CORRESPONDENCE

Generation of Human Induced Pluripotent Stem Cells from Liver Progenitor Cells by Only Small Molecules

To the Editor:

The team of Liu et al. generated endoderm-derived human induced pluripotent stem (iPS) cells from primary hepatocytes.[1] However, they generated human iPS cells by using viral transgenes.[1] Clinical applications of human iPS cells require avoiding viral transgenes. On the other hand, the reprogramming of human cells with only small molecules has yet to be reported. Therefore, we tried to reprogram human liver progenitor cells with only two small molecules.[2] First, octamer3/4-positive human liver progenitor cells were treated with 2′O-methyl-microRNA-145 as microRNA-145 inhibitor (100 nmol/L for 96 hours; after that, 50 nmol/L for 72 hours) and transforming growth factor-beta ligand (100 pM for 48 hours) in human embryonic stem (hES) cell medium.[2] As a result, we could generate human iPS cells from human liver progenitor cells only by use of small molecules.[2] The human iPS cells were similar to hES cells in morphology, proliferation, surface antigens, gene expression, and epigenetic status of pluripotent cell-specific genes.[2] Furthermore, these cells could differentiate into cell types of the three germ layers *in vitro* and in teratomas.[2] Therefore, we designated the human iPS cells as chemicals-human induced pluripotent stem (ChiPS) cells.[2]

On the other hand, although Liu et al. did not show the risk evaluation of malignant transformations for the human iPS cells lines that they generated,[1] we performed the risk evaluation.[2]

It was reported that cancer risk for patients with Down syndrome was less than healthy individuals, and the microvessel density (MVD) within severe combined immunodeficient (SCID) mice in which human iPS cells derived from patients with Down syndrome were transplanted was also less than the MVD in SCID mice in which human iPS cells derived from healthy people were transplanted.[3] Therefore, according to the method of Baek et al.,[3] by using MVD within SCID mice in which ChiPS cell lines as human iPS cell lines were transplanted, we performed the risk evaluation of malignant transformations for the cell lines. As a result, the MVD in our study[2] was equal to the case[3] of patients with Down syndrome.

Furthermore, we tried to differentiate human normal hepatocytes from ChiPS cells as human iPS cells, according to the method of Liu et al.[1] As a result, we could generate mature hepatocytes 21 days after the initiation of differentiation (Fig. 1). Moreover, according to the method of Liu et al.,[1] although we evaluated cytochrome P450 (CYP450) metabolism in ChiPS cell–derived mature hepatocytes, the CYP3A4 and CYP1A2 activity appeared to be the same as in the case of the ihH10 cell line that Liu et al.[1] generated.

In conclusion, human iPS cells that Liu et al.[1] or we[2] generated would be useful for the study of liver disease pathogenesis. However, our ChiPS cells[2] would have an advantage in clinical applications of human iPS cells.

Hisashi Moriguchi[1,2,3]
Raymond T. Chung[2]
Makoto Mihara[1]
Chifumi Sato[3]
[1]*Department of Plastic and Reconstructive Surgery, The University of Tokyo Hospital, Tokyo, Japan*
[2]*Gastrointestinal Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA*
[3]*Department of Analytical Sciences, Tokyo Medical and Dental University, Tokyo, Japan*

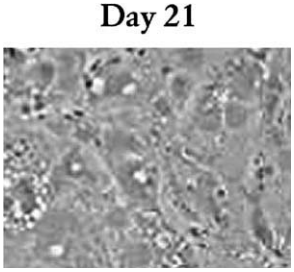

Fig. 1. Mature hepatocytes at 21 days after differentiation initiation from ChiPS cells as human iPS cells.

References

1. Liu H, Ye Z, Kim Y, Sharkis S, Jang YY. Generation of endoderm-derived human induced pluripotent stem cells from primary hepatocytes. Hepatology 2010;51:1810-1819.
2. Moriguchi H, Chung RT, Mihara M, Sato C. The generation of human induced pluripotent stem (iPS) cells from liver progenitor cells by only small molecules and the risk for malignant transformations of the cells. ACSC 2010; in press. Available at: http://www.jstage.jst.go.jp/browse/acsc.
3. Baek KH, Zaslavsky A, Lynch RC, Britt C, Okada Y, Siarey RJ, et al. Down's syndrome suppression of tumour growth and the role of the calcineurin inhibitor DSCR1. Nature 2009;459:1126-1130.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.23851
Potential conflict of interest: Nothing to report.

