close biochemical monitoring as well as vigorous treatment of the precipitating cause. Paediatric LTx may be indicated for chronic liver disease from Types 1 and 4 glycogenoses.[4-6] There is a need for future studies to ascertain if uncooked cornstarch and a high protein diet would be able to prevent long-term complications of GSD-6 and 9.[19]

Galactosaemia

As galactose is normally converted to glucose, the less common deficiency of the enzyme galactose-1-phosphate uridyl transferase results in accumulation of galactose-1-phosphate in the blood. This autosomally recessive trait results in hypoglycaemia and acidosis in the neonate.[1] Progressive hepatosplenomegaly, cataracts, renal tubular defects, and mental retardation occur.[21] Early hepatic changes include fatty infiltration and hepatic necrosis proceeding to pseudoglandular transformation of hepatocytes, hepatic fibrosis, and cirrhosis in patients who survive. Treatment is with a galactose-free diet, which if started early, results in normal development. Untreated patients die within a few days. Prenatal diagnosis and diagnosis of the carrier state are possible by measurement of the level of galactose-1-phosphate in the blood.[3,22]

Fructosaemia

Absorbed fructose is chiefly metabolised in the liver to lactic acid or glucose. Three defects of metabolism occur and all are inherited as AS traits. Fructosuria, a benign condition, is due to a fructokinase deficiency. Fructose intolerance is due to fructose-1-phosphate aldolase deficiency caused by mutations of the *ALDOB* gene located at 9q22.3. Fructose-1-phosphate accumulates after fructose ingestion, resulting in symptoms of hypoglycaemia. Hepatomegaly and renal tubular defects occur but are reversible on a fructose-free diet.[3] Fructose 1,6-diphosphate deficiency leads to a failure of gluconeogenesis, and hepatomegaly. Steatosis and necrosis of hepatocytes in the early stages progresses to intralobular fibrosis, and ultimately cirrhosis, and its complications. Vomiting and hepatomegaly are almost always present as is a failure to thrive. In the first 2 months of life jaundice and a bleeding diathesis with deranged coagulation is found. The diagnosis is confirmed by the regression of symptoms when fructose is withdrawn from the diet and the demonstration of low activity of fructose-1-phosphate aldolase in liver or intestinal mucosa. If the patient survives, and fructose and sucrose are excluded from the diet, progress is excellent with regression of the liver damage.[1,23]

Congenital Disorder of Glycosylation Type 2A

It is a very rare inherited metabolic disorder where a GLcNAc transferase 2-enzyme defect causes defective carbohydrate compounds to be attached to glycoproteins and thus impairing their function.[24] The symptoms include dysmorphic features, psychomotor retardation, hypotonia, underdeveloped cerebellum, and seizures. Mannose supplementation may relieve the symptoms although hepatic fibrosis may persist.[25]

AMINO ACID DISORDERS

Hereditary Tyrosinaemia

This disorder is characterised by the accumulation in serum and urine of tyrosine and its metabolites. This is caused by toxic metabolites which accumulate because of deficiency of

fumarylacetoacetase, the last enzyme in the tyrosine catabolic pathway. It is associated with severe liver damage, initially in the form of fatty infiltration but proceeding to cirrhosis. Rickets and hypoglycaemia are frequent complications, and galactosaemia and fructosaemia are main differentials.[26] In its acute form the disease presents in the first 6 months of life with vomiting, diarrhoea, hepatosplenomegaly, ascites, a bleeding diathesis, and severe failure to thrive. The diagnosis is suspected on the basis of clinical features and a low urine succinyl acetone is a diagnostic marker of this disease. This test can be used as part of neonatal screening along with the grossly abnormal liver function tests, and raised serum alpha-fetoprotein levels. 2-[2-nitro-(trifluoromethyl) benzoyl]-cyclohexanedione-1,3-dione is a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase and has been shown to efficiently prevent tyrosine degradation and production of succinylacetone.[27] Treatment includes restriction of dietary tyrosine, phenylalanine, and methionine and their careful plasma monitoring, correction of hypoglycaemia and electrolyte imbalance, and large doses of vitamin D to heal the rickets. Although treatment corrects the abnormal biochemical findings, most cases die in infancy or early childhood from liver failure.[1,26] LTx should be considered early in childhood to prevent HCC, which develops in a third of patients by 2 years.[4-6]

UREA-CYCLE DISORDERS

Genetically determined deficiency has been identified for five separate enzymes in the liver involved in the conversion of ammonia to urea. The diagnosis is suspected by the finding of a high blood ammonia, but established by the demonstration of deficiency of the particular enzyme involved.[3] The principal pathological change is brain damage, but the liver usually shows an increase in fat and glycogen. The clinical features include a dislike of protein-containing food and vomiting, and irritability or lethargy following ingestion of such foods. Dietary protein restriction beginning immediately after birth should therefore be the basis of their treatment. The protein requirement is highest during the first months of life and a balance has to be struck between providing sufficient protein for growth and control of hyperammonaemia.[1,28] Paediatric LTx may be indicated in these urea cycle defects.[6]

Table 3: Glycogen storage diseases

Type	Affected tissue	Enzyme defect	Clinical features	Tissue needed for diagnosis*	Outcome
1 (Von Gierke's disease)	Liver, intestine, kidney	Glucose-6-phosphatase	Hepatomegaly, hypoglycaemia, stunted growth, obesity, hypotonia	Liver	If patients survive initial hypoglycaemia, prognosis is good; hyperuricaemia is a late complication
2 (Pompe's disease)	Liver, muscle, heart	Lysosomal α-glucosidase	Heart failure, cardiomyopathy	Leukocytes, liver, muscle	Death in first 6 months; juvenile and adult variants seen
3 (Forbes' disease)	Liver, muscle (abnormal glycogen structure)	Amylo-1, 6-glucosidase	Like Type I	Leukocytes, liver, muscle	Good prognosis
4 (Andersen disease)	Liver (abnormal glycogen structure)	1,4-α-glucan branching enzyme	Failure to thrive, hepatomegaly, cirrhosis and its complications	Leukocytosis, liver, muscle	Death in first 3 years
5 (McArdle disease)	Muscle only	Phosphorylase	Muscle cramps and myoglobinuria after exercise (in adults)	Muscle	Normal lifespan; exercise must be avoided

*tissue obtained is used for the biochemical assay of the enzyme.

GLYCOPROTEIN DISORDERS

Hepatic Aspects of Alpha-1 Antitrypsin (A1AT) Deficiency

A1AT inhibits tissue damaging serine proteases and over 30 alleles of A1AT can now be distinguished by the absence of the alpha-1 band in isoelectric focusing. The gene is located on the long arm of chromosome 14 (14q 31-32). These are inherited as an AS disorder with co-dominant expression as each allele contributes 50% of the total circulating enzyme inhibitor.[1,3] In the deficient individual uninhibited action of proteases may cause progressive liver disease. A1AT deficiency was first discovered in the 1960s in a family with early-onset emphysema. Liver disease occurs in individuals who are homozygous for the variant (ZZ) (incidence 1:3,400 in the UK), or who do not have glycoprotein in their serum (Pi null phenotype). Antenatal diagnosis is possible by foetal blood sampling at 17-week gestation, at which time termination of the pregnancy is possible. Genetic counselling poses particular difficulties because not all ZZ foetuses will develop liver or lung disease.[29] Liver disease is usually first detected in infancy by the appearance of a conjugated hyperbilirubinaemia with pale stools, dark urine, hepatomegaly, splenomegaly, and failure to thrive. 25% of jaundiced infants die of cirrhosis in early childhood, while 40-50% have chronic persisting liver disease with features of compensated cirrhosis.[30] At present, no treatment has been shown to modify liver disease associated with A1AT deficiency but recent data suggest the autophagy (process that removes abnormal proteins in cells) - enhancing drug, carbamazepine, to be beneficial.[31] Hepatoma may occur as a complication of the cirrhosis and LTx is indicated in the 10-15% with PiZZ phenotype who develop features of decompensation in late childhood or early adolescence. The 5-year post-transplant survival is 80%.[4-6]

Hepatic Aspects of Cystic Fibrosis (CF)

CF is the commonest recessively inherited disorder (abnormality of chromosome 7) in Caucasians, with a carrier frequency of 1 in 20-25. In America, Western Europe, and Australia, 1 in 2,000 births are homozygous for the CF gene, but the carrier frequency in mongoloid races is considerably less and CF is unknown in blacks.[1,3] It is a genetically determined disorder of exocrine secretory glands which causes them to produce tenacious viscoid secretions. The abnormal mucus stagnates in small ducts, causing destruction of cells draining into or associated with them, a process aggravated by infection. The respiratory system and pancreas are principally involved, with a high sweat sodium concentration over 60 mmol/l, but other organs with mucus-producing glands in their duct systems, such as the biliary system, are also affected.[32] The main cause of chronic liver disease is thought to be inspissated biliary secretions causing focal biliary obstruction and fibrosis, and in 20% of cases, the gall bladder is small with an inability to concentrate bile. Drugs, infection, and abnormal immune mechanisms have all been implicated in causing chronic liver damage. A biliary type of cirrhosis may appear in the first year of life, but more often becomes evident in late childhood or early adolescence. Obstructive jaundice is rare and only a minority of patients die of liver disease in the first few years of life. Decompensated cirrhosis with ascites is rare except when *cor pulmonale* develops.[3,32] No treatment which influences the underlying process is available. Symptomatic treatment with ursodeoxycholic acid is useful in the short term. There may be genetic modifiers of CF-associated liver disease in the future.[33] LTx is indicated for the 15% who develop cirrhosis, but the risks are increased by associated cardiopulmonary disease, malnutrition, and chronic infection.[6]

LYSOSOMAL STORAGE DISEASES (LSDS) (SPHINGOLIPIDOSES)

LSDs are due to IEM which are inherited in a sex-linked recessive manner. Sphingolipids are degraded by a series of lysosomal enzymes, and accumulate when there is a deficiency of these enzymes. Many of the sphingolipidoses can be diagnosed by demonstrating the enzyme deficiency in the appropriate tissue. Enzyme replacement therapy for lysosomal acid lipase deficiency, although not a cure, can allow improved metabolism and partially prevent disease progression, as well as potentially reverse some symptoms.[34]

Fabry's Disease (FD)

FD is a rare genetic lysosomal storage disease, inherited in an X-linked manner, involving dysfunctional metabolism of sphingolipids due to an alpha-galactosidase A deficiency causing a wide range of systemic symptoms. It causes glycolipid, and globotriaosylceramide (Gb3, GL-3) to accumulate within the blood vessels, other tissues,

and organs, leading to an impairment of their functions. There is potential replacement therapy with Fabrazyme (agalsidase beta), although most patients eventually develop renal problems in early adult life.[35]

Gaucher's Disease (GD)

In GD there is an accumulation of glucocerebroside in the reticuloendothelial system, particularly the liver, bone marrow, and spleen. There is a high incidence in Ashkenazi Jews (1 in 3,000 births). Acute GD presents in infancy or childhood with rapid onset of hepatosplenomegaly with neurological involvement, due to Gaucher's cells in the brain. The outlook is poor but treatment with recombinant glucocerebrosidase may be beneficial.[36]

Niemann-Pick Disease

Nieman-Pick Type 2 is the second most common genetic cause of liver disease in infancy in the UK after A1AT deficiency. This is due to the accumulation of sphingomyelin and foam cells in reticuloendothelial macrophages in many organs particularly the liver, spleen, bone marrow, and lymph nodes. Over 50% of cases present with hepatitis in infancy or intrauterine ascites. A third of these die by 6 months, while the survivors unfortunately develop features of progressive, ultimately fatal, neurological involvement from 2 years of age. LTx does not arrest the disease but diagnosis is essential for genetic counselling. Prenatal diagnosis by amniocentesis is possible and success derived in the experimental use of (2-hydroxypropyl)-β-cyclodextrin.[37]

CONCLUSIONS

Paediatric metabolic disorders arise invariably from IEM, which are mostly inherited in an AS manner. The implications are that they are rare but lethal as it is manifested in the homozygote form. For selected metabolic disorders, paediatric LTx have proved successful. With the advancement in amniocentesis techniques, there is a growing role for gene therapy.

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REFERENCES

1. Schwarzenberg SJ, Sharp HL. Pediatric gastroenterology. Update on metabolic liver disease. Pediatr Clin North Am. 1996;43(1):27-56.

2. Galton DJ (ed.), Molecular genetics of common metabolic disease (1985), Edward Arnold: London.

3. Olpin SE. Metabolic disorders presenting as liver disease. Paediatr Child Health. 2010;20:1-6.

4. Burdelski M, Rogiers X. Liver transplantation in metabolic disorders. Acta Gastroenterol Belg. 1999;62(3): 300-5.

5. Mazariegos G et al. Liver transplantation for pediatric metabolic disease. Mol Genet Metab. 2014;111(4):418-27.

6. Zhang KY et al. Liver transplantation for metabolic liver disease. Clin Liver Dis. 2007;11:265.

7. Roberts EA, Schilsky ML; American Association for Study of Liver Diseases (AASLD). Diagnosis and treatment of Wilson disease: an update. Hepatology. 2008;47(6):2089-111.

8. Sampson EL et al. Young onset dementia. Postgrad Med J. 2004;80: 125-39.

9. Forbes N et al. Evidence for synergistic effects of PRNP and ATP7B mutations in severe neuropsychiatric deterioration. BMC Med Genet. 2014;15:22.

10. Brewer GJ, Askari FK. Wilson's disease: clinical management and therapy. J Hepatol. 2005;42 Suppl(1):S13–21.

11. Holtzman NA, Gaumnitz BM. Studies on the rate of release and turnover of ceruloplasmin and apoceruloplasmin in rat plasma. J Biol Chem. 1970;245(9): 2354-8.

12. Tanner MS. Role of copper in Indian childhood cirrhosis. Am J Clin Nutr. 1998;67(5 Suppl):1074S-1081S.

13. Crownover BK, Covey CJ. Hereditary hemochromatosis. Am Fam Physician. 2013;87(3):183–90.

14. Andrews NC. Disorders of iron metabolism. N Engl J Med. 1999;341(26):1986–95.

15. Tziomalos K, Perifanis V. Liver iron content determination by magnetic resonance imaging. World J Gastroenterol. 2010;16(13):1587-97.

16. Day CP. Non-alcoholic fatty liver disease: a massive problem. Clin Med. 2011;11:176-8.

17. Giorgio V et al. Pediatric non alcoholic fatty liver disease: old and new concepts on development, progression, metabolic insight and potential treatment targets. BMC Pediatr. 2013;13:40.

18. Smit GPA et al, "The Glycogen Storage Diseases And Related Disorders," Fernandes J et al (eds.), Inborn metabolic diseases: diagnosis and treatment (2006) 4th edition, Springer Berlin Heidelberg, pp. 101–19.

19. Beauchamp NJ et al. Glycogen storage disease type IX: high variability in clinical phenotype. Mol Genet Metab. 2007;92:88–99.