Reply:

We thank Moriguchi et al. for their interest in our study[1] recently published in Hepatology. We share the view that tumor risk evaluation for induced pluripotent stem (iPS) cells is important and that clinical applications of human iPS cells require safe iPS generation. Our article is the first to demonstrate the feasibility of deriving human iPS cells from cells of human endoderm origin (i.e., human primary hepatocytes that are very short lived *in vitro* and thus are considered to be hard to reprogram into iPS cells) and redirecting these endoderm-derived iPS cells into multistage hepatic cells.

Because we are fully aware of the importance of tumor risk evaluation for iPS cells and their derivatives, *in vivo* transplantation studies with our human endoderm iPS cells have been performed. So far, we have not detected any malignant transformation with hepatic cells differentiated from iPS cells when they have been transplanted into immunodeficient mouse models of chronic liver diseases.

More importantly, we have also derived virus-free and integration-free human iPS cells (Fig. 1A) with an established nonviral approach,[2] and we have been able to differentiate these virus-free iPS cells into hepatic endoderm cells (Fig. 1B). Therefore, we have demonstrated that the hepatic differentiation protocol that we used in our previous study[1] is also applicable to the hepatic differentiation of virus-free and integration-free human iPS cells (Fig. 1).

Although we have observed reliable iPS generation with the nonviral method (Fig. 1), it is less efficient than the viral protocol; the reprogramming process has been significantly longer with the

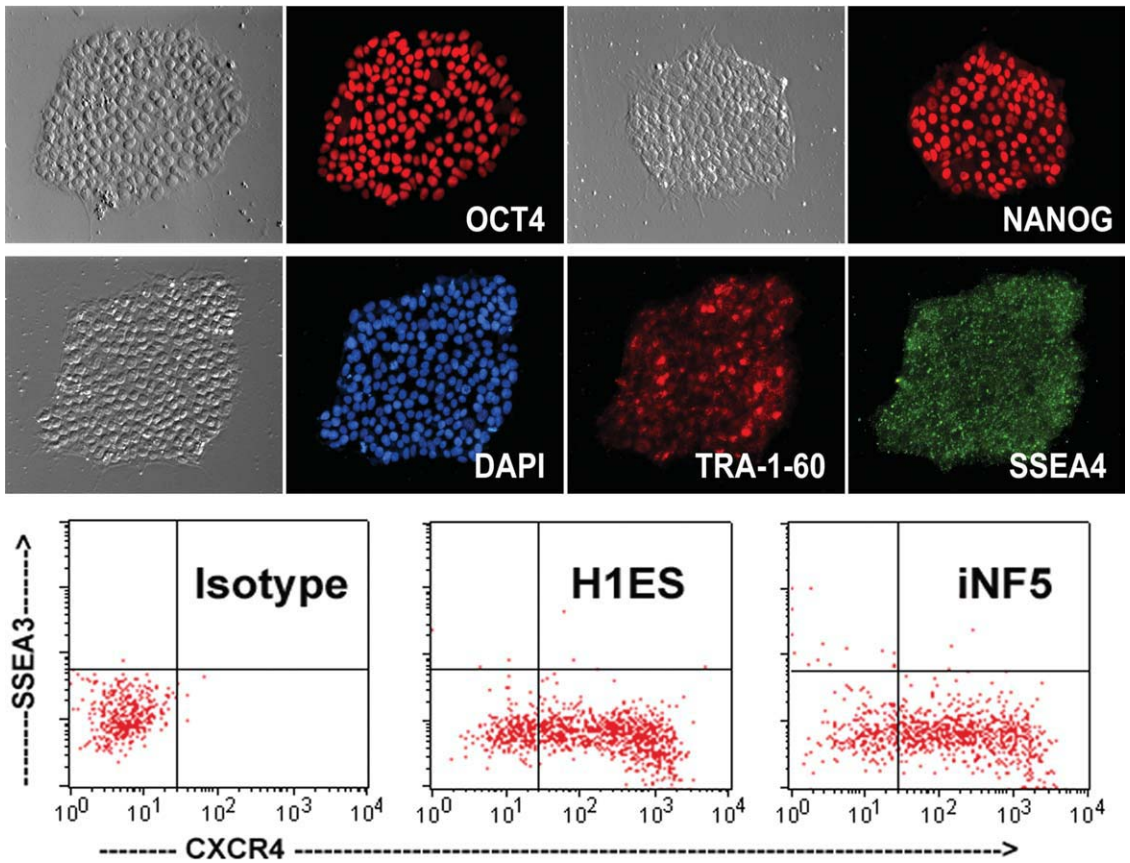

Fig. 1. (A) Representative immunofluorescence analysis of one of the integration-free human iPS cell lines (iNF5) growing on Matrigel. The clear expression of embryonic stem cell surface antigens SSEA4 and TRA-1-60 and nuclear transcription factors OCT4 and NANOG can be observed. (B) Differentiation of iNF5 cells into hepatic cells: efficient endoderm induction of integration-free human iPS cells. Fluorescence-activated cell sorting analysis showed that approximately 80% of the induced cells expressed the definitive endoderm marker CXCR4 on day 4 after activin A treatment. Abbreviations: CXCR4, chemokine (C-X-C motif) receptor 4; DAPI, 4′,6-diamidino-2-phenylindole; OCT4, octamer 4; SSEA, stage-specific embryonic antigen.

nonviral method (17-35 days for the detection of iPS colonies) versus the viral method that we reported (6-14 days for the detection of iPS colonies).[1] Therefore, novel reprogramming technologies that can efficiently generate safe iPS cells from mature cell types will be highly beneficial to the field.

Hua Liu, M.D., Ph.D.
Yonghak Kim, M.S.
Saul Sharkis, Ph.D.
Zhaohui Ye, Ph.D.
Yoon-Young Jang, M.D., Ph.D.
Johns Hopkins University School of Medicine
Baltimore, MD

References

1. Liu H, Ye Z, Kim Y, Sharkis S, Jang YY. Generation of endoderm-derived human induced pluripotent stem cells from primary hepatocytes. Hepatology 2010;51:1810-1819.
2. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science 2009;324:797-801.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.23871
Potential conflict of interest: Nothing to report.

Twelve-Week Posttreatment Follow-Up Predicts a Sustained Virological Response to Pegylated Interferon and Ribavirin Therapy

To the Editor:

The article by Martinot-Peignoux and colleagues[1] nicely demonstrates that testing for serum hepatitis C virus (HCV) RNA with the highly sensitive transcription-mediated amplification assay [lower limit of detection (LOD) = 9.6 IU/mL] 12 weeks after the end of a treatment period with pegylated interferon (PegIFN) and ribavirin (Rbv) is as effective as the standard 24-week posttreatment completion measurement for assessing sustained virological response (SVR) in patients with chronic hepatitis C. Although we

Table 1. Serum HCV RNA Outcome During the 24 Weeks Posttreatment Follow Up

Serum HCV RNA (Follow-Up)	Patients (n)	HCV RNA(−)	SVR	PPV (95% CI)
Amplicor (LOD = 50 IU/mL)	431			
Week 4		285	259	91% (86.9%-93.9%)
Week 12		259	259	100% (98.6%-100%)
Week 24		259	259	100% (98.6%-100%)
TaqMan (LOD = 15 IU/mL)	72			
Week 4		38	32	84% (69%-94%)
Week 12		32	32	100% (89%-100%)
Week 24		32	32	100% (89%-100%)

acknowledge that anticipating the response to therapy is likely to positively affect the management of HCV patients, we think that it is important to externally validate these results even with less sensitive commercially available HCV RNA assays. To answer this question, we analyzed two cohorts of consecutively treated naïve patients with chronic hepatitis C in whom different HCV RNA assays were used to determine the viral response during treatment and follow-up. The first cohort, consisting of 431 patients of any HCV genotype treated with either PegIFN-alfa2a/Rbv or PegIFN-alfa2b/Rbv (Milan Safety Tolerability study),[2] was tested during and after treatment with a qualitative HCV RNA assay with a lower LOD of 50 IU/mL (COBAS Amplicor HCV test version 2.0, Roche Diagnostics).[3] The second cohort consisted of 72 HCV-1 and HCV-4 patients who were consecutively treated with PegIFN-alfa2a/Rbv and evaluated with a real-time polymerase chain reaction assay with an LOD of 15 IU/mL (COBAS TaqMan 2.0, Roche Diagnostics).[4] Both cohorts were followed up for at least 12 months after treatment completion, and they showed no hepatitis relapse after the achievement of SVR by the 24-week posttreatment time point, the SVR rates being 60% and 44%, respectively. With both assays, undetectable HCV RNA during week 12 of the posttreatment follow-up had a 100% positive predictive value (PPV) for SVR, with 95% confidence intervals (CIs) that very closely mimicked those reported by Martinot-Peignoux et al. in their study (Table 1). In our experience, during week 12 of follow-up, HCV RNA testing using assays less sensitive than the one used by Martinot-Peignoux and colleagues provided reliable estimates of SVR to PegIFN/Rbv therapy in naïve patients. Whether this holds true also for the retreatment of patients who have failed a previous course of interferon-based therapy remains to be established.