20. Alshak NS et al. Hepatocellular adenoma in glycogen storage disease type IV. Arch Pathol Lab Med. 1994;118(1):88-91.

21. Murphy M et al. Genetic basis of transferase-deficient galactosaemia in

Ireland and the population history of the Irish Travellers. Eur J Hum Genet. 1999;7(5):549–54.

22. Berry GT et al, "Disorders Of Galactose Metabolism," Fernandes J et al (eds.), Inborn metabolic diseases: diagnosis and treatment (2006) 4th edition, Springer Berlin Heidelberg, pp. 121-30.

23. Steinmann B, Santer R, "Disorders of Fructose Metabolism," Saudubray JM et al (eds.), Inborn metabolic diseases: diagnosis and treatment (2012) 5th edition, Springer Berlin Heidelberg, pp. 157–65.

24. Haeuptle MA, Hennet T. Congenital disorders of glycosylation: an update on defects affecting the biosynthesis of dolichol-linked oligosaccharides. Hum Mutat. 2009;30:1628-41.

25. Mention K et al. Development of liver disease despite mannose treatment in two patients with CDG-Ib. Mol Genet Metab. 2008;93:40-3.

26. Chakrapani A, Holme E, "Disorders Of Tyrosine Metabolism," Fernandes J et al (eds.), Inborn metabolic diseases: diagnosis and treatment (2006) 4th edition, Springer Berlin Heidelberg, pp. 233-43.

27. Holme E, Lindstedt S. Tyrosinaemia type I and NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione). J Inherit Metab Dis. 1998;21(5):507-17.

28. Stanbury JB et al. (eds.), The metabolic basis of inherited disease (1982) 5th edition, McGraw-Hill: New York.

29. Fairbanks KD, Tavill AS. Liver disease in alpha 1-antitrypsin deficiency: a review. Am J Gastroenterol. 2008;103:2136-41.

30. Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. N Engl J Med. 1976;294(24):1316-21.

31. Puls F et al. Autophagy-enhancing drug carbamazepine diminishes hepatocellular death in fibrinogen storage disease. J Hepatol. 2013;59:626-30.

32. Herrmann U et al. Cystic fibrosis-associated liver disease. Best Pract Res Clin Gastroenterol. 2010;24(5):585-92.

33. Colombo C. Liver disease in cystic fibrosis. Curr Opin Pulm Med. 2007;13(6):529-36.

34. Du H et al. Enzyme therapy for lysosomal acid lipase deficiency in the mouse. Hum Mol Genet. 2001;10(16): 1639-48.

35. Wilcox WR et al; International Fabry Disease Study Group. Long-term safety and efficacy of enzyme replacement therapy for Fabry disease. Am J Hum Genet. 2004;75(1):65-74.

36. Tekoah Y et al. Glycosylation and functionality of recombinant β-glucocerebrosidase from various production systems. Biosci Rep. 2013;33(5)pii:e00071.

37. Ottinger EA et al. Collaborative development of 2-hydroxypropyl-β-cyclodextrin for the treatment of Niemann-Pick type C1 disease. Curr Top Med Chem. 2014;14(3):330-9.

DIAGNOSIS AND MANAGEMENT OF OCCULT HEPATITIS B VIRUS INFECTION: A SHORT REVIEW

Javier Martínez González, *José Luis Lledó Navarro, Enrique Rodríguez de Santiago, Agustín Albillos Martínez

Gastroenterology and Hepatology Service, Hospital Universitario Ramón y Cajal, Madrid, Spain
**Correspondence to jllledo63@yahoo.es*

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ABSTRACT

Occult hepatitis B virus infection (OBI) is a challenging clinical entity. It is defined as the presence of viral DNA in the liver or serum of subjects who test negative for the hepatitis B surface antigen. Molecular evidence of OBI consists of covalently closed circular DNA persisting in the nuclei of hepatocytes after infection. Immunocompetent individuals have a lower risk of complications than immunosuppressed subjects. However, under certain scenarios, OBI acquires clinical manifestations that include transmission of the infection via blood or organ transplantation, chronic liver disease progression, hepatocellular carcinoma, and virus reactivation when a state of immunosuppression develops. This review updates the clinical aspects of the diagnosis and management of OBI.

Keywords: Occult hepatitis B virus infection, diagnosis, management.

INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem. Estimates indicate that around a third of the world's population have past or present HBV infection with 350 million persons chronically infected.[1] The clinical spectrum of chronic HBV infection ranges from a healthy carrier state to more advanced liver disease (LD), such as cirrhosis or hepatocellular carcinoma (HCC).[1,2] HBV infection is effectively the leading cause of cirrhosis and HCC worldwide.[2] During the natural course of HBV infection, constant interaction between host factors (immune system) and the virus serves to explain the different stages of HBV infection: immune tolerance, immune clearance, inactive carrier state, reactivation stage, and hepatitis B surface antigen (HBsAg)-negative (occult) stage. These stages are not necessarily unidirectional, sequential, and stable, and any changes in the immune system and/or HBV can modify the state of infection.[1-3] The stages leading to occult HBV infection (OBI) have been widely addressed. This review focuses on the occult stage of HBV infection and discusses present knowledge of its definition, diagnosis, clinical scenarios, and management.

DEFINITION

OBI is defined as the presence of the HBV genome in the liver tissue of patients, with detectable or undetectable serum HBV DNA, who test negative for HBsAg.[4] The molecular rationale for OBI is the conversion of HBV DNA to covalently closed circular DNA (cccDNA). After binding to different proteins, cccDNA becomes a stable and durable mini-chromosome that persists indefinitely within hepatocyte nuclei.[5]

Two different serological patterns of OBI have been defined according to detected serum markers of HBV.[4] This includes seropositive OBI, which represents 80% of cases. This term is used to describe OBI patients who test positive for the anti-hepatitis B core antibody (anti-HBc) and/or for the anti-hepatitis B surface antibody (anti-HBs), yet lack detectable HBsAg in serum. This situation may arise when acute hepatitis B resolves after months of HBsAg carriage, or when, after years of chronic HBsAg positive infection, the patient tests negative for the antigen. Along with seronegative OBI, which represents 20% of cases. These patients test negative for both antibodies (anti-HBc and anti-

HBs) and usually only very low levels of HBV DNA are detected. This serological pattern may reflect the time between infection and the detection of antibodies known as the 'window period' (pre-seroconversion), or clearance of hepatitis B antibodies. This seronegative OBI pattern should always be considered, since any person lacking serum HBV antigens or antibodies could be a seronegative OBI patient.

PREVALENCE

The prevalence of OBI is difficult to assess and varies according to factors such as: endemic disease level, sub-population examined, the methods used to assess OBI, or tissue tested (liver versus serum).[6] In the following sections, we describe reported OBI prevalence for the different populations examined to date.

The transmission route for HBV and hepatitis C virus (HCV) is similar in that a high prevalence of OBI may be expected in HCV patients. Effectively, HCV patients show the highest reported prevalence of OBI. In one study, it was detected that 33% of HCV patients had OBI compared to 14% of controls.[7] However, the impact of OBI in patients with HCV remains unclear. It has so far been established that OBI worsens the clinical course of HCV infection, as more inflammatory activity, more fibrosis, and an increased rate of cirrhosis and HCC have been observed in HCV patients with OBI.[8,9]

Risk factors for a haemodialysis patient to develop OBI include an increased number of blood transfusions, frequent invasive procedures, and immunosuppression. Several studies have shown that 0-36% of patients on haemodialysis, and nearly 10% of patients on continuous ambulatory peritoneal dialysis, suffer from OBI.[6,10] Reported OBI prevalence in HIV patients have been 0-89%.[11] This wide range reflects difficulties in assessing OBI. The pathological explanation could be cellular immune deficiency (decreased CD4).[11] There is little evidence linking LD of unknown origin to OBI. However, some authors report that 19-31% of patients with cryptogenic LD present with OBI.[12,13] OBI prevalence in blood donors varies among the Unites States, Europe, and Asia. In Europe, despite improvements in screening for blood donation, HBV DNA is detected in 0-1.6% of cases.[6] Thus, HBV remains the most frequent transfusion-transmitted viral infection.

Few studies have examined this issue in the general population. In one study, OBI was detected in 18% of subjects with serological evidence of previous HBV infection and in 8% of HBV seronegative individuals.[13] Other authors have reported similar prevalence. Despite a lack of data, we would expect high variation among different populations.

DIAGNOSIS

OBI is diagnosed when HBV DNA is detected in the liver or in blood samples of patients who test HBsAg negative.[2,4] Although the gold standard is liver tissue testing, this is not usually feasible; most often the diagnosis of OBI is based on the results of a blood test.[14] However, there is frequent discrepancy between the detection of HBV DNA in the liver or blood. Usually, when a blood sample tests positive for HBV DNA, a liver sample will also return a positive result, but the reverse is not always true. Thus, HBV DNA may be detected in the liver, but not necessarily in blood. Of course, since HBV DNA occurs inside the nuclei of hepatocytes, if a liver sample is negative for HBV DNA, a blood sample will also be negative.[14]

It is important to define the optimal methodology to quantify HBsAg and HBV DNA.[14-16] Most HBsAg commercial assays are able to detect all genotypes and subtypes of the wild-type virus, but some may miss mutations in the S region. Hepatologists should bear this in mind, because some patients might be diagnosed with OBI, when they only have an undetectable mutation in their HBsAg (false OBI).[16] To detect HBV DNA, it is very important to use a highly sensitive and specific assay because OBI is usually associated with low levels of HBV DNA. An international consensus[3] introduced a cut-off value for serum HBV DNA (<200 IU/ml). This means that cases of individuals whose HBV DNA levels are similar to those with evident overt HBV infection are generally due to infection with HBV-escape mutants and should be labelled as 'false OBI'.[2] It is recommended that the assay has a detection limit of <10 copies/ml. With current technologies (nested-polymerase chain reaction (PCR), real time-PCR, and transcription based mediated amplification), it is possible to reduce the lower detection limit to >5 copies/ml, which clearly improves sensitivity.[15,16]

The use of anti-HBc antibody for OBI diagnosis has been addressed in some studies. This is the first antibody to appear. It is considered a sign of active or past infection depending on the other HBV

serum markers. It can be found in almost every patient with a previous contact with HBV, even in HBV carriers without other responses.[17] Despite not being an ideal marker, the risk of occult hepatitis associated with anti-HBc seropositivity has been demonstrated extensively, and the presence of anti-HBc antibodies can be considered a sentinel marker of occult HBV infection.[18] In fact, it has been recommended by a panel of experts as a surrogate marker to identify potential seropositive OBI patients when sensitive HBV DNA tests are not available.[4] Hepatologists should nonetheless be aware that the absence of this antibody (anti-HBc) does not rule out OBI (seronegative-OBI).

MANAGEMENT

The clinical significance of OBI has not been fully established. However, OBI should be carefully assessed in certain clinical scenarios: HBV infection transmission (via blood transfusion or solid organ transplantation), LD progression, HCC onset, and HBV reactivation (Figure 1).

Risk of Transmission

Blood transfusion. The introduction of sensitive assays has meant that HBV transmission through blood transfusion is a rare event. However, some subjects who screen negative for HBV in the usual serum marker test, could be at risk for HBV transmission. These are: a) seronegative OBI patients (who test negative for all antigens and antibodies, yet have DNA detectable in blood), and b) patients infected with an S-escape mutant virus that is able to actively replicate but whose mutant HBsAg is not detected by routinely available diagnostic assays. This is the most frequent situation leading to cases of hepatitis B related to blood transfusion.[19]

Today, OBI is the main cause of post-transfusion hepatitis B. Hence the risk of transmission is probably greater than for HCV or HIV. However, not all patients transfused with blood containing HBV DNA will suffer from hepatitis B, due to previous vaccination of the recipient, immune complexes, presence of defective virions, transient periods of viraemia in OBI patients, etc. Recipients with anti-HBs antibodies make the possibility of transmission insignificant, although the cut-off level of these antibodies is still a matter of debate.[20]

Some studies have shown that the frequency of serum HBV DNA positivity in HBsAg negative donors is related to HVB prevalence, which differs between countries and clearly affects serum markers for screening.[20] The management of these patients and ways to screen samples differ between world regions, and the type of suspect patient.

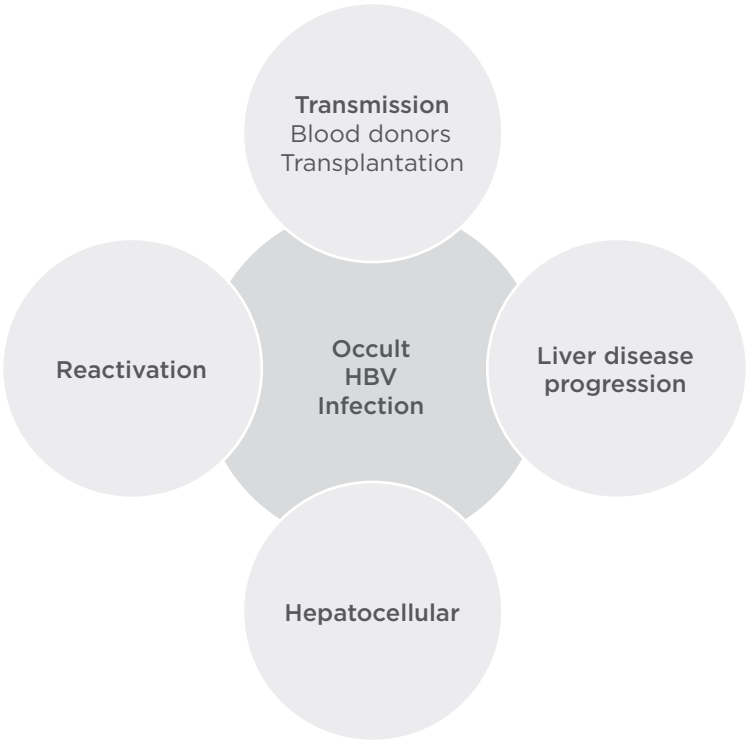

Figure 1: Current clinical scenarios related to occult hepatitis B virus (HBV) infection.