Alessio Aghemo, M.D.
Maria Grazia Rumi, M.D.
Stella De Nicola, M.D.
Massimo Colombo, M.D.
A. M. Migliavacca Center for Liver Disease
First Division of Gastroenterology
Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Ca' Granda, Ospedale Maggiore Policlinico
Universitá degli studi di Milano, Italy

References

1. Martinot-Peignoux M, Stern C, Maylin S, et al. Twelve weeks post-treatment follow-up is as relevant as 24 weeks to determine the sustained virologic response in patients with hepatitis C virus receiving pegylated interferon and ribavirin. Hepatology 2010;51.
2. Rumi MG, Aghemo A, Prati GM, D'Ambrosio R, Donato MF, Soffredini R, et al. Randomized study of peginterferon-alpha2a plus ribavirin vs peginterferon-alpha2b plus ribavirin in chronic hepatitis C. Gastroenterology 2010;138:108-115.
3. Doglio A, Laffont C, Caroli-Bosc FX, Rochet P, Lefebvre J. Second generation of the automated Cobas Amplicor HCV assay improves sensitivity of hepatitis C virus RNA detection and yields results that are more clinically relevant. J Clin Microbiol 1999;37:1567-1569.
4. Kawai S, Yokosuka O, Kanda T, Imazeki F, Maru Y, Saisho H. Quantification of hepatitis C virus by TaqMan PCR: comparison with HCV Amplicor Monitor assay. J Med Virol 1999;58:121-126.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.23685
Potential conflict of interest: Nothing to report.

Sorafenib Therapy in Patients with Hepatocellular Carcinoma Before Liver Transplantation

To the Editor:

There is great interest in the role of neoadjuvant therapies in patients with hepatocellular carcinoma (HCC) awaiting liver transplantation. The recent study by Vitale et al.[1] in Hepatology considers the role of sorafenib in this setting, and this approach is highlighted in the review of sorafenib by Finn.[2] Sorafenib inhibits multiple pathways implicated in HCC pathogenesis, most notably vascular endothelial growth factor (VEGF)–stimulated angiogenesis through inhibition of the receptor tyrosine kinase activity of VEGF receptors.

Although this study addresses an important and highly relevant clinical question, there are developing concerns regarding the use of anti-VEGF therapies in this setting. It is recognized that despite effective blockade of angiogenesis, there is inevitable tumor progression (reviewed by Bergers and Hanahan[3]). There is now emerging evidence from preclinical mouse models that anti-VEGF therapy in the form of receptor tyrosine kinase inhibition promotes invasion and increases the metastatic potential of tumors.[4,5] In the study by Pàez-Ribes et al.,[4] treatment with sunitinib (a multiple-receptor tyrosine kinase inhibitor similar to sorafenib) for as little as 1 week increased invasiveness and metastases in models of pancreatic neuroendocrine tumors and glioblastoma. In models of both breast cancer and malignant melanoma, when mice were pretreated with either sorafenib or sunitinib, both agents promoted metastases and shortened survival.[5] It is important to note that the authors also found more rapid development of metastases in models in which anti-VEGF therapy was given as adjuvant therapy. The mechanisms driving tumor progression in this environment are not well understood but may rely on the generation of tumor hypoxia, the expression of alternative growth factors, and/or the induction of an epithelial-to-mesenchymal transition.[3]

These preclinical data argue that neoadjuvant treatment with sorafenib, rather than slowing disease progression, may increase tumor invasiveness and metastatic potential during therapy and the recurrence of HCC after liver transplantation. The study by Vitale et al.[1] is based on the assumption that the hazard ratio of disease progression with sorafenib treatment is known. However, because the clinical studies of sorafenib[6,7] address the use of this drug in

patients with advanced disease, this may not be representative of the efficacy of sorafenib in the population with HCC being considered for liver transplantation. It would be interesting to know to what extent increased HCC recurrence and consequent decreases in survival rates after transplantation would influence overall outcomes in this model.

Although a study of sorafenib as neoadjuvant therapy for patients with HCC is appropriate,[1,2] it is imperative that such a study be adequately designed to assess disease progression while patients are receiving sorafenib treatment, the tumor phenotype in the explant, and the overall outcomes of patients receiving this therapy.

IAN A. ROWE, MBChB MRCP(UK)
Hepatitis C Virus Research Group
Centre for Liver Research
Institute of Biomedical Research
University of Birmingham
Birmingham, United Kingdom

References

1. Vitale A, Volk ML, Pastorelli D, Lonardi S, Farinati F, Burra P, et al. Use of sorafenib in patients with hepatocellular carcinoma before liver transplantation: a cost-benefit analysis while awaiting data on sorafenib safety. HEPATOLOGY 2010;51:165-173.
2. Finn RS. Drug therapy: sorafenib. HEPATOLOGY 2010;51:1843-1849.
3. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 2008;8:592-603.
4. Pàez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Viñals F, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell 2009;15:220-231.
5. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell 2009;15:232-239.
6. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008; 359:378-390.
7. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25-34.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.23702
Potential conflict of interest: Nothing to report.