The use of anti-HBs antibodies in HBsAg assays is strongly recommended to detect false OBI patients. In cases of pre-seroconversion period donation (HBsAg and anti-HBc negative), only HBV DNA detection serves to diagnose OBI. In regions where the prevalence of HBV is high (usually coincidental with low-level vaccination areas), OBI may be detected using nucleic acid amplification techniques. In countries with a low prevalence of HBV, screening based on sensitive HbsAg and anti-HBc assays appears to be sufficient.[4,19,20]

Organ transplantation. As a consequence of virus reactivation during immunosuppression, grafts from HBsAg negative and anti-HBc positive donors can transmit HBV to recipients.[21,22] This risk is particularly high in liver transplantation compared to kidney, heart, or bone marrow transplantation, and is also greater in recipients testing negative for all serum HBV markers. It is uncertain how many HBV infections following transplant are really *de novo* or transmitted from seronegative OBI donors.[21,23]

The management of these patients is not clearly established. What has been determined is that immunisation prior to transplant creates an anti-HBs antibody response that can modulate or abort infection. Moreover, prophylaxis in HBsAg-negative transplanted patients receiving livers from anti-HBc-positive donors prevents the efficient reactivation of OBI.[24] Immunoglobulin alone or in combination with lamivudine has been used for many years, although lamivudine monotherapy may be similarly efficient yet more cost-effective.[25,26] More recent evidence suggests that the new nucleotide analogs (tenofovir and entecavir) can also be used to prevent the reactivation of HBV.[27] Despite these advances, prophylaxis cannot always prevent HBV infection or reactivation, and there is much debate over whether OBI can impact the long-term outcome of orthotopic liver transplantation (OLT).[22,28] A possible role of OBI has been proposed in progression of post-OLT LD to cirrhosis in patients with HCV infection.[24]

Risk of Disease Progression

Chronic LD progression. OBI per se is thought to be inoffensive in immune-competent individuals, but if other causes of LD co-exist, then minimal lesions produced by OBI infection might negatively influence the outcome of the disease.[8,9]

OBI is observed in patients with cryptogenic chronic LD, suggesting its possible aetiologic role.[12,13] HBV genomes may persist over time in the liver of subjects who have recovered from self-limited acute hepatitis.[29,30] It has been reported that these OBI patients show normal liver enzyme activity, but their biopsies show a mild necroinflammation that may persist for years and possibly lead to liver fibrosis and the development of cryptogenic cirrhosis.[6] In effect, the results of a recent meta-analysis suggest that OBI is linked to disease progression in HCV cirrhotic patients, enhanced inflammatory activity, augmented fibrosis and cirrhosis, higher anti-HCV antibodies titres, a reduced sustained virological response, and an increased risk of HCC. In addition, more LD-related deaths observed in HCV patients with OBI than those without OBI.[8,9,31,32] However, these findings have been disputed by other authors. Thus, current available data do not support a conclusive role of OBI in LD progression or even related it to a worse outcome in patients with HCV.[6] Well-designed large prospective studies with homogeneous cohorts and uniform selection criteria are needed to determine the true impacts of OBI in HCV patients.

HCC. HBV is an oncogenic virus clearly related to HCC development. Both epidemiological and molecular studies have identified correlation between OBI and HCC. The hypothesis is that when the virus persists as OBI, both direct and indirect HBV oncogenic mechanisms are maintained. HBV acts directly via the integration of viral DNA into the host genome, and indirectly through persistent necroinflammation produced by viral replication with effects on the progression of other LDs as previously detailed.[32,33] In a recent meta-analysis, OBI emerged as an important risk factor for HCC development regardless of HCV.[33] OBI could explain HCC in patients with no known LD. However, more work is needed to elucidate the relationship between OBI and HCC development. In the meantime, there is insufficient evidence to justify testing for OBI in HBsAg-negative patients with HCC.[32-35]

HBV reactivation. Occult HBV does appear to be safe in immune-competent subjects.[32] However, these patients will be at risk when immune-suppressed.[1,36-39] Interest in this particular scenario has been triggered by the expanding use of potent immunological therapies, which can induce fulminant hepatitis with a mortality between 20-80%. According to European Association For The Study Of The Liver (EASL) guidelines, all patients scheduled for chemotherapy and/or immunotherapy must be tested for ALT and

HBV DNA before, during, and some months after treatment.[1]

A risk of virus reactivation is well documented in HBsAg-positive patients undergoing chemotherapy for onco-haematologic disease, or other immunosuppressive drugs.[1,37,38] There is consensus support that these patients require anti-HBV prophylaxis with an antiviral agent to prevent viral reactivation.[1,4] However, although less frequent, virus reactivation can also occur in patients with OBI,[32] which have HBsAg-negative. The reactivation has been linked to haemato-oncological malignancy such as leukaemias, lymphomas, and bone marrow transplantation/ haematopoietic stem cell transplantation, with 10-50% of reactivations in anti-HBc-positive patients. Chemotherapy, especially if steroids are added to any other chemotherapy schedule, is also related with a probability of reactivation between 40-75%. Potent immunosuppressive drugs (rituximab, alemtuzumab, or infliximab) are related with a risk of reactivation around 25-50% in anti-HBc positive patients. The risk of reactivation in HIV patients is low and does not, at present, justify prophylaxis.[34,38-41]

In patients with OBI testing DNA-negative and anti-HBc positive, there are insufficient data to support routine prophylaxis, and it is recommended that antiviral therapy be delayed until HBV DNA becomes detectable.[37-41] However, this type of decision should be based on each individual´s serologic pattern and treatment risk (Tables 1 and 2). A prudent approach to managing oncological patients with OBI is to initiate antiviral HBV prophylaxis therapy prior to chemotherapy. Lamivudine, despite its low genetic barrier, remains the first choice for the prophylaxis of OBI reactivation because of its low cost and the low or absent level of HBV viraemia in OBI. Prophylaxis should be continued for at least 6-12 months after stopping immunosuppressive treatment. In cases of longer treatments (over 12 months), higher HBV DNA levels, or advanced LD, entecavir, or tenofovir are the agents of choice.[42] In HBV DNA-negative, anti-HBc-positive oncological patients, surveillance testing for HBV DNA and/or HBsAg every 1/2 months is recommended. An exception to endorsing strict surveillance would be the presence of a high-risk situation, such as rituximab treatment, bone marrow stem cell transplantation, or steroids added to the chemotherapy schedule.[38-41] In these cases, the EASL guidelines suggest starting with anti-HBV drugs (lamivudine for therapy <6-12 months, and tenofovir or entecavir if treatment is anticipated to be longer or baseline HBV DNA is above 2,000 IU/ml).[1] The reason for this prudent approach when managing OBI patients and those testing HBV DNA-negative and anti-HBc positive is that antiviral therapy is usually unsuccessful after ALT becomes elevated.

Table 1: Risk treatments in reactivation of hepatitis B virus.

High-risk treatments	Medium-risk treatments
Rituximab	Anti-tumour necrosis factor drugs
Treatment of onco-haematological diseases (Non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, acute myeloid leukaemia, acute lymphoblastic leukaemia, multiple myeloma, Waldenstrom macroglobulinaemia, plasmacytoma, aplastic anaemia, myelodysplastic syndrome, bone marrow transplantation, breast cancer, lung cancer, nasopharyngeal cancer.)	Thiopurines
Use of steroids at any dose added to any chemotherapy (Cyclophosphamide, chlorambucil, cisplatin, vincristine, vinblastine, doxorubicin, epirubicin, daunorubicin, bleomycin, mitomycin C, actinomycin D, cytarabine, fluorouracil, gemcitabine, thioguanine, alemtuzumab, folinic acid, colaspase, docetaxel, etoposide, fludarabine, interferon, procarbazine.)	Isolated immunosupressive drugs (azatioprine, methotrexate)

Table 2: Management of occult hepatitis B virus infection (OBI) in patients treated with immunusuppression.

Serum markers	DNA	Diagnosis	Risk Treatment	Recommendation
HbsAg negative Anti-HBc positive	positive	OBI	High or Medium	Prophylaxis*
	negative	Past HBV	High	Prophylaxis*
			Medium	Surveillance^

*Lamivudine 2-3 weeks before initiation of immunosupressive or chemotherapy treatment, until 6-12 months after stopping treatment. In cases of longer treatment, high viral load or advanced liver disease, tenofovir or entecavir are recommended. ^Test ALT and HBV DNA every 1-2 months.
HBsAg: hepatitis B surface antigen; anti-HBc: hepatitis B core antibody; HBV: hepatitis B virus; ALT: alanine transaminase.

SUMMARY

OBI is a new challenge in virology. It is diagnosed when HBV DNA is detected in the liver or serum of patients who are HBsAg negative. Molecular evidence of OBI consists of covalently closed circular DNA persisting in the nuclei of hepatocytes after infection. OBI affects HCV and HIV patients, patients on haemodialysis, transplanted patients, patients with other chronic LDs, and also the general population. Immunocompetent subjects do not seem to suffer from OBI. However, there are certain scenarios where OBI has to be kept in mind. These include organ transplantation, especially liver transplantation, with the risk of transmission of the infection and virus reactivation during immunosuppression. Moreover, OBI is thought to be related with some cases of HCC and progression of chronic LD. OBI is of relevance when patients are immunosuppressed because reactivation can occur clearly affecting the prognosis.

REFERENCES

1. European Association for the Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol. 2012;57:167-85.

2. Nabil Z. An overview of occult hepatitis B virus infection. World J Gastroenterol. 2011;17:1927-38.

3. Ganem D, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med. 2004;350:1118–29.

4. Raimondo G et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol. 2008;49:652-7.

5. Levrero M et al. Control of cccDNA function in hepatitis B virus infection. J Hepatol. 2009;51:581-92.

6. Gutiérrez-García ML et al. Prevalence of occult hepatitis B virus infection. World J Gastroenterol. 2011;17:1538-42.

7. Cacciola I et al. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. N Engl J Med. 1999;341: 22-6.

8. Squadrito G et al. Impact of occult hepatitis B virus infection on the outcome of chronic hepatitis C. J Hepatol. 2013;59:696-700.

9. Fernandez-Rodriguez CM et al. Influence of occult hepatitis B virus infection in chronic hepatitis C outcomes. World J Gastroenterol. 2011;17:1558-62.

10. Fabrizi F et al. Occult hepatitis B virus infection in dialysis patients: a multicentre survey. Aliment Pharmacol Ther. 2005;21:1341-7.

11. Cohen Stuart JW et al. Occult hepatitis B in persons infected with HIV is associated with low CD4 counts and resolves during antiretroviral therapy. J Med Virol. 2009;81:441-5.

12. Chemin I et al. High incidence of hepatitis B infections among chronic hepatitis cases of unknown aetiology. J Hepatol. 2001;34:447-54.

13. Raimondo G et al. Occult hepatitis B virus in liver tissue of individuals without hepatic disease. J Hepatol. 2008;48: 743-6.

14. Torbenson M, Thomas DL. Occult hepatitis B. Lancet Infect Dis. 2002;2: 479-86.

15. Hollinger FB, Sood G. Occult hepatitis B virus infection: a covert operation. J Viral Hepat. 2010;17:1-15.

16. Gerlich WH et al. Deficiencies in the standardization and sensitivity of diagnostic tests for hepatitis B virus. J Viral Hepat. 2007;14 Suppl 1:16-21.

17. Ocaña S et al. Diagnostic strategy for occult hepatitis B virus infection. World J Gastroenterol. 2011;17:1553-7.

18. Urbani S et al. The role of anti-core antibody response in the detection of occult hepatitis B virus infection. Clin Chem Lab Med. 2010;48:23-29.

19. Candotti D, Allain JP. Transfusion-transmitted hepatitis B virus infection. J Hepatol. 2009;51:798-809.

20. Reesink HW et al. Occult hepatitis B infection in blood donors. Vox Sang. 2008;94:153-66.

21. Prieto M et al. De novo hepatitis B after liver transplantation from hepatitis B core antibody-positive donors in an area with high prevalence of anti-HBc positivity in the donor population. Liver Transpl.

2001;7:51-8.

22. Abdelmalek MF et al. Subclinical reactivation of hepatitis B virus in liver transplant recipients with past exposure. Liver Transpl. 2003;9:1253-7.

23. Cheung CK et al. Occult hepatitis B virus infection of donor and recipient origin after liver transplantation despite nucleoside analogue prophylaxis. Liver Transpl. 2010;16:1314-23.

24. Cholongitas E et al. Liver grafts from anti-hepatitis B core positive donors: a systematic review. J Hepatol. 2010;52: 272-9.

25. Chang MS et al. Prevention of de novo hepatitis B in recipients of core antibody-positive livers with lamivudine and other nucleos(t)ides: a 12-year experience. Transplantation. 2013;95:960-5.

26. Wright AJ et al. Lamivudine compared with newer antivirals for prophylaxis of hepatitis B core antibody positive livers: a cost-effectiveness analysis. Am J Transplant. 2014;14:629-34.

27. Cholongitas E, Papatheodoridis GV. Review of the pharmacological management of hepatitis B viral infection before and after liver transplantation. World J Gastroenterol. 2013;19:9189-97.

28. Angelico M et al; Liver Match Investigators. Hepatitis B-core antibody positive donors in liver transplantation and their impact on graft survival: evidence from the Liver Match cohort study. J Hepatol. 2013;58:715-23.

29. Bläckberg J, Kidd-Ljunggren K. Occult hepatitis B virus after acute self-limited infection persisting for 30 years without sequence variation. J Hepatol. 2000;33:992-7.

30. Yuki N et al. Long-term histologic and virologic outcomes of acute self-limited hepatitis B. Hepatology. 2003;37:1172-9.

31. Covolo L et al. Occult hepatitis B virus and the risk for chronic liver disease: a meta-analysis. Dig Liver Dis. 2013;45: 238-44.

32. Squadrito G et al. The clinical significance of occult HBV infection. Ann Gastroenterol. 2014;27:15-9.

33. Shi Y et al. Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis. Liver Int. 2012;32:231-40.

34. Lledó JL et al. Management of occult hepatitis B virus infection: an update for the clinician. World J Gastroenterol. 2011;17:1563-8.

35. Hu KQ. Occult hepatitis B virus infection and its clinical implications. J Viral Hepat. 2002;9:243-57.

36. Manzano-Alonso ML, Castellano-Tortajada G. Reactivation of hepatitis B virus infection after cytotoxic chemotherapy or immunosuppressive therapy. World J Gastroenterol. 2011;17:1531-7.

37. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology. 2009;50:661-2.

38. Mindikoglu AL et al. Hepatitis B virus reactivation after cytotoxic chemotherapy: the disease and its prevention. Clin Gastroenterol Hepatol. 2006;4:1076-81.

39. Marzano A et al; Italian Association for the Study of the Liver. Prophylaxis and treatment of hepatitis B in immunocompromised patients. Dig Liver Dis. 2007;39:397-408.

40. Pei SN et al. Reactivation of hepatitis B virus following rituximab-based regimens: a serious complication in both HBsAg-positive and HBsAg-negative patients. Ann Hematol. 2010;89:255-62.

41. Coppola N et al. Reactivation of overt and occult hepatitis B infection in various immunosuppressive settings. J Med Virol. 2011;83:1909-16.

42. Sagnelli E et al. Clinical impact of occult hepatitis B virus infection in immunosuppressed patients. World J Hepatol. 2014;6:384-93.