Possible Role of Adipocytokines in the Development of Nonalcoholic Steatohepatitis–Related Hepatocellular Carcinoma

To the Editor:

We read with great interest the article by Ascha et al.[1] on the incidence and risk factors for hepatocellular carcinoma (HCC) in patients with nonalcoholic steatohepatitis (NASH). The authors state that patients with NASH cirrhosis have a greatly increased risk of HCC and that the most significant factor related to this increase seems to be alcohol consumption. Although this study is important and intriguing in a number of ways and provides insight into the risk factors involved in HCC development, we would like to add one additional comment to the authors' discussion that is noteworthy in several respects.

In their article,[1] the authors attempt to discuss comprehensively the incidence and risk factors for NASH-related and hepatitis C virus–related cirrhosis and HCC. Although it is obvious that evaluating the mechanisms for these diseases is beyond the scope of their study, it would have been worthwhile for the authors to mention a potential underlying mechanism for these conditions that could lead to a better understanding of the results achieved in their report. For this reason, we would like to draw attention to the possible role of adipocytokines in the development of HCC, which could elucidate the risk factors associated with the development of HCC.

Among the great number of mechanisms that have been proposed to explain the link between NASH-related cirrhosis and HCC, adipocytokine dysregulation offers new and promising perspectives for a wide range of liver diseases. Adipose tissue is a major source of bioactive substances known as adipocytokines, which include resistin, leptin, tumor necrosis factor α, and adiponectin.[2] Hypertrophied adipocytes in obesity release chemokines that induce the accumulation of macrophages in visceral adipose tissue, which then produce nitric oxide and proinflammatory cytokines. These inflammatory changes induce adipocytokine dysregulation, which is characterized by a decrease in insulin-sensitizing and anti-inflammatory adipocytokines and an increase in proinflammatory adipocytokines.[2] Disturbed adipocytokine secretion due to inflammatory changes in obese adipose tissue might, therefore, promote hepatic steatosis, inflammation, fibrogenesis, or hepatocarcinogenesis of the liver.[3] Moreover, alcohol, which induces cell death and inflammation in the liver, can cause the development of HCC in NASH patients because of the liver injury already triggered by adipocytokine dysregulation.

In conclusion, on the basis of the aforementioned mechanisms, it is reasonable to expect increased rates of HCC in NASH patients, especially those with increased alcohol consumption. Further clinical and experimental research is warranted to elucidate the association between adipocytokines and liver disease.

YAVUZ BEYAZIT, M.D.[1]
MURAT KEKILLI, M.D.[1]
TUGRUL PURNAK, M.D.[2]
MEVLUT KURT, M.D.[1]
[1]*Department of Gastroenterology*
Turkiye Yuksek Ihtisas Hospital
Ankara, Turkey
[2]*Department of Gastroenterology*
Ankara Numune Education and Research Hospital
Ankara, Turkey

References

1. Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. HEPATOLOGY 2010;51:1972-1978.
2. Kamada Y, Takehara T, Hayashi N. Adipocytokines and liver disease. J Gastroenterol 2008;43:811-812.
3. Schäffler A, Schölmerich J, Büchler C. Mechanisms of disease: adipocytokines and visceral adipose tissue—emerging role in nonalcoholic fatty liver disease. Nat Clin Pract Gastroenterol Hepatol 2005;2:273-280.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.23701
Potential conflict of interest: Nothing to report.

"Normal" Alanine Aminotransferase and Christopher Boorse

To the Editor:

I read with great interest the article by Lee et al.[1] in HEPATOLOGY regarding the healthy upper limit of normal of serum alanine aminotransferase (ALT) for Korean liver donors with histologically normal livers. Like Prati et al.,[2] they echo the claim for lowering the healthy upper limit of normal threshold of ALT. However, I have a few comments.

Serum ALT is an easily available, low-cost screening tool for detecting silent liver disease.[3] To screen for disease, we must know what is healthy. In this respect, as in most clinical laboratory tests, we have taken recourse to the biostatistical theory of health as articulated by Christopher Boorse,[4] who defined *health* ("freedom from disease") as "the statistical normality of function, i.e., the ability to perform all typical physiological functions with at least typical efficiency." *Normal function* means the statistically typical contribution of all the organism's parts and processes to the organism's overall goals of survival and reproduction. The group with respect to which a contribution is considered statistically typical is the reference class, "a natural class of organism of uniform functional design" and specifically an age group of a sex of a race of a species.[4] Thus, the American Gastroenterological Association has defined the normal range of ALT as the mean ± 2 standard deviations of the ALT levels of the normal population,[5] with ALT ranges often being stratified by sex but not by age.

The disadvantage of the biostatistical theory model is that *normal*, often interchangeably used with *healthy*, will vary according to the chosen reference class,[6] such as voluntary blood donors and laboratory technicians.[2,3] The choice of the reference class causes interlaboratory variability in the reference range of ALT.[7] Metabolically abnormal individuals presumed to have a high risk of underlying nonalcoholic fatty liver disease were excluded from the reference class in Prati et al.'s study,[2] but they were found to have normal liver histology, albeit with statistically higher ALT levels, and were included in this study.[1] Moreover, is the chosen reference class representative of the general population? Voluntary blood donors represent the healthiest subset of the general population, and this is reflected by their significantly lower mortality and incidence of cancer and transfusion-transmittable viral infections in comparison with the general population; this is due to self-selection (altruism) and strict screening guidelines.[8,9] Liver donors also undergo similarly strict selection procedures. Should reference ranges of ALT obtained from such cohorts be used for the general population? Finally, why did the authors exclude 627 individuals with simple steatosis from their reference class? Individuals with simple steatosis do not have different long-term outcomes vis-à-vis an age-matched and sex-matched general population.[10]

Another way of defining healthy levels involves outcome studies, which are based on the development of adverse events during long-term follow-up (e.g., blood pressure).[11] Here, *disease* is defined as "a state that places individuals at increased risk of adverse consequences."[12] An increased ALT level, even within the present normal range, is definitely a predictor of future development of metabolic syndrome[13] and has been associated with increased overall, cardiovascular, and liver disease–related mortality in some but not all studies.[11] The future publication of outcome studies will guide us further in this respect.

Finally, race has never been used to select the reference class for ALT. The significant genetic component in ALT variability among twins, even after adjustments for age, sex, body mass index, and alcohol consumption,[14] points to the possibility that normal values of ALT will vary according to race, and this may be an explanation for the slight difference in the upper limit of normal of "normal" ALT levels between Koreans and Italians.[1,2]

KSHAUNISH DAS
Associate Professor
Division of Gastroenterology
School of Digestive and Liver Diseases
Institute of Post Graduate Medical Education and Research
Kolkata, India

References

1. Lee JK, Shim JH, Lee HC, Lee SH, Kim KM, Lim Y, et al. Estimation of the healthy upper limits for serum alanine aminotransferase in Asian populations with normal liver histology. HEPATOLOGY 2010;51:1577-1583.
2. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 2002;137:1-10.
3. Kaplan MM. Alanine aminotransferase levels: what's normal? Ann Intern Med 2002;137:49-51.
4. Boorse C. Health as a theoretical concept. Philos Sci 1977;44:542-573.
5. Green RM, Flamm S. AGA technical review on the evaluation of liver chemistry tests. Gastroenterology 2002;123:1367-1384.
6. Kingma E. What is it to be healthy? Analysis 2007;67:128-133.
7. Neuschwander-Tetri B, Unalp A, Creer M. Influence of local reference populations on upper limits of normal for serum alanine aminotransferase levels. Arch Intern Med 2008;168:663-666.
8. Edgren G, Tran TM, Hjalgrim H, Rostgaard K, Shanwell A, Titlestad K, et al. Improving health profile of blood donors as a consequence of transfusion safety efforts. Transfusion 2007;47:2017-2024.
9. Glynn SA, Kleinman SH, Schreiber GB, Busch MP, Wright DJ, Smith JW, et al. Trends in incidence and prevalence of major transfusion-transmissible viral infections in US blood donors, 1991 to 1996. Retrovirus Epidemiology Donor Study (REDS). JAMA 2000;284:229-235.
10. Angulo P. Long-term mortality in nonalcoholic fatty liver disease: is liver histology of any prognostic significance? HEPATOLOGY 2010;51: 373-375.
11. Duofour DR. Alanine aminotransferase: is it healthy to be "normal"? HEPATOLOGY 2009;50:1699-1701.
12. Temple LFK, McLeod RS, Gallinger S, Wright JG. Defining disease in the genomics era. Science 2001;293:807-808.
13. Fraser A, Ebrahim S, Harris R, Smith GD, Sattar N, Lawlor DA. Alanine aminotransferase, γ-glutamyltransferase, and incident diabetes. The British Women's Heart and Health Study and Meta-Analysis. Diabetes Care 2009;32:741-750.
14. Rahmioglu N, Andrew T, Cherkass L, Surdulescu G, Swaminathan R, Spector T, et al. Epidemiology and genetic epidemiology of the liver function test proteins. PLoS ONE 2009;4:e4435.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.23731
Potential conflict of interest: Dr. Das is a consultant for Novartis.