OCCULT HBV INFECTION REACTIVATION IN NON-HODGKIN'S LYMPHOMA: AN UPDATE ON PREVALENCE AND MANAGEMENT

***Valerio Rosato,[1] Mario Masarone,[2] Ernesto Claar,[3] Marcello Persico[2]**

1. Internal Medicine and Hepatology Department, Second University of Naples, Naples, Italy
2. Internal Medicine and Hepatology Unit, University of Salerno, Salerno, Italy
3. Internal Medicine Department, Hepatology Unit, Ospedale Evangelico Villa Betania, Naples, Italy
**Correspondence to valeriorosato@gmail.com*

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ABSTRACT

Occult hepatitis B virus infection (OBI) is characterised by the persistence of hepatitis B virus (HBV) genome in the liver, without any evidence of overt infection: without HBV surface antigen (HBsAg) and HBV DNA detectable in the serum, or fugacious spots of very low levels of viraemia. OBI, a possible phase in the natural history of chronic hepatitis B, is mainly due to the strong suppression of viral replication by host's immunity. Although every condition inducing a strong immunosuppression may cause an OBI reactivation, onco-haematological patients, particularly those affected by non-Hodgkin's lymphoma (NHL), are at the highest risk of this occurrence. This is mostly due to the primary involvement of the immune system that characterises these diseases, and the strong immunosuppressive treatments used for their cure. OBI reactivation represents a life-threatening risk, because of the possible development of an overt acute hepatitis that may lead to hepatic failure. Prophylaxis with lamivudine can prevent OBI reactivation and, when it occurs, the prompt administration of an antiviral therapy with nucleos(t)ide analogues can stop it. Currently, no valid serological tests for occult HBV detection are available, in this way every HBsAg-negative patient undergoing treatment for NHL is to be considered at risk of a 'probable OBI reactivation'. The estimation of the real extent of this occurrence in a NHL setting is a difficult challenge, mostly due to the difficulty of obtaining a definitive diagnosis (which involves the availability of a liver biopsy performed before its development) and the high variability of the literature reports on this issue. In fact, the data concerning this prevalence range from 2.3-27.7% among the different papers, according to different study designs, different diagnostic criteria, different study populations, and different geographical areas of origin of the patients. The aim of this review is to browse the available knowledge about occult HBV infection amongst NHL patients, focusing on the prevalence of OBI reactivations, their identification, and their management.

Keywords: Occult HBV infection, non-Hodgkin's lymphoma, hepatitis B virus reactivation, immunosuppression.

INTRODUCTION

Occult hepatitis B virus infection (OBI) is one of the most difficult challenges in the field of hepatology. In fact, it is difficult to diagnose, it can be present in patients that are totally seronegative for hepatitis B virus (HBV) markers, and when it occurs in a patient that needs a strong immunosuppressive treatment (i.e. for non-Hodgkin's lymphoma [NHL]), it represents a life-threatening risk of overt HBV reactivation. OBI represents a possible phase of the natural history of chronic hepatitis B,[1] and it is characterised by long-lasting persistence and low-level replication of HBV genomes in the liver, without the evidence of overt HBV infection. It is defined by the presence of HBV DNA in the liver with undetectable or occasionally detectable (with fugacious 'spots' of viraemia of very low levels)

HBV DNA in the serum, in individuals with negative testing for HBV surface antigen (HBsAg) and positive testing for HBV core antibodies (HBcAb).[2] It has been episodically reported also in 'totally negative' patients (HBsAg/HBcAb negative) and even in HBV surface antibody (HBsAb) positive patients.[3] In other cases, the presence of a HBV infection, with the undetectability of HBsAg, is caused by the so-called 'S-escape mutants' that produce a modified HBsAg not identified by the standard commercially available diagnostic kits.[4] This last entity is not to be considered an OBI. In fact, OBI is caused by viruses with no genetic mutations, their replication being strongly suppressed by the host's immuno-surveillance.[5] In immunocompetent subjects, occult infection is totally asymptomatic, without any amount of damage of the liver.[5] Nevertheless, every condition inducing a strong immunosuppression, such as immunosuppressive therapies used in post-transplant and autoimmune diseases, or chemotherapies for solid and haematologic malignancies, has been indicated to eventually cause an OBI reactivation, in the same way it can cause the reactivation of an overt HBV infection.[2] In the real practice, onco-haematological malignancies, and in particular B cell NHL and their treatments, have demonstrated to represent the highest risk of OBI reactivation.[6] The aim of this paper is to review the available data about occult HBV in patients with NHL, focusing on the prevalence of reactivations, its identification, and management.

METHODS

To estimate the prevalence of OBI reactivation, we performed a computer based literature search in PubMed, Scopus, Google Scholar, and MEDLINE databases using the medical subject headings "hepatitis B" and "lymphoma". We limited the research to English language publications and excluded reviews and case reports. Eligible studies were limited to those focusing on HBsAg-negative/HBcAb positive patients receiving treatment for lymphoma. From 856 studies only 16 were selected as eligible. Despite this narrow selection, prevalence of OBI reactivation varies greatly between the selected studies.

OBI DIAGNOSIS AND VIROLOGY

The occult infection status depends on the peculiar life cycle of the HBV and the ability to convert its viral genome into a covalently closed circular DNA (cccDNA), a long lasting HBV intermediate that persists in the nucleus of the hepatocyte as a stable chromatinised episome.[7] The cccDNA is a stable and long-term persistent molecule, therefore, in a long half-life cell as the hepatocyte, this peculiar form of infection may persist for its lifetime, even in the absence of replicating HBV DNA strains in the cytoplasm.[8] The ability of HBV to develop an occult infection does not appear to be related to its genetic variability. In fact, an occult carrier may transmit HBV through blood transfusion or organ transplantation inducing a classic acute hepatitis B in the recipients,[9,10] and during OBI reactivation, the carriers may show a typical hepatitis B serological profile with titratable HBsAg and HBV DNA in the serum.[3] According to Taormina statements,[11] we can distinguish two types of OBI carriers on the basis of the HBV antibody profile: seropositive-OBI (HBcAb and/or HBsAb positive) and seronegative-OBI (HBcAb and HBsAb negative) individuals. Seronegative-OBI is reported to occur in about 20% of the cases.[5] It represents the main challenge forthe prevention of reactivation during chemosuppressive and immunosuppressive therapies due to the fact that no HBV markers are present to suspect such a condition.

OBI diagnosis: At present, no valid serological tests for occult HBV detection are available. Indeed, the gold standard for OBI diagnosis is the analysis of DNA extracts from liver tissues. It must be performed by the use of highly sensitive techniques such as nested polymerase chain reaction (PCR) or real-time PCR, conducted with specific primers for different HBV genomic regions (core, X, and S genes), complementary to highly conserved (genotype shared) nucleotide sequences, in order to find the low quantities of nuclear cccDNA.[11] To obtain the diagnosis of OBI, at least two of the three genes are to be found on the liver specimens of a HBsAg negative patient that is suspected to carry this infection. Obviously, only in a minority of cases, liver tissue specimens (collected before the reactivation) are available for this diagnostic analysis, and the liver biopsy is not always feasible in NHL patients. On account of the fluctuating profile of detectable viraemia in OBI, serially collected samples should be tested. A serological assay for HBcAb should be considered less than an ideal surrogate marker for identifying potential seropositive OBI, indeed not all HBcAb positive individuals are found to be

HBV DNA positive and this test may provide false-positive results.[11]

OBI reactivation diagnosis: By now, a precise definition of OBI reactivation has not yet found a consensus in literature. In most of the cases, it is reported as the reappearance of HBsAg or a *de novo* detection of HBV DNA in the serum, in patients where previous negativity of both of these virological markers can be demonstrated. Besides this evidence, the presence of almost two of three genes ('core', X, and S) of HBV DNA sequence in liver specimens, collected any time before the reactivation itself,[12,13] must also be demonstrated. As reported above, the availability of liver samples is limited and, moreover, these specimens must have been stored in liquid nitrogen to allow DNA extraction. For these reasons, the most common approach used by the clinicians to address this issue is to define the presence of a 'probable OBI reactivation' when an overt HBV acute reactivation occurs in a patient with previous evidences of a 'resolved HBV infection' (i.e. an HBsAg negative, HBcAb positive individual). In this way, when it is not possible to directly confirm the presence of HBV on the liver tissue prior to reactivation and/or any sign of a previous contact with HBV (i.e. in totally seronegative patients), we should consider every reactivation occurring in patients who are HBsAg negative prior to starting immunosuppressive treatments, as 'probable OBI', recalling also the possibility of a *de novo* infection.[2] In this context, it appears clear that the real prevalence of this 'elusive' infection in the general population is difficult to know, and it is not easy to estimate the probability of reactivation in the onco-haematological setting.

OBI Reactivation in NHL

As mentioned above, it is well known that the presence of OBI can represent a life-threatening risk in patients who undergo cytotoxic chemotherapies. This is due to the fact that an overt HBV reactivation (with titratable HBsAg and HBV DNA levels) may occur for the loss of the host's immnosurveillance. Even if this occurrence is less frequent in individuals with OBI than in HBsAg positive patients,[14] it represents a non-neglectable risk. Rarely, it presents itself as a fibrosing cholestatic hepatitis that can occur at any time from the beginning of immunosuppression, and it is characterised by a direct cell damage produced by an intense intrahepatic production of viral antigens.[3] However, in the majority of cases the hepatic damage is related to T cell immune reaction against the viral replication, which starts at the end of chemotherapy, when the immune competence is restored. This can lead to the development of an acute hepatitis that may range from a simple lobular hepatitis with mild alanine transaminase (ALT) elevation and only minimal lesions to a fulminant liver failure (LF).[15] Compared to the other cancer patients, onco-haematological patients, and particularly those affected by NHL, have the highest risk of HBV reactivation.[6] This is caused by the primary involvement of the immune system peculiar to this group of lymphoproliferative diseases[16] and also by the strongly immunosuppressive treatment regimens.[2] For example, the majority of treatment regimens contain high doses of corticosteroids that are administered for long periods. This type of administration (high-dose-long-time) as well as establishing an immunosuppressive state, may directly stimulate HBV replication through the glucocorticoid responsive element, a transcriptional enhancer element present in HBV viral genome.[17] Nevertheless, the drug that has attracted more attention in this field is certainly rituximab. Rituximab is a chimeric mouse-human monoclonal antibody (mAb) that has been introduced in CD20-positive NHL therapy since 1997, in addition to conventional chemotherapy. This drug radically changed the natural history of NHL, giving a great improvement of patient outcomes in both indolent and aggressive NHL, becoming the major part of the standard of care for these diseases.[18,19] It is also to be noted that the use of rituximab is not limited to haematological malignancies but its use is also spreading in a wide range of diseases in which there is an autoimmune involvement of B lymphocytes (i.e. rheumatoid arthritis, inflammatory bowel diseases, etc.).[20]

After a few hours of infusion, rituximab induces a strong depletion of B lymphocytes and this condition persists for 6-8 months, in which the patient remains immunosuppressed.[20] It is very interesting that this drug, a strong inhibitor of B lymphocytes, is considered the most potent inductor of the HBV reactivation, even if the host immune control on HBV has been historically considered to be exerted by T lymphocytes. Probably, B lymphocytes intervene in the control of HBV replication by enhancing the cytotoxic response of CD8 T lymphocytes through their activity as antigen-presenting cells.[21] The

combination of rituximab with cyclophosphamide, hydroxydaunorubicin, oncovin, and predniso(lo)ne (R-CHOP protocol) provide the highest risk of reactivation both in patients with overt infection and those with occult infection.[22] In support of these hypotheses, before the introduction of rituximab in the standard of care for lymphoproliferative disease, OBI reactivation was a rare and anecdotal event and was mostly widespread among patients undergoing haematopoietic stem cell transplantation.[16,23-25]

Ofatumumab, used for treatment of onco-haematological diseases, is another anti-CD20 mAb considered a potential factor responsible for reactivation of OBI.[26] OBI reactivation has also been occasionally observed in patients with rheumatologic diseases, undergoing treatments with 'biologic' drugs (anti-CD20 and/or anti-tumour necrosis factor-alpha) or high doses of corticosteroids.[27,28] Moreover, besides NHL and rheumatologic patients, other high-risk categories of OBI reactivation consist of subjects undergoing haematopoietic stem cell transplantation (in which itwas described that HBsAg inverse-seroconversion often occurs, although often without a clinically typical acute hepatitis[29,30]), and liver or kidney transplantation from HBcAb positive donors.[31,32] There are also few data about the real risk of OBI reactivation in patients undergoing chemotherapy for solid tumours[33] and transarterial chemoembolisation for hepatocarcinoma.[2] In this review our attention has been focused on OBI reactivation in NHL patients.

OBI Reactivation Prevalence in NHL Setting

Approximately 350 million people worldwide are chronically infected with HBV, but many of them do not develop a chronic active hepatitis and remain asymptomatic. Moreover, in high endemic areas it is estimated that the prevalence of isolated HBcAb positivity is higher than 60% and, therefore, it is estimated that almost 40% of the world population has been in contact with or is a carrier of HBV.[34-36] As mentioned above, OBI reactivation can occur in patients with evidences of a 'previously resolved' HBV infection. With 'previously resolved infection' we refer to various clinical situations ranging from the complete immunity (HBsAb positivity), the isolated HBcAb positivity, and the total seronegativity for HBV markers. Considering this scenario, to estimate the prevalence of OBI reactivation is a difficult challenge. The reports in the literature, in fact, seem to confirm this statement, showing a high variability in the prevalence among various patient cohorts, also analysing only the NHL setting.

This variability can be, in part, accounted for by the different designs of the studies, but also to the patient characteristics themselves. The studies differ mainly in the characteristics of the population on which it is estimated the prevalence of reactivation: in some cases it is calculated on the general HBsAg negative population, obtaining lower prevalence rates, in other cases it is evaluated exclusively among HBsAb negative/HBcAb positive patients, thus resulting in higher prevalence rates. Although in recent years the research has been focused on anticore positive patients, in whom the reactivation is more frequent, it must be stressed that >20% of occult carriers are negative for all serum markers of HBV[5] and the risk of reactivation between these patients is not negligible. Therefore, in our view, the reports that analyse all HBsAg negative patients may be considered more reliable on assessing this issue. The methods to diagnose HBV reactivation varied widely across the different studies and thus also the definition of OBI reactivation. In the majority of the studies, especially those in a small population, a 'pre-clinical HBV reactivation' is considered, that is defined as the reappearance of detectable HBV DNA in the serum, even without an abnormal ALT level. On the contrary, large population studies very often use a 'clinical reactivation' definition, usually described as the derangement of ALT/aspartate transaminase (at least 2/3 times upper normal values), with HBsAg and HBV DNA detectable in the serum. Finally, among the reviewed studies, only a few papers reported that an analysis of DNA extracts from liver tissues was performed, thus assessing the 'true prevalence of OBI'.[37] As previously said, after the OBI reactivation, the patient can develop an acute hepatitis that can range from simple lobular hepatitis with minimal lesions to fulminant LF, or have non-clinical complication. As will be widely discussed in the section of the management, currently the treatment should be started in the preclinical phase of reactivation to ensure the best results.

Undoubtedly, the most talked about risk factor for the OBI reactivation is the treatment that patients receive. As said previously, many evidences in the literature identify as the main risk factor for the OBI reactivation therapy with anti CD20 mAb (rituximab). In 2006, Hui et al.[13] performed a study on 244 HBsAg-negative

NHL patients receiving systematic chemotherapy with or without rituximab. Among the 8 (3.3%) who developed HBV reactivation, 7 were treated with rituximab, and multivariate analysis demonstrated that rituximab was a risk factor for HBV reactivation in HBsAg negative patients.[13]

Table 1: Reported prevalence of occult hepatitis B virus (HBV) reactivation in non-Hodgkin's lymphoma patients.

Study	Country of origin	No. of patients HBsAg negative	No. of patients HBcAb positive	Prevalence of OBI reactivation No. (%)a	Type of therapy during reactivation	Definition of reactivation	Study limitations
Hui et al.[13]	China	244	152	8/244 (3.27%)	7 +rituximab 1 -rituximab	Clinical[b]	Retrospective
Persico et al.[37]	Italy	52	18	5/18 (27.7%)	Not reported	Preclinical[c] and histological[d]	Small numbers
Hanbali et al.[41e]	USA	26		7/26 (26.9%)	7 +rituximab	Clinical	Retrospective, small numbers, only rituximab
Targhetta et al.[38]	Italy	638	74 (+rituximab) 245 (-rituximab)	2/245 (0.6%) 2/74 (2.7%)	2 +rituximab 2 -rituximab	Clinical	Retrospective
Pei et al.[42e]	Taiwan	95		4/95 (4.2%)	4 +rituximab	Clinical	Retrospective, only rituximab[e]
Yeo et al.[12]	China	80	46	5/80 (6.2%)	5 +rituximab	Clinical	
Fukushima et al.[43]	Japan	127	48	2/48 (4.1%)	1 +rituximab 1 -rituximab	Clinical and Preclinical	Retrospective
Francisci et al.[44]	Italy	56	13	3/13 (23%)	Not reported	Preclinical	Type of therapy not reported, small numbers
Ji et al.[39]	China	368	45 (-rituximab) 43 (+rituximab)	1/43 (2.3%)	1 +rituximab	Clinical	Retrospective
Méndez-Navarro et al.[45]	USA		25	0/25 (0%)		Clinical	Retrospective, small numbers
Koo et al.[46e]	Singapore		62	2/62 (3.2%)		Clinical	Only rituximab
Watanabe et al.[47]	Japan		45 (24 + rituximab)	5/24 (20.8%)	5 +rituximab	Preclinical	Retrospective
Oh et al.[48e]	Korea		67	2/67 (3%)	+rituximab	Preclinical	Retrospective, only rituximab
Huang et al.[50e]	Taiwan		39	7/39 (17.9%)	+rituximab	Preclinical	Only rituximab
Masarone et al.[40]	Italy	460		10/460 (2.2%)	5 +rituximab 5 -rituximab	Clinical	Retrospective
Hsu et al.[49e]	Taiwan		150	17/150 (11.3%)	17 rituximab	Clinical	Only rituximab

a. Prevalence as it has been reported by the study.
b. Increase in alanine transaminase (ALT) at least 3-times upper normal values with HBV DNA and/or HBsAg detectable in the serum.
c. HBV DNA detectable in the serum even without an abnormal ALT level.
d. HBV DNA detectable with polymerase chain reaction on the liver specimen.
e. Study performed only on patients undergoing rituximab treatment.
HBsAg: HBV surface antigen; HBcAb: HBV core antibodies; OBI: occult HBV infection.

Similarly in 2009, Yeo et al.[12] reported a study in 80 HBsAg-negative patients with diffuse large B cell lymphoma receiving treatment with R-CHOP or CHOP regimen, between these HBV reactivation occurred in 5 (6.2%) of the patients who received rituximab treatment. In 2008, Targhetta et al.[38] retrospectively analysed 311 HBsAg negative-HBcAb positive lymphoma patients, of which 241 received chemotherapy and 74 had immunochemotherapy with anti-CD20 mAb. Reactivation occurred in 4 patients, 2 (0.8%) in the standard chemotherapy group, and 2 (2.7%) in the rituximab group, confirming a greater incidence of HBV reactivation under anti-CD20 antibody treatment.[38] On the contrary in 2010, Ji et al.[39] performed a retrospective study on 88 HBcAb positive NHL patients during chemotherapy. Among them, 43 received R-CHOP regimen and only one of these (2.3%) had hepatitis associated with HBV reactivation. Therefore probably due to the small numbers, no statistically significant risk factors were identified.[39] Recently, our group reported a study in 460 HBsAg negative patients undergoing anti-neoplastic treatment protocols that included rituximab or high immunosuppressive drugs. We found HBV reactivation in 10 (2.2%) patients, 5 treated with rituximab and 5 without, so we concluded that the risk of HBV reactivation was not exclusively associated to rituximab.[40] This result is in contrast with recent reports of a slightly higher prevalence of OBI reactivation in rituximab-treated patients. We concluded that this surprising finding was mostly due to the retrospective nature of the study, which included a large cohort of patients treated in the past 10 years, an era in which rituximab was less widely included in chemotherapy protocols. For this reason the prevalence of OBI reactivation in non-rituximab therapies was higher. We found also that OBI reactivation in non-rituximab treatment occurred exclusively in patients treated with highly immunosuppressive ('dose-dense') regimens, enlightening the fact that the risk of reactivation may not strictly be correlated with rituximab, but rather with the strong immunosuppression, regardless of the type of treatment used. However, the prevalence of occult HBV infection, reported by the studies examined for this review, range from 2.3% to 27.7%.[12,13,37-50] The data are reported in detail in Table 1.[12,13,37-50]

MANAGEMENT OF OBI AND PREVENTION OF THE REACTIVATION

While HBV reactivation in patients with overt infection (HBsAg positive) is a well-recognised clinical entity on which international guidelines give highly defined recommendations for its management, the best strategy for the prevention of OBI reactivation is less clear. In fact, even if pre-emptive antiviral therapy with nucleoside/nucleotide analogues (NUCs) has demonstrated to be efficacious in preventing HBV reactivation in NHL settings,[37,51-55] OBI is not easy to diagnose and, in this way, any HBcAb positive/HBsAg negative patient could be a possible occult infection carrier. The core of the problem is that not all HBcAb positive patients have an occult infection, that there are also OBI carriers that are HBcAb negative, and, finally, that not all OBI carriers who experiment an immuno suppression have a HBV reactivation. To these statements it must be added that no reliable serum markers to predict OBI reactivation are available at the moment. Moreover, even if we consider only HBsAg negative/HBcAb positive patients (in whom there is the higher probability to find an OBI), the prevalence of this type of patient is too high to suggest a universal prophylaxis strategy with NUCs in all immunosuppressive therapy. For this particular issue, international guidelines gave their recommendations: American Association for the Study of the Liver Disease suggests a periodical monitoring of serum HBsAg and HBV DNA and commence antiviral therapy at the first sign of HBV reactivation.[56] Likewise European Association for Study of the Liver[1] recommends ALT monitoring and eventually HBV DNA assays every 2-3 months. The Italian Association for the Study of the Liver[57] proposes two different strategies for patients undergoing chemotherapy: for mild haematological therapies (standard protocol without mAbs) HBsAg monitoring is advised, whereas in intense immunosuppression therapy (i.e. protocol including mAbs and/or strongly immunosuppressive therapies, i.e. 'dose-dense' regimen) recommends universal pre-emptive therapy (universal prophylaxis). Nevertheless, it has to be noted that the strength of these recommendations is low and further studies to address this issue are encouraged. In the already mentioned above paper, our group retrospectively evaluated 498 NHL patients, comparing these two strategies, and performed a cost-effectiveness analysis by calculating the costs of universal prophylaxis

versus strict monitoring (which was the method used in the study patients), in a time interval of a standard rituximab-containing chemotherapy protocol (about 12 months). From this analysis, the 'monitoring' approach resulted in being significantly more cost-effective in respect to universal prophylaxis.[40] Considering that very often NHL patients need more than one therapy cycle to obtain NHL remission, or undergo long-term 'maintenance' therapies with rituximab, the universal prophylaxis may be less justifiable. Nonetheless, the principal limitation to the 'monitoring' approach is the possible failure of rescue antiviral therapy that has been reported in other papers in literature.[12,42,58-60]

Lamivudine, the first-generation nucleoside analogue, is the drug that is generally used as the 'first approach' for the prophylaxis of OBI. Even if it has a 'low genetic barrier' (namely a high rate of resistance development to it by the emergence of HBV mutant species that are also able to replicate in the presence of the drug), this choice is justified by the low price, the low toxicity, and the low risk of the occurrence of resistance in such patients that carry very low HBV DNA levels. The use of a potent NUC with a 'high genetic barrier' (a scarce or null tendency to develop resistance), i.e. entecavir or tenofovir, does not seem to have significant advantages in preventing OBI reactivation[61] and therefore should not be used during the prophylaxis. Nevertheless, in the unfortunate case of a reactivation occurrence, the treatment with entecavir or tenofovir should be preferred, especially if there is an ALT rise. In fact, some reports show the capacity of these NUCs to avoid the fulminant course in case of acute hepatitis.[2] In this sense, since in OBI NUCs are generally commenced after reactivation, the most potent NUCs, such as entecavir or tenofovir, may be considered as first-line treatment. However, the possibility of an occult infection with lamivudine-resistant viral strains able to determine viral reactivation even under lamivudine prophylaxis cannot be excluded, so it is recommended to continue careful HBV DNA monitoring during antiviral treatment with lamivudine, in order to switch to tenofovir if HBV DNA becomes detectable.[62] If, during treatment with lamivudine, HBV DNA becomes detectable or it increases, it is advisable to switch to tenofovir, which has proven effective in suppressing lamivudine resistant mutants.

CONCLUSION

From this brief review we can conclude that OBI reactivation in NHL patients undergoing strong immunosuppressive therapies, and, in particular, rituximab containing protocols, represents a unique challenge for the hepatologist who is called to not only make a difficult diagnosis but also to make quick decisions on a management that is not widely acknowledged. Further studies are desirable to address the various issues that are still open in this particular field of hepatology.

REFERENCES

1. Papatheodoridis G et al. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol. 2012;57:167-85.

2. Raimondo G et al. Occult HBV infection. Semin Immunopathol. 2013;35:39-52.

3. Marinone C et al. HBV disease: HBsAg carrier and occult B infection reactivation in haematological setting. Dig Liver Dis. 2011;43 Suppl 1:S49-56.

4. Stramer SL et al. Nucleic acid testing to detect HBV infection in blood donors. N Engl J Med. 2011;364:236-47.

5. Torbenson M, Thomas DL. Occult hepatitis B. Lancet Infect Dis. 2002;2: 479-86.

6. Coppola N et al. Reactivation of overt and occult hepatitis B infection in various immunosuppressive settings. J Med Virol. 2011;83:1909–16.

7. Bock CT et al. Structural organization of the hepatitis B virus minichromosome. J Mol Biol. 2001;307:183-96.

8. Zoulim F. New insight on hepatitis B virus persistence from the study of intrahepatic viral cccDNA. J Hepatol. 2005;42:302-8.

9. Chazouillères O et al. "Occult" hepatitis B virus as source of infection in liver transplant recipients. Lancet. 1994;343:142–6.

10. Japanese Red Cross Non-A, Non-B Hepatitis Research Group. Effect of screening for hepatitis C virus antibody and hepatitis B virus core antibody on incidence of post-transfusion hepatitis. Lancet. 1991;338:1040–1.

11. Raimondo G et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol. 2008;49:652–7.

12. Yeo W et al. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. J Clin Oncol. 2009;27:605–11.

13. Hui CK et al. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. Gastroenterology. 2006;131:59–68.

14. Shouval D, Shibolet O. Immunosuppression and HBV reactivation. Semin Liver Dis. 2013;33:167–77.

15. Xunrong L et al. Hepatitis B virus (HBV) reactivation after cytotoxic or immunosuppressive therapy--pathogenesis and management. Rev Med Virol. 2001;11:287–99.

16. Liang R. How I treat and monitor viral hepatitis B infection in patients receiving intensive immunosuppressive therapies or undergoing hematopoietic stem cell

transplantation. Blood. 2009;113:3147-53.

17. Tur-Kaspa R et al. Hepatitis B virus DNA contains a glucocorticoid-responsive element. Proc Natl Acad Sci U S A. 1986;83:1627–31.

18. Schulz H et al. Immunochemotherapy with rituximab and overall survival in patients with indolent or mantle cell lymphoma: a systematic review and meta-analysis. J Natl Cancer Inst. 2007;99(9):706-14.

19. Coiffier B et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood. 2010;116:2040-5.

20. Cooper N, Arnold DM. The effect of rituximab on humoral and cell mediated immunity and infection in the treatment of autoimmune diseases. Br J Haematol. 2010;149:3-13.

21. Raimondo G. Therapy of occult hepatitis B virus infection and prevention of reactivation. Intervirology. 2014;57: 189-95.

22. Yeo W, Chan HL. Hepatitis B virus reactivation associated with anti-neoplastic therapy. J Gastroenterol Hepatol. 2013;28:31–7.

23. Lalazar G et al. Screening, prevention and treatment of viral hepatitis B reactivation in patients with haematological malignancies. Br J Haematol. 2007;136:699-712.

24. Onozawa M et al. Progressive disappearance of anti-hepatitis B surface antigen antibody and reverse seroconversion after allogeneic hematopoietic stem cell transplantation in patients with previous hepatitis B virus infection. Transplantation. 2005;79:616-9.

25. Ceccarelli L et al. Late hepatitis B virus reactivation after lamivudine prophylaxis interruption in an anti-HBs-positive and anti-HBc-negative patient. J Infect. 2012;65:180-3.

26. Mitka M. FDA: increased HBV reactivation risk with ofatumumab or rituximab. JAMA. 2013;310:1664.

27. Lee YH et al. Hepatitis B virus (HBV) reactivation in rheumatic patients with hepatitis core antigen (HBV occult carriers) undergoing anti-tumor necrosis factor therapy. Clin Exp Rheumatol. 2013;31:118–21.

28. Giardina AR et al. No detection of occult HBV-DNA in patients with various rheumatic diseases treated with anti-TNF agents: a two-year prospective study. Clin Exp Rheumatol. 2013;31:25–30.

29. Onozawa M et al. Progressive disappearance of anti-hepatitis B surface antigen antibody and reverse seroconversion after allogeneic hematopoietic stem cell transplantation in patients with previous hepatitis B virus infection. Transplantation. 2005;79:616-9.

30. Vigano M et al. Risk of hepatitis B surface antigen seroreversion after allogeneic hematopoietic SCT. Bone Marrow Transplant. 2011;46:125–31.

31. Cholongitas E et al. Liver grafts from anti-hepatitis B core positive donors: a systematic review. J Hepatol. 2010;52: 272-9.

32. Chen GD et al. Outcomes and risk factors for hepatitis B virus (HBV) reactivation after kidney transplantation in occult HBV carriers. Transpl Infect Dis. 2013;15:300–5.

33. Saitta C et al. Risk of occult hepatitis B virus infection reactivation in patients with solid tumours undergoing chemotherapy. Dig Liver Dis. 2013;45:683–6.

34. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat. 2004;11(2):97-107.

35. Liu CJ et al. Epidemiology of HBV infection in Asian blood donors: emphasis on occult HBV infection and the role of NAT. J Clin Virol. 2006;36 Suppl 1:S33-44.

36. Goldstein ST et al. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. Int J Epidemiol. 2005;34(6):1329-39.

37. Persico E et al. Occult hepatitis B virus infection in patients with non-Hodgkin lymphoma: the need for early diagnosis in anti-Hbc positive patients. Gut. 2007;56:1470-1.