Optimal Dosage of Ribavirin

To the Editor:

We read with interest the article by Diago et al.,[1] who attempted to identify factors predictive of sustained virological response (SVR) in patients with hepatitis C virus genotype 2 or 3 who were treated for only 16 weeks with peginterferon alfa-2a and flat doses of ribavirin. After a 5-year-long debate about the value of abbreviated antiviral therapy for naive patients infected with hepatitis C virus genotype 2 or 3, a general agreement seems to have been reached. An abbreviated regimen may be an option in patients with rapid virological response (RVR), lower body weight, and an absence of advanced fibrosis. This proposal, which was originally based on our data,[2] has found successive support in the trial by Dalgard et al.[3] and recently in repetitive analyses of results generated by the ACCELERATE study.[4]

What remains to be further stressed is the optimal dosage of ribavirin to be administered when short-term therapy is being contemplated. In two studies, a flat dose of 800 mg daily was administered, and the highest difference in SVR rates between patients treated for a short duration and patients treated for the standard duration was reported.[3,5] On the contrary, when higher doses were given (1000-1200 mg daily), this difference was not significant or was barely significant.[1,2] In Diago et al.'s reanalysis,[1] only patients with RVR were considered, and the authors found body weight to be inversely related to either the SVR or the relapse rate. Indeed, among patients with low body weights (<65 kg) who were assigned to 16 or 24 weeks of treatment, SVR rates were 89% and 93%, respectively, and the respective relapse rates were 7% and 3%. The resulting differences were not significant, and this implies that when the ribavirin dosage (mg/kg of body weight) is given at a higher dosage than that conventionally suggested, short-term therapy is as effective as therapy of the standard duration. As a matter of fact, in the subset of patients with high body weights (>65 kg), the administration of 800 mg of ribavirin would not be enough to protect them from lower SVR rates and higher relapse rates. We have recently stressed the relevance of administering adequate doses of ribavirin (>15.2 mg/kg of body weight) when a short treatment duration is being considered for genotype 2 and 3–infected patients without advanced fibrosis/cirrhosis who achieve RVR,[6] and the data presented by Diago et al. reinforce our original findings.

Consequently, determining the ribavirin dosage by body weight is the first step in further consideration of predictors of SVR. This point has not been addressed in the reanalysis of the ACCELERATE study presented by Diago et al.,[1] who have evaluated only the impact of ribavirin exposure on SVR rates.

Alessandra Mangia, M.D.[1]
Angelo Andriulli, M.D.[2]
[1]*Liver Unit, Ospedale Casa Sollievo della Sofferenza San Giovanni Rotondo, Italy*
[2]*Gastroenterology, Ospedale Casa Sollievo della Sofferenza San Giovanni Rotondo, Italy*

References

1. Diago M, Shiffmann M, Bronowicki J, Zeuzem S, Rodriguez-Torrez M, Pappas S, et al. Identifying hepatitis C virus genotype 2/3 patients who can receive a 16-week abbreviated course of peginterferon alfa-2a (40 KD) plus ribavirin. Hepatology 2010;51.
2. Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, et al. Peginterferon alfa-2b and ribavirin for 12 vs 24 weeks in HCV genotype 2 or 3. N Engl J Med 2005;352:2609-2617.
3. Dalgard O, Bjoro K, Ring-Larsen H, Bjornsson E, Holberg-Petersen M, Skovlnd E, et al. Pegylated interferon alfa and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. Hepatology 2008;47:35-43.
4. Shiffmann ML, Suter F, Bacon BR, Nelson D, Harley H, Solà R, et al., for the ACCELERATE Investigators. Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 and 3. N Engl J Med 2007;357:124-134.
5. Lagging M, Langeland N, Pedersen C, Färkkilä M, Buhl MR, Mørch K, et al., for the NORDynamIC Study Group. Randomized comparison of 12 or 24 weeks of peginterferon alpha-2a and ribavirin in chronic hepatitis C virus genotype 2/3 infection. Hepatology 2008;47:1837-1845.
6. Mangia A, Dalgard O, Minerva N, Verbaan H, Bacca D, Björo K, et al. Ribavirin dosage in HCV genotypes 2 and 3 patients completing short therapy with peg-interferon alfa-2b and ribavirin. Aliment Pharmacol Ther; doi:10.1111/j.1365-2036.2010.04290.x.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.23742
Potential conflict of interest: Nothing to report.