38. Targhetta C et al. Hepatitis B virus-related liver disease in isolated anti-hepatitis B-core positive lymphoma patients receiving chemo- or chemo-immune therapy. Haematologica. 2008;93:951-2.

39. Ji D et al. Low incidence of hepatitis B virus reactivation during chemotherapy among diffuse large B-cell lymphoma patients who are HBsAg-negative/HBcAb-positive: a multicenter retrospective study. Eur J Haematol. 2010;85(3):243-50.

40. Masarone M et al. Management of the HBV reactivation in isolated HBcAb positive patients affected with Non Hodgkin Lymphoma. BMC Gastroenterol. 2014;14:31.

41. Hanbali A, Khaled Y. Incidence of hepatitis B reactivation following rituximab therapy. Am J Hematol. 2009;84(3):195.

42. Pei SN et al. Reactivation of hepatitis B virus following rituximab-based regimens: a serious complication in both HBsAg-positive and HBsAg-negative patients. Ann Hematol. 2010;89:255-62.

43. Fukushima N et al. Retrospective and prospective studies of hepatitis B virus reactivation in malignant lymphoma with occult HBV carrier. Ann Oncol. 2009;20(12):2013-7.

44. Francisci D et al. Management of hepatitis B virus reactivation in patients with hematological malignancies treated with chemotherapy. Infection. 2010;38: 58-61.

45. Méndez-Navarro J et al. Hepatitis B screening, prophylaxis and re-activation in the era of rituximab-based chemotherapy. Liver Int. 2011;31(3):330-9.

46. Koo YX et al. Risk of hepatitis B virus (HBV) reactivation in hepatitis B surface antigen negative/hepatitis B core antibody positive patients receiving rituximab-containing combination chemotherapy without routine antiviral prophylaxis. Ann Hematol. 2011;90(10):1219-23.

47. Watanabe M et al. Re-appearance of hepatitis B virus following therapy with rituximab for lymphoma is not rare in Japanese patients with past hepatitis B virus infection. Liver Int. 2011;31(3):340-7.

48. Oh MJ, Lee HJ. A study of hepatitis B virus reactivation associated with rituximab therapy in real-world clinical practice: a single-center experience. Clin Mol Hepatol. 2013;19(1):51-9.

49. Hsu C et al; Taiwan Cooperative Oncology Group. Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: a prospective study. Hepatology. 2014;59(6):2092-100.

50. Huang YH et al. Randomized controlled trial of entecavir prophylaxis for rituximab-associated hepatitis B virus reactivation in patients with lymphoma and resolved hepatitis B. J Clin Oncol. 2013;31(22):2765-72.

51. Ziakas PD et al. Effect of prophylactic lamivudine for chemotherapy-associated hepatitis B reactivation in lymphoma: a meta-analysis of published clinical trials and a decision tree addressing prolonged prophylaxis and maintenance. Haematologica. 2009;94(7):998-1005.

52. Loomba R et al. Systematic review: the effect of preventive lamivudine on hepatitis B reactivation during chemotherapy. Ann Intern Med. 2008;148(7):519-28.

53. Hsu C et al. A revisit of prophylactic lamivudine for chemotherapy-associated hepatitis B reactivation in non-Hodgkin's lymphoma: a randomized trial. Hepatology. 2008;47(3):844-53.

54. Kohrt HE et al. Systematic review: lamivudine prophylaxis for chemotherapy-induced reactivation of chronic hepatitis B virus infection. Aliment Pharmacol Ther. 2006;24(7):1003-16.

55. Persico M et al. Efficacy of lamivudine to prevent hepatitis reactivation in hepatitis B virus-infected patients treated

for non-Hodgkin lymphoma. Blood. 2002;99(2):724-5.

56. Lok AS, McMahon BJ. Chronichepatitis B: update 2009. Hepatology. 2009;50(3):661-2.

57. Marzano A et al; Italian Association for the Study of the Liver. Prophylaxis and treatment of hepatitis B in immunocompromised patients. Dig Liver Dis. 2007;39(5):397-408.

58. Umemura T et al; Japan de novo Hepatitis B Research Group. Mortality secondary to fulminant hepatic failure in patients with prior resolution of hepatitis B virus infection in Japan. Clin Infect Dis. 2008;47(5):e52-6.

59. Koo YX et al. Hepatitis B virus reactivation and role of antiviral prophylaxis in lymphoma patients with past hepatitis B virus infection who are receiving chemoimmunotherapy. Cancer. 2010;116(1):115–21.

60. Evens AM et al. Rituximab-associated hepatitis B virus (HBV) reactivation in lymphoproliferative diseases: meta-analysis and examination of FDA safety reports. Ann Oncol. 2011;22(5):1170-80.

61. Chen FW et al. Entecavir versus lamivudine for hepatitis B prophylaxis in patients with haematological disease. Liver Int. 2013;33:1203-10.

62. Pollicino T et al. Molecular and functional analysis of occult hepatitis B virus isolates from patients with hepatocellular carcinoma. Hepatology. 2007;45:277-85.

HOMOZYGOSITY FOR THE C282Y SUBSTITUTION IN THE *HFE* GENE: THE INCOMPLETE PENETRANCE AND VARIABLE EXPRESSIVITY

Dilum Ekanayake,[1] Clinton Roddick,[1] Murtaza Khanbhai,[1] *Lawrie W. Powell[1,2]

1. School of Medicine, The University of Queensland, Brisbane, Australia
2. Centre for the Advancement of Clinical Research, Royal Brisbane and Women's Hospital, Brisbane, Australia
**Correspondence to lawrie.powell@qimr.edu.au*

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ABSTRACT

The syndrome of hepatic cirrhosis diabetes and skin pigmentation ('Bronze diabetes') has been well documented, including its propensity to lead to hepatocellular cancer. However, this picture of advanced disease is much less common nowadays with increased awareness and early diagnosis. However, in addition to this, it has been increasingly recognised that in contrast to other diseases inherited as autosomal recessive traits, subjects carrying the genetic predisposition infrequently develop overt disease. This is due only in part to physiological and pathological blood loss, and further relevant genetic mutations have been anticipated. Indeed, an international consortium has recently identified that the genetic variant (*GNPAT*) has been identified as predisposing to iron overload related disease. Further mutations can be anticipated and will assist in early diagnosis and treatment as well as identifying subjects predisposed to significant iron overload.

Keywords: Haemochromatosis, iron metabolism, *HFE* haemochromatosis, secondary iron overload.

INTRODUCTION

The clinical syndrome of haemochromatosis has been recognised in its advanced state for >100 years, and in the last two decades clinical and molecular research into this disease has accelerated.[1] Following identification of the mutation in the *HFE* gene, which is responsible for >90% of cases of classic haemochromatosis,[2] it was recognised that the genotypic predisposition is common among those of Northern European ancestry but that the incidence of biochemical abnormalities and clinical disease is less frequent. The recognition of the *HFE* mutation has allowed other forms of iron overload to be diagnosed as distinct entities, typically related to mutations in other iron-regulatory molecules. Largely as a result of the identification of the *HFE* gene, but also because of activities of patient support groups such as Haemochromatosis Australia, there is now a much better awareness of the disorder and subjects and their family members are increasingly recognised at an early stage, e.g. simply with raised serum ferritin (SF) levels and transferring saturation. It has also been increasingly recognised, that in contrast to other diseases inherited as autosomal recessive traits, subjects homozygous for C282Y do not always develop overt disease. In fact, it is now established that approximately only 75% of homozygous subjects develop increased SF levels, only 50% biochemical abnormalities, only 25% increased liver iron, and fewer than 5% develop iron overload related diseases (Figure 1[3-5]).

This was emphasised by Beutler et al.[6] in 2005, when he stated that "*HFE* mutations are necessary but not sufficient to cause haemochromatosis". This has led to a global search for further genes and mutations that could lead to iron overload. As discussed below, several mutations in iron transport molecules have been clearly identified and more

recently a mutation in *GNPAT* has been identified as predisposing to iron overload related disease. It should also be noted that certain transferrin genotypes or polymorphisms have been shown to affect disease expressivity,[7] in addition to which heterozygous mutations in non-*HFE* genes have also been shown to affect disease severity in C282Y homozgotes. Further mutations can be anticipated. Recent research in the field of iron metabolism has deepened our understanding of the molecular processes of iron transport and regulation and how this is disturbed in haemochromatosis. At the same time, population studies better describe the risk to an individual with a genetic mutation, and clinical investigations have improved the tools available for assessment and monitoring. One aspect of this condition that has changed the least is the treatment, with the ancient practice of bloodletting still the main therapy available. With new therapeutic agents under trial, this to may soon be changing. This review focuses on the recent developments in the field of genetic haemochromatosis, including the molecular basis of iron metabolism, relevant genetic mutations, and advances in investigations and therapy, and places these in a global perspective.

IRON ABSORPTION AND THE ROLE OF HEPCIDIN

Iron is absorbed from the gastrointestinal tract in one of two ways depending on whether it is in the haem or non-haem form. Transporters involved in haem iron absorption are not fully understood, however two carriers have been implicated – haem carrier protein 1 and haem responsive gene 1 protein.[8] In contrast, non-haem iron absorption is well characterised. Dietary ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}) by ferrireductases mainly, duodenal cytochrome B.[8] Activity of duodenal cytochrome B is facilitated by ascorbate, which acts as an electron donor. This allows ferrous iron uptake through the divalent metal transporter.[8,9] Once inside the cell, iron may be stored in the polymeric protein, ferritin. Once there, it is released into the circulation through ferroportin – a transport protein on the basolateral surface of enterocytes, macrophages, and other cells.[10] Ferroportin further interacts with feroxidases: hephaestin and ceruloplasmin to oxidise ferrous iron back to ferric, prior to release into circulation.[11] Ferroportin is the only known iron channel allowing export of iron.[12] Once released into the serum, free iron will bind transferrin (Tf). This complex can be carried to and taken up by cells expressing transferrin receptor 1 (TfR1).[8,13] Transferrin saturation is utilised in iron sensing pathways and acts as negative feedback to regulate ferroportin expression, through hepcidin signalling, in hepatocytes, macrophages, and other cells.

Hepcidin is the key regulator of iron metabolism. It is a small 25 amino acid protein synthesised in the liver and exerts its action by regulating ferroportin expression.[14-16] Hepcidin binds to an extracellular loop of ferroportin facilitating endocytosis and proteolysis of the channel, thus reducing the number of transporters available for iron export.[8,17] Normally, excess iron stimulates hepcidin expression in the hepatocytes, leading to a subsequent decrease in serum iron.[12] Regulation of hepcidin is not fully understood, however multiple pathways of regulation have been described. Experimental evidence suggests that hepcidin regulation occurs at a transcriptional level.[18] Mediators influencing hepcidin levels include the *HFE* gene product, transferrin receptor 2 (TfR2), haemojuvelin (HJV), bone morphogenic protein 6 (BMP-6), and matriptase-2.[19] The HFE protein is thought to be expressed primarily in Kupffer cells and bile duct epithelia, and exerts its effects on hepatocytes to induce hepcidin production.[20] It has been suggested that *HFE* interacts with TfR1 and as serum iron increases, it displaces *HFE* allowing it to interact with TfR2 and mediate hepcidin production.[19] HJV is thought to act as a ligand for BMP-2, 4, and 6 leading to increased hepcidin mRNA expression.[21] It has been shown that *HFE* interacts with HJV, suggesting that they form a complex together.[19,22] BMP-6 is thought to play a significant role in iron metabolism given that mouse models with BMP-6 ablation show very low levels of hepcidin mRNA expression.[22]

NOMENCLATURE OF IRON OVERLOAD STATES

The naming of haemochromatosis subtypes is based on the molecules affected. To date four main subdivisions named I, II, III, and IV, which affect the molecules *HFE*, HJV/hepcidin, TfR2, and ferroportin respectively, have been described in literature.[23,24] Further to this, Types 2 and 4 are subdivided into 2A, 2B, 4A, and 4B. As haemochromatosis involving *HFE* is by far the most prevalent, the alternative classification of Type 1 as *HFE* associated haemochromatosis and Types 2-4 as non-*HFE* haemochromatosis still persists.

Types 1-3 are autosomal recessive and affect hepcidin synthesis and regulation where Type 4 differs, being both autosomal dominant and not having a principal effect on hepcidin.[25] The phenotypic expression of typical disease likewise is similar between Types 1-3 and is described in the next section.

HFE (Type 1)

Type 1 haemochromatosis resulting from mutations in the *HFE* gene is the commonest and best established subtype. With an autosomal recessive inheritance pattern it shows the typical phenotype commonly associated with disease. Often presenting with nonspecific symptoms of fatigue and lethargy progressing to hepatic fibrosis, endocrine dysfunction, and cardiomyopathy.[26] Principally three mutations, C282Y, H63D, and S65C, at various loci in the *HFE* gene have been associated with disease. Of these, only the C282Y homozygotes, heterozygotes, and H63D/C282Y compound heterozygotes are of clinical interest. Recently, the role of S65C in disease has been shown to be limited to a mild risk factor. Further to which, compound heterozygosity is only considered clinically relevant in the presence of other risk factors such as heavy alcohol consumption. Even though the role of the membrane protein encoded by *HFE* remains unclear, it is known that aberrations in this gene cause iron overload by interfering with iron sensing in the liver.[8] This leads to decreased hepcidin regulation by iron and subsequent increased gastrointestinal iron uptake.

NON-HFE

Juvenile Haemochromatosis (Types 2A and 2B)

Characterised by earlier onset (typically by the second and third decades) Type 2 is a more severe form of haemochromatosis with higher mortality than Type 1.[27] Two distinct molecular origins have been identified; Types 2A and 2B affecting the *HJV* and hepcidin (*HAMP*) genes respectively.[28] *HJV* is a crucial modulator in the BMP/SMAD pathway of hepcidin regulation, and exerts a stronger influence over this pathway than the *HFE* gene product. Rather than influencing hepcidin release, Type 2B represents a mutation in *HAMP* itself.[29,30]

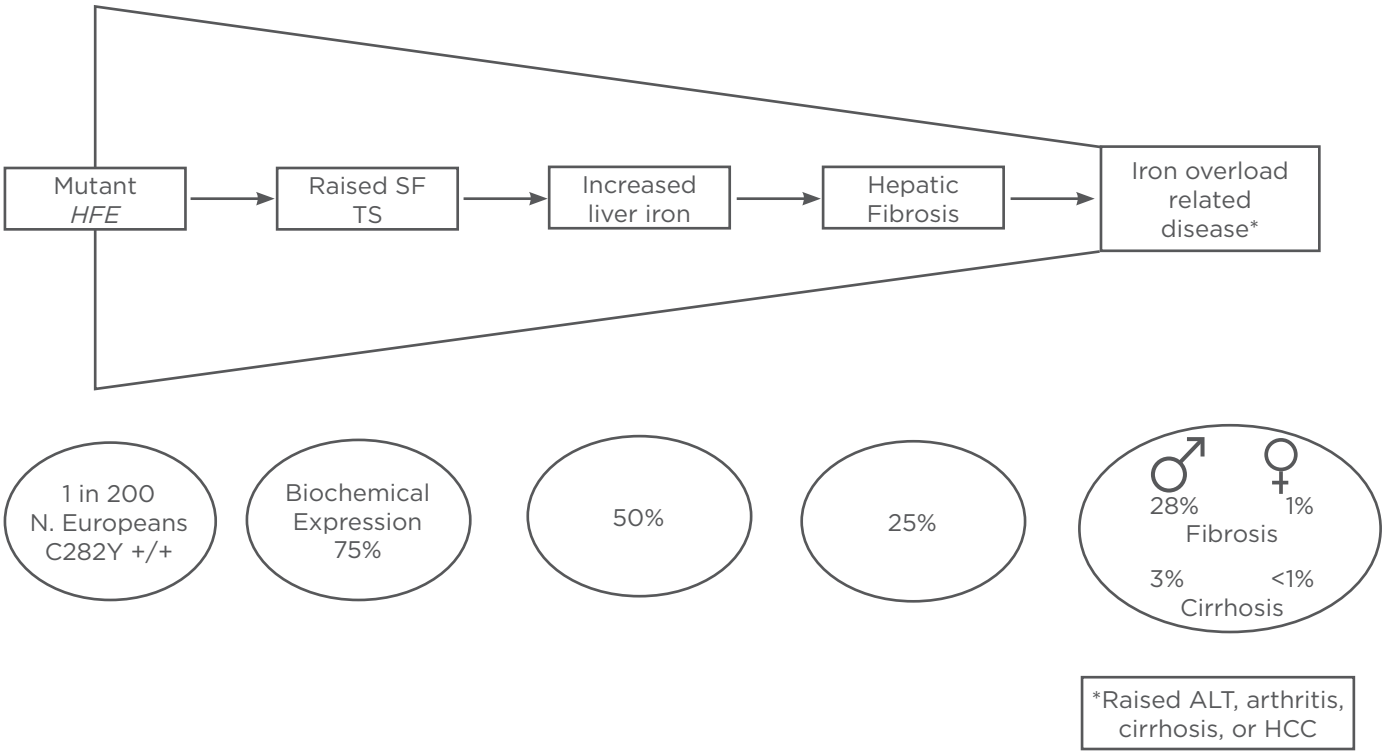

Figure 1: The natural history and disease burden of HFE associated haemochromatosis.
In addition to the above, note that in practice HFE genotyping is usually carried out in selected patients with elevated transferrin saturation (TS) or serum ferritin (SF) levels.
HCC: hepatocellular carcinoma; ALT: alanine transaminase.
Reproduced with permission[3-5]

Both subtypes are inherited in an autosomal recessive pattern. Clinically rapid iron deposition overload preferentially damages the heart and endocrine organs and causes diabetes, cardiomegaly, and hypogonadotropic hypogonadism.[25,26,31] Presentation is abdominal pain, hypogonadism, and arrhythmia with untreated patients commonly dying of congestive cardiac failure or arrhythmia.[26,32]

TfR2 Haemochromatosis (Type 3)

Described in 2000 TfR2, haemochromatosis was the first subtype attributed to mutations outside of *HFE*.[33] It is rare with symptomatic severity of falling between those of Types 1 and 2. Onset can be both adult and juvenile and clinical symptoms are similar to typical *HFE* haemochromatosis. TfR2 is homologous to TfR1 and is suspected to function as an iron sensing molecule in the liver. Defects in TfR2 are thought to affect the BMP/SMAD pathway and cause aberrant iron regulation by hepcidin.[34]

Ferroportin Haemochromatosis (Type 4A and B)

Ferroportin is the only known exporter of iron from cells in the body. It is also involved in the pathogenesis of the only autosomal dominant subtype of haemochromatosis. Ferroportin disease also differs in its clinical presentation. Both 4A and 4B are caused by mutations in the gene encoding *FPN*.[32] However, clinical presentations of 4A and 4B vary considerably. Type 4B is associated with mutations that affect the affinity of ferroportin for hepcidin and mediate hepcidin resistance. In 4A, an inactivation of ferroportin leads to loss of iron export function, causing increased hepatic and tissue iron deposition with low serum transferrin. Normal-low serum Tf, high SF, iron-loaded macrophages, and iron-deficiency anaemia[32,35] is the common biochemical picture. Complications are mild, with minimal sinusoidal fibrosis (extending to hepatocytic fibrosis with advanced age), but phlebotomy is poorly tolerated.[25,32,36] Conversely, 4B is a gain-of-function mutation, with ferroportin becoming insensitive to hepcidin.[31,35] Type 4B shows a more typical clinical picture with elevated transferrin saturation.[23]

THE TYPICAL NATURAL HISTORY AND DISEASE BURDEN OF HAEMOCHROMATOSIS

Gan et al.[37] outlines a four-stage process of iron overload disease: 1) Genetic predisposition to iron overload disease, and mildly raised serum TF; 2) Asymptomatic iron overload (0-5 g of parenchymal iron overload, >300 ug/l SF); 3) Nonspecific symptoms of iron overload (lethargy, arthralgia, malaise, etc.); 4) Iron-overload related disease (diabetes, cirrhosis, arthritis) Clinical complications of untreated iron overload are bronze pigmentation, insulin-dependent diabetes mellitus, hypopituitarism, and subsequent hypogonadism, arthropathies (classically the second and third metacarpophalangeal joints), cardiomyopathy, and hepatic cirrhosis.[25,38] Laboratory measures of liver function may show raised alanine transaminase (ALT) and aspartate aminotransferase (AST).[6] However, Adams et al.[39] note, that the stereotypical presentation of 'bronze diabetes' is quite rare (1%). Instead, it is more common to detect disease either on familial screens or when patients present with nonspecific symptoms.[25] Hepatic iron deposition histologically starts in the acinar zones 1 and 2 (peri-portal), and eventually spreads to central veins. With disease progression iron granules become denser and larger, and begin to involve kupffercells.[35] As mentioned above, identical pathophysiology is seen in Types 2 and 3. Hepatocellular carcinoma (HCC) is a late stage complication of cirrhosis in 6% of male and 1.5% of female patients.[37] A 30-year study by Fracanazani et al.[40] found that of 70 recorded deaths, 56% were due to HCC. Additionally, there is an increased risk of colorectal and breast cancer in C282Y homozygotes, and a tripled risk of colorectal cancer in H63D homozygotes also positive for mismatch repair mutations.[37,41]

HFE haemochromatosis appears to have a risk window. A Norwegian study screened 65,000 adults for iron overload, identifying new cases in 177 males and 92 females. 46% of the male cases were in the 40-49 year range, with an additional 34% being 50-59 years. Females were older, with 25% being 50-59 years and 23% being 60-69 years.[42] This matches work by other groups, which suggest an onset of symptoms around the fourth to fifth decades of life, with women being affected several years older.[25,26,42,43] At the time of diagnosis, men with stage 0-1 fibrosis averaged 40 years, and women 42 years. Men and women with stage 2-4 averaged 45 years and 50 years respectively.[7] There is no generalised association between serum iron and age,[41] but patients with raised SF show a correlation, with patients reaching 500-1,000 ug/l by 41.8 years and plateauing >3,000 ug/l by 54.7 years of age.[7] Most cases manifest by 55

years, and it is rare for SF to rise significantly after this age.[5] Not all C282Y homozygotes progress to end-organ disease. In many SF remains normal.[7,39-41] The Melbourne Collaborative Cohort Study showed that only 50% of patients with SF 300-1,000 ug/l progressed to clinical iron overload (>1,000 ug/l) in a 12 year study.[41] Unfortunately, the rarity of non-HFE strains precludes us from knowing their penetrance, but it is believed to vary between types.[31]

The incomplete penetrance of disease in persons with associated genetic predispositions means that from genotype to phenotype, iron overload and subsequent disease cannot be explained by any single mutation. This is demonstrated in the variable disease prevalence and progression, exemplified by 60-80% of C282Y homozygotes displaying raised SF, but only 24-43% of men, and 1-14% of women, showing clinical iron overload.[6,7,38,41] Known risk factors cause one of either: increased iron availability, reduction of hepcidin activity, or acceleration of hepatic fibrosis.[25,26,37,44] Protective factors include consumption of non-citrus fruit, female sex, Ca2+ channel-blockers, occult/menstrual bleeding, and chronic malabsorptive states.[25,26,35,44] However, Fracanzani et al.[40] found 27% of *HFE* and 39% of non-*HFE* hereditary haemochromatosis had no known environmental risk factors implicating other unknown genetic influences.

MANAGEMENT AND TREATMENT

Treatment for haemochromatosis with venesection and phlebotomy has remained unchanged over the years. Early intervention, prior to the onset of symptoms, improves patient prognosis.[45] Furthermore, venesection in symptomatic individuals improves certain symptoms, such as skin pigmentation, while not having an effect on others such as cirrhosis and arthropathy.[45] Current guidelines suggest that phlebotomy should be used when SF is above the reference range, but further study into the area is required. The aims of venesection are 2-fold, firstly, to directly reduce serum iron by depleting haemoglobin levels, and secondly, to mobilise stored iron from tissues to replace the depleted circulating levels. The current treatment guidelines suggest yearly follow-up in patients whose SF levels are within normal range.

Table 1: Types of hereditary haemochromatosis.

TYPE	GENE	FUNCTION	PREVALENCE	PENETRANCE	ASSOCIATED FEATURES
TYPE 1	*HFE*	Hepcidin upregulation	Most common form worldwide; varies by race	Autosomal recessive: 2-28% penetrance	Classical haemochromatosis
TYPE 2A	*HJV* (haemojuvelin)	Hepcidin upregulation	Rare. More common than Type 2B	Autosomal recessive	Severe, early onset. Associated with hypogonadal hypogonadism and cardiomyopathy.
TYPE 2B	*HAMP* (hepcidin)	Inhibition of enterocyte iron uptake	Rare	Autosomal recessive	
TYPE 3	*TFR2* (transferrin receptor 2)	Hepatic transferrin, possible hepcidin upregulation.	Rare. Commonest form in Japan, also seen in Italy and Brazil.	Autosomal recessive: high, but possibly confounded by observer bias.	Can be either juvenile or adult onset. Most cases are adult, with a slightly earlier and more severe course than Type 1.
TYPE 4A	*SLC40A1* (ferroportin)	Iron export	Rare	Autosomal dominant: high	Reduced end-organ damage and serum iron. Increased tissue sequestration. Reduced serum levels.
TYPE 4B	Ferroportin	Iron export	Rare	Autosomal dominant	Similar to classical haemochromatosis.

However, in those with elevated SF, venesection is to be used to bring SF down to maintenance levels.[46] There has been some debate in the literature with regards to the ideal level at which SF should be maintained. With reports that overzealous treatment may have unintended deleterious effects, the traditional suggestion of maintaining ferritin levels below 50 ug/l has been updated. Although the issue has not been settled conclusively, current guidelines suggest maintenance levels between 50-100 ug/l.[25,46] It is known that 1 unit of blood contains approximately 200-250 mg of iron. The amount of iron removed at each venesection, however, is highly variable.[45] This means that venesection intervals and treatment regimens need to be personalised to each patient informed on a case by case basis. The aforementioned treatment strategies work best with Types 1-3 and also with Type 4B. However, due to the aberrant iron export from cells in Type 4A patients may not tolerate venesection well.[47] Hence management of patients with Type 4A haemochromatosis is not so straightforward.

NEW RESEARCH AND INTERNATIONAL CONSORTIUM

Once the *HFE* locus was identified[48] it became apparent that it alone did not explain all cases of haemochromatosis. Parallels have been drawn between C282Y in haemochromatosis and Wilson's disease. However C282Y is a common polymorphism where *ATP7B* mutations in Wilson's disease are rare and usually deleterious for protein function. Studies resulted in the eventual identification of hepcidin, Transferrin receptor 2, HJV, and ferroportin.[29,30,33,48,49] Although mutations in these genes are much less common than in *HFE* they do explain many cases of haemochromatosis that are non-*HFE* or *HFE* negative (Table 1) and thus cases that were previously puzzling clinically. However, once again, clinicians looking after patients with iron overload were puzzled. In stark contrast to other familial liver diseases that are inherited as autosomal recessive traits, virtually all subjects with genetic mutations leading to the other diseases developed full blown disease, e.g. Wilson's disease. In 2010, an international consortium was formed to study the 'black swans' of this disease i.e. rare cases of advanced disease that stand out from the majority.[50] This group of eight centres fom the USA,Canada, and Australia is now funded by the National Institutes of Health and, so far, has discovered one significant gene (*GNPAT*) which appears to predispose to significant expression of this disease. The precise function and possible role *GNPAT* plays in iron metabolism is currently not known. Further modifying genes may be anticipated which assist clinicians in predicting those individuals predisposed to severe disease.

SUMMARY

The low penetrance of symptomatic haemochromatosis in those with HFE mutations and the worldwide prevalence of haemochromatosis in the absence of HFE mutations both have clarified the necessity to study other factors contributing to disease. The study of those who show iron overload without an underlying change in HFE has led to the discovery and classification of the non-HFE or HFE negative haemochromatosis; juvenile haemochromatosis, ferroportin disease, and transferrin receptor 2 associated haemochromatosis. Unfortunately, the reasons behind the incomplete penetrance of disease phenotype in those with HFE mutations has not yet been fully explained. Even though various environmental factors have been shown to influence the natural progression, it has become evident that further genetic factors must also play a part. To this end the discovery of GNPAT as a significant player in disease expression opens up the door to future investigation into this area. It also opens up future avenues for early detection and treatment of haemochromatosis.

REFERENCES

1. Von Recklinghausen F. Uber haemochromatose. Tageblatt Versammlung Dtsche Naturforscher Arzte Heidelberg. 1889;62:324-5.
2. McDonald CJ et al. Iron storage disease in Asia-Pacific populations: the importance of non-HFE mutations. J Gastroenterol Hepatol. 2013;28(7): 1087-94.
3. Wood MJ et al. The global burden of iron overload. Hepatol Int. 2009;3(3): 434-44.
4. Allen KJ et al. Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med. 2008;358(3):221-30.
5. Powell LW et al. Screening for hemochromatosis in asymptomatic subjects with or without a family history. Arch Intern Med. 2006;166(3):294-301.
6. Beutler E et al. Penetrance of

845G--> A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet. 2002;359(9302):211-8.

7. Guillemot J et al. Implication of the proprotein convertases in iron homeostasis: proprotein convertase 7 sheds human transferrin receptor 1 and furin activates hepcidin. Hepatology. 2013;57(6):2514-24.

8. Ganz T. Systemic iron homeostasis. Physiol Rev. 2013;93(4):1721-41.

9. Kanamori Y et al. Hepcidin expression in liver cells: evaluation of mRNA levels and transcriptional regulation. Gene. 2014;546(1):50-5.

10. Przybyszewska J, Zekanowska E. The role of hepcidin, ferroportin, HCP1, and DMT1 protein in iron absorption in the human digestive tract. Prz Gastroenterol. 2014;9(4):208-13.

11. Anderson GJ et al. The ceruloplasmin homolog hephaestin and the control of intestinal iron absorption. Blood Cells Mol Dis. 2002;29(3):367-75.