Treating Fatty Liver for the Prevention of Cardiovascular Diseases

To the Editor:

We read with great interest the article by Speliotes et al.,[1] who have demonstrated that fatty liver is associated with the main features of metabolic syndrome (MetS) independently of visceral fat in the well-known population of the Framingham Heart Study. The study potently shows that the quantity of liver fat, as measured by multidetector computed tomography, predicts the presence of the typical glucose and lipid metabolic disturbances of MetS in an independent manner. Interestingly, the study also demonstrates that fatty liver is associated with an increase in blood pressure. Because of the cross-sectional nature of the study, in their conclusion, the authors show caution in interpreting their findings as proof of concept of the pivotal role of liver fat in the pathophysiology of cardiovascular events in MetS. Nonetheless, we suggest that their findings could have been strengthened if the authors had taken into account recent findings coming from translational research. In particular, Korenblat et al.[2] first showed that insulin action in the liver, muscle, and adipose tissue is directly related to the intrahepatic triglyceride (IHTG) content in obese subjects, and these findings were further extended by Fabbrini et al.,[3] who elegantly demonstrated that the IHTG content predicts the impairment of insulin action in the liver, adipose tissue, and muscle better than visceral fat. Consistently, an increase in whole-

body adiposity without an increase in IHTG is not associated with augmented metabolic dysfunction.[4] The aforementioned studies did not investigate the molecular pathways involved in the disturbed crosstalk between the adipose tissue, liver, and muscle, and the studies in mice provided contrasting evidence in this respect. Nevertheless, taken together, these data indicate that the treatment of fatty liver should be a main target for the prevention of cardiovascular events, which are the hallmark of MetS.[5] Currently, nonalcoholic fatty liver disease is strongly divided into the categories of nonalcoholic fatty liver and nonalcoholic steatohepatitis. Nonalcoholic fatty liver is not a progressive liver disease, whereas nonalcoholic steatohepatitis is characterized by inflammatory and fibrogenic behavior and may have a cirrhotic and tumorigenic evolution. Although this classification is well justified when patients at risk for liver-related mortality are being identified, it should not lead to nonalcoholic fatty liver being considered a benign condition because it is associated with increased cardiovascular mortality and overall mortality.[6] Noteworthily, the presence of fatty liver is completely ignored in the international consensus on MetS[5] and in the guidelines on diabetes management from the American Diabetes Association.[7] Strikingly, the decrease in IHTG with physical activity leads to a marked improvement in systemic insulin resistance independently of the decrease in visceral adipose tissue.[8] Thus, we suggest that fatty liver management should be a main goal in the treatment of MetS. Finally, we agree with the authors' conclusions that prospective clinical studies will further clarify whether IHTG measurement can be a prompt predictor of the cardiometabolic risk. If this is the case, shall we call it fatty liver syndrome?

Federico Salamone, M.D.[1]
Fabio Galvano, Ph.D.[2]
Giovanni Li Volti, M.D., Ph.D.[2]
[1]*Department of Internal Medicine, University of Catania, Catania, Italy*
[2]*Department of Biological Chemistry, University of Catania, Catania, Italy*

References

1. Speliotes EK, Massaro JM, Hoffmann U, Vasan RS, Meigs JB, Sahani DV, et al. Fatty liver is associated with dyslipidemia and dysglycemia independent of visceral fat: the Framingham Heart Study. Hepatology 2010;51:1979-1987.
2. Korenblat KM, Fabbrini E, Mohammed BS, Klein S. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. Gastroenterology 2008;134:1369-1375.
3. Fabbrini E, Magkos F, Mohammed BS, Pietka T, Abumrad NA, Patterson BW, et al. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. Proc Natl Acad Sci U S A 2009;106:15430-15435.
4. Magkos F, Fabbrini E, Mohammed BS, Patterson BW, Klein S. Increased whole-body adiposity without a concomitant increase in liver fat is not associated with augmented metabolic dysfunction. Obesity (Silver Spring) 2010.
5. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120:1640-1645.
6. Angulo P. Long-term mortality in nonalcoholic fatty liver disease: is liver histology of any prognostic significance? Hepatology 2010;51:373-375.
7. American Diabetes Association. Standards of medical care in diabetes—2010. Diabetes Care 2010;33(Suppl 1):S11-S61.
8. van der Heijden GJ, Wang ZJ, Chu ZD, Sauer PJ, Haymond MW, Rodriguez LM, et al. A 12-week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, Hispanic adolescents. Obesity (Silver Spring) 2010;18:384-390.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.23741
Potential conflict of interest: Nothing to report.