12. Donovan A et al. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab. 2005;1(3): 191-200.

13. Silvestri L et al. The extrahepatic role of TFR2 in iron homeostasis. Front Pharmacol. 2014;5:93.

14. Krause A et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett. 2000;480(2-3):147-50.

15. Park CH et al. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem. 2001;276(11):7806-10.

16. Pigeon C et al. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem. 2001;276(11):7811-9.

17. Rochette L et al. The iron-regulatory hormone hepcidin: a possible therapeutic target? Pharmacol Ther. 2014;pii:S0163-7258(14)00166-1.

18. Flanagan JM et al. In vivo imaging of hepcidin promoter stimulation by iron and inflammation. Blood Cells Mol Dis. 2007;38(3):253-7.

19. D'Alessio F et al. The hemochromatosis proteins HFE, TfR2, and HJV form a membrane-associated protein complex for hepcidin regulation. J Hepatol. 2012;57(5):1052-60.

20. Bridle KR et al. Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. Lancet. 2003;361(9358):669-73.

21. Babitt JL et al. Modulation of bone morphogenetic protein signaling in vivo regulates systemic iron balance. J Clin Invest. 2007;117(7):1933-9.

22. Camaschella C. BMP6 orchestrates iron metabolism. Nat Genet. 2009;41(4): 386-8.

23. Bardou-Jacquet E et al. Non-HFE hemochromatosis: pathophysiological and diagnostic aspects. Clin Res Hepatol Gastroenterol. 2014;38(2):143-54.

24. Wallace DF, Subramaniam VN. Non-HFE haemochromatosis. World J Gastroenterol. 2007;13(35):4690-8.

25. Kanwar P, Kowdley KV. Diagnosis and treatment of hereditary hemochromatosis: an update. Expert Rev Gastroenterol Hepatol. 2013;7(6):517-30.

26. Siddique A, Kowdley KV. Review article: the iron overload syndromes. Aliment Pharmacol Ther. 2012;35(8): 876-93.

27. De Gobbi M et al. Natural history of juvenile haemochromatosis. Br J Haematol. 2002;117(4):973-9.

28. Pietrangelo A. Hereditary hemochromatosis—a new look at an old disease. N Eng J Med. 2004;350(23): 2383-97.

29. Roetto A et al. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat Genet. 2003;33(1):21-2.

30. Papanikolaou G et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat Genet. 2004;36(1):77-82.

31. Bardou-Jacquet E et al. Variable age of onset and clinical severity in transferrin receptor 2 related haemochromatosis: novel observations. Br J Haematol. 2013;162(2):278-81.

32. Camaschella C, Poggiali E. Rare types of genetic hemochromatosis. Acta Haematol. 2009;122(2-3):140-5.

33. Camaschella C et al. The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nat Genet. 2000;25(1):14-5.

34. Worthen CA, Enns CA. The role of hepatic transferrin receptor 2 in the regulation of iron homeostasis in the body. Front Pharmacol. 2014;5:34.

35. Bassett ML et al. The changing role of liver biopsy in diagnosis and management of haemochromatosis. Pathology. 2011;43(5):433-9.

36. Brissot P. Hereditary hemochromatosis. Hematology. 2013;18(6):370-1.

37. Gan EK et al. Natural history and management of HFE-hemochromatosis. Semin Liver Dis. 2011;31(3):293-301.

38. Kanwar P, Kowdley KV. Metal storage disorders: Wilson disease and hemochromatosis. Med Clin North Am. 2014;98(1):87-102.

39. Adams PC. The natural history of untreated HFE-related hemochromatosis. Acta Haematol. 2009;122(2-3):134-9.

40. Fracanzani AL et al. Hemochromatosis in Italy in the last 30 years: role of genetic and acquired factors. Hepatology. 2010;51(2):501-10.

41. Nadakkavukaran IM et al. Screening for hereditary haemochromatosis. Pathology. 2012;44(2):148-52.

42. Asberg A et al. Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,238 persons. Scand J Gastroenterol. 2001;36(10):1108-15.

43. Pietrangelo A. Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. Gastroenterology. 2010;139(2):393-408, 408.e1-2.

44. Adams PC et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Eng J Med. 2005;352(17):1769-78.

45. Bacon BR. Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. Hepatology. 2011;54(1):328-43.

46. European Association for the Study Of the Liver. EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol. 2010;53(1):3-22.

47. Pietrangelo A. The ferroportin disease. Blood Cells Mol Dis. 2004;32(1):131-8.

48. Feder JN et al. A novel MHC class I–like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996;13(4):399-408.

49. Montosi G et al. Autosomal-dominant hemochromatosis is associated with a mutation in the ferroportin (SLC11A3) gene. J Clin Invest. 2001;108(4):619-23.

50. Emond MJ et al. Exome sequencing identifies genes and variant alleles associated with severity of iron overload in hemochromatosis HFE C282Y homozygotes. Blood. 2013;122(21):179.

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HOW I DIAGNOSE AND TREAT ACUTE (FULMINANT) LIVER FAILURE

Dr Kenneth J. Simpson

Senior Lecturer in Hepatology, University of Edinburgh & Honorary Consultant Physician, Scottish Liver Transplant Unit, Royal Infirmary, Edinburgh, UK.

INTRODUCTION

Acute (fulminant) liver failure (ALF) is a rare but devastating clinical condition resulting in massive hepatocyte cell death. The clinical syndrome is characterised by hepatic encephalopathy (HE) in the presence of significant liver injury as evidenced by deranged coagulation and elevated serum transaminases. ALF can rapidly progress to multi-organ failure and death. The causes of ALF vary according to geographical area; paracetamol or acetaminophen hepatotoxicity (POD) is more common (70-80% cases series) in the developed world, and in contrast, viral hepatitis is more frequently found in other countries. However, in most case series' the cause of ALF cannot be identified in a significant proportion of patients, so-called seronegative or nonA-E hepatitis. The ALF syndrome is classified further according to the period between the development of clinical jaundice and HE; hyperacute ALF (≤7 days), acute ALF (8-28 days), and sub-acute ALF (5-12 weeks). POD characteristically causes hyperacute ALF and is associated with greater risk of fatal complications such as cerebral oedema (CO), but relatively increased spontaneous survival. In contrast, viral and seronegative hepatitis causes acute or subacute ALF, the risk of CO is lower but the chances of spontaneous survival are relatively reduced.

How do I Recognise Patients with ALF?

Because POD is the most common cause of ALF in the UK, this seems a rather easy-to-answer question. However, in the UK the proportion of cases following single time point suicidal consumption of excessive amounts of paracetamol is becoming less frequent. Suicidal consumption of paracetamol and subsequent hepatotoxicity follows a recognisable clinical course; the patients usually present because they have taken an overdose, a significant elevation of serum transaminases develops, reaching a peak level of several 100-fold increase by 3 days, increasing coagulopathy, acidosis, kidney injury, and HE follow with multi-organ failure and death. In contrast, after accidental consumption of excessive paracetamol patients present significantly unwell, possibly unconscious, hypothermic, hypotensive with significant acidosis, kidney injury, and hypoglycaemia. Serum transaminases may not be significantly elevated in such cases, but paracetamol may still be detectable in the blood and the patients are usually significantly jaundiced with coagulopathy. Paradoxically, patients with accidental overdoses have a significantly worse outcome compared with suicidal overdose.

In contrast, patients with acute or sub-acute ALF can be difficult to differentiate from those with decompensated chronic liver or acute-on-chronic liver failure. These patients may have jaundice, encephalopathy, ascites, and portal hypertension. Often radiological imaging shows a small and shrunken liver, due to parenchymal collapse rather than cirrhotic nodules. A relatively short clinical course may be the only clue and such cases require a high index of suspicion to be identified. Where there is doubt, liver biopsy is essential, although this may have to be acquired via the transjugular route due to the coagulopathy and ascites. Wilson's disease may present with ALF; this is an extraordinarily rare but characteristic clinical syndrome. The patients are usually in their late teens, presenting with encephalopathy, very high serum bilirubin due to haemolytic anaemia, and low serum alkaline phosphatase levels. These patients have liver cirrhosis, but are managed in similar fashion to other cases of ALF.

How do I Manage Patients without HE or with Low-Grade HE?

Patients with significant acute liver injury and coagulopathy require management in high dependency or intensive care environments.

Close monitoring of organ systems, particularly renal, cardiovascular, and neurological systems, is essential. Frequent biochemical monitoring of blood glucose, phosphate, coagulation, creatinine, and other liver functions is required, sometimes as frequently as 4-times per day. Blood glucose may be measured even more frequently in hyperacute cases, where 2-hourly measurements would not be excessive. Careful attention to fluid balance, correction of electrolyte disturbance (especially hyponatraemia), and avoidance of significant hypotension are important. Coagulation monitoring mostly utilises measuring the prothrombin time (PT) and impressive prolongation of PT can be observed. Routine correction of PT should be avoided as this is an important prognostic marker. More complete assessment of the coagulation and fibrinolytic systems in ALF has revealed a balance that results in no increased risk of spontaneous bleeding. In fact, invasive monitoring can be safely inserted in patients with ALF and prolonged PT. We only routinely administer coagulation factors when inserting intracranial pressure monitors. N-acetylcysteine may be helpful in both POD and other causes of ALF. Specific treatments such as anti-viral therapy, transjugular intrahepatic stent shunts, or delivery may be appropriate for viral hepatitis, acute Budd-Chiari syndrome, and pregnancy associated ALF, respectively. Early psychiatric involvement, before the development of HE in cases of POD, are often crucial in assisting decisions regarding emergency liver transplantation (LTx) should a patient deteriorate.

When do I Decide to Refer to a Transplant Centre?

There are few data to guide decisions regarding transfer of patients to liver transplant centres. Transfer of patients following the development of HE can be risky, with the significant potential for deepening HE, development of CO, or cardiovascular instability. Patients with HE should be ventilated for transfer. Waiting until patients have achieved national criteria for transplantation in referring units before transfer should be avoided; this limits assessment in transplant centres and may fatally delay listing for emergency LTx. Early discussion with a transplant centre should be undertaken where there is doubt. Published guidelines from the British Society of Gastroenterology suggest early acidosis (pH <7.3), rising international normalised ratio (>3.5 and rising), or PT (>number of hours after the overdose), HE, rising serum creatinine (>200 µmol/l), or hypoglycaemia are worrying clinical features and should prompt discussion with transplant units in patients with POD. Elevated arterial lactate (>3.5 mmol/l) especially if not responsive to fluid resuscitation is another sign of potential for deterioration in patients following POD. Recently we have suggested that sequential organ failure assessment (SOFA) scoring may assist transfer decisions in patients with POD; SOFA scores of <7 are rarely associated with significant extra-hepatic organ failure and HE.

Timing transfer of patients with non-paracetamol ALF is more problematic and again early discussion with transplant centres is advocated. Rising bilirubin and shrinking liver volume are signs of potential deterioration in patients with non-POD severe liver injury. Patients who have otherwise reached the UK non-paracetamol ALF transplant listing criteria, but without HE should be discussed. Infection in these patients can cause rapid and fatal deterioration. Within the UK there are discussions regarding potential for emergency transplant listing in such cases in the absence of HE.

How do I Manage Patients with ALF in the Intensive Therapy Unit (ITU)?

Managing patients with ALF in ITU is a complex and challenging topic. Locally a team of physicians and intensivists manages our patients. Several guidelines have been published, EASL are due to publish more up-to-date guidelines shortly. Early renal replacement therapy, protective ventilation strategies, and cardiovascular support (with norepinepherine) are often required. Continued controversy regards prophylactic antibiotic therapy and use of intracranial pressure monitoring. Locally we use prophylactic antibiotic and anti-fungal therapy in all ventilated patients. Our hyperacute ALF patients have intracranial pressure monitoring guided by arterial ammonia concentration; significantly elevated ammonia is associated with increased risk of raised intracranial pressure in hyperacute ALF. We use mannitol and hypertonic saline as our first-line treatment of CO. Use of plasmapharesis may improve survival, but this trial has not been published as a full paper yet. There is continued hope for the development of an effective liver support device for use in ALF cases; a meta-analysis of the currently published data suggested survival advantage but the introduction of liver support into routine clinical management presents a considerable logistical challenge.

How do I Decide if Patients with ALF need Emergency LTx?

A recent provocative review challenged conventional criteria for emergency LTx in patients with ALF, especially in POD-ALF. LTx has never been the subject of a randomised trial in ALF. Improved spontaneous survival in POD cases with careful ITU management have been reported in one specialist unit, but it is not clear if this occurs elsewhere. In the UK the modified King's College criteria are used to identify patients for emergency LTx, but these were first published in 1989. Two recent systematic reviews and meta-analysis of these transplant criteria have highlighted reduced sensitivity and also some reduction in specificity over the intervening years. A national review of emergency LTx criteria has been commissioned in the UK and this group is due to report soon.

Currently my approach to deciding candidacy for LTx of an individual ALF patient depends on collecting as much information regarding the patients previous history before onset of HE; alcohol, drug, and previous psychiatric issues are most relevant in POD patients and may exclude up to 25-30% of cases from further consideration. Some patients are just too unstable to consider transplantation (25-30%), a situation that is more common in POD cases. I will not list POD cases without HE that achieve the 'lactate criteria' (arterial lactate >24 hours post-overdose >3.5 mmol/l on admission or >3.0 mmol/l after overdose and after fluid resuscitation). I am also reluctant to use these criteria alone when listing POD cases after they have developed HE and prefer to see other evidence of clinical deterioration. Otherwise I would use the conventional King's College criteria to decide if patients have better survival with emergency LTx.

FURTHER READING

Craig DG et al. The systemic inflammatory response syndrome and sequential organ failure assessment scores are effective triage markers following paracetamol (acetaminophen) overdose. Aliment Pharmacol Ther. 2011;34(2):219-28.

Lee WM et al. Introduction to the revised American Association for the Study of Liver Diseases Position Paper on acute liver failure 2011. Hepatology. 2012;55(3):965-7.

Devlin J, O'Grady J. Indications for referral and assessment in adult liver transplantation: a clinical guideline. British Society of Gastroenterology. Gut. 1999;45 Suppl 6:VI1-VI22.

Craig DG et al. Review article: the current management of acute liver failure. Aliment Pharmacol Ther. 2010;31(3):345-58.

Craig DG et al. Systematic review: prognostic tests of paracetamol-induced acute liver failure. Aliment Pharmacol Ther. 2010;31(10):1064-76.

McPhail MJ et al. Meta-analysis of performance of Kings's College Hospital Criteria in prediction of outcome in non-paracetamol-induced acute liver failure. J Hepatol. 2010;53(3):492-9.

Stutchfield BM et al. Is there a role for extracorporeal liver support as a bridge to liver transplantation in acute liver failure? Transplantation. 2011;92(8):e44-5.

O'Grady J. Timing and benefit of liver transplantation in acute liver failure. J Hepatol. 2014;60(3):663-70.

Bernal W et al. Lessons from look-back in acute liver failure? A single centre experience of 3300 patients. J Hepatol. 2013;59(1):74-80.