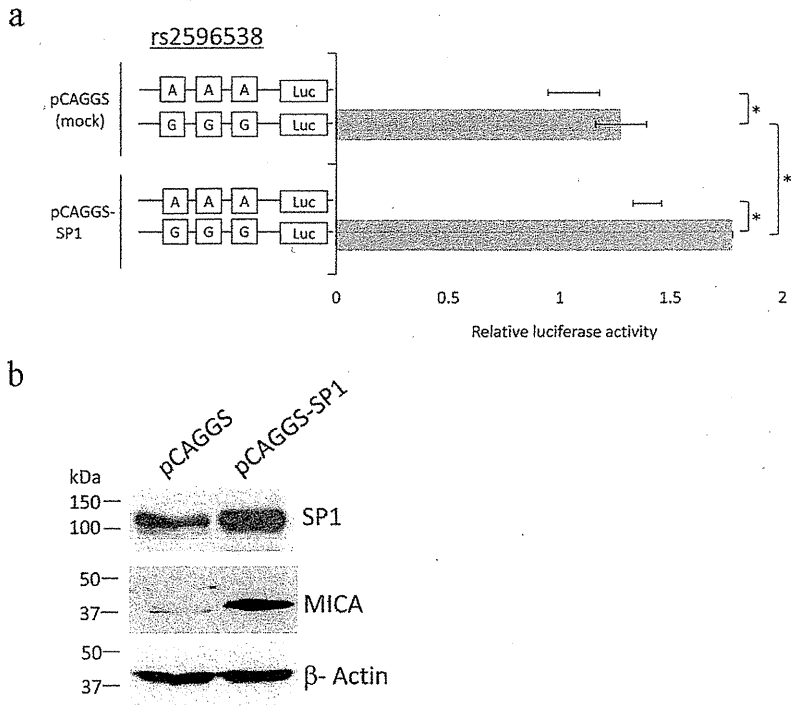

Figure 3. Transcriptional regulation of MICA by SP1 through genomic region including SNP rs2596538. (**A**) Reporter assay using constructs including 3 copies of 31 bp DNA fragment flanking SNP rs2596538. Reporter constructs are transfected into HLE cells with pRL-TK and pCAGGS or pCAGGS-SP1 vector. The value of relative luciferase activity was calculated as the firefly luciferase intensity divided by the renilla luciferase intensity. The data represent the mean ± SD value of 4 independent studies. (*$P<0.05$, Student's *t*-test) (**B**) MICA expression in HLE cells after transfection with pCAGGS or pCAGGS-SP1 vector. β-actin is served as a protein loading control.
doi:10.1371/journal.pone.0061279.g003

We also evaluated the effect of ectopically expressed SP1 on the MICA expression in HLE cells. Western-blot analysis showed that MICA protein expression was significantly increased after the SP1 over-expression (Fig. 3b). These results provided a strong evidence that the G allele has higher transcriptional potential that can be inducible by SP1.

Association of SNP rs2596538 with HCC risk and sMICA level in HCV-induced HCC patients

To further investigate the role of SNP rs2596538 in human carcinogenesis, we investigated the association of SNP rs2596538 with HCV-induced HCC in 721 HCV-HCC cases and 5,486 HCV-negative controls that had been genotyped using Illumina HumanHap610-Quad Genotyping BeadChip in our previous study [6]. We performed imputation analysis by using haplotype data from 1000 genome database [20] and found that an A allele of SNP rs2596538 was considered to be a risk allele for HCV-related HCC (Table 3, odds ratio = 1.343, $P = 1.82\times10^{-5}$). The functional SNP rs2596538 exhibited a stronger association with the HCC risk than the marker SNP rs2596542 (2.46×10^{-5}). We also analyzed the relationship between the SNP rs2596538 and the sMICA level among 246 HCV-induced HCC patients and found a significant association with the P-value of 0.00616 (Fig. 4). These results were concordant with our functional analyses in which the G allele exhibited a higher affinity to SP1 and revealed a higher transcriptional activity.

Table 3. Association of SNP rs2596542 and SNP rs2596538 with HCV-induced HCC.

SNP ID	Relative position[a]	A1	OR	*P* value
rs2596542	−4815	A	1.339	2.46×10^{-5}
rs2596538	−2778	A	1.343	1.82×10^{-5}

Note: Genotype data of 721 HCV-HCC cases and 5,486 HCV-negative controls were imputed using 1000 genomes as reference. A1, risk allele; OR, odds ratio for the risk allele calculated by considering the protective allele as a reference.
[a]Relative position to exon 1 of the *MICA* gene.
doi:10.1371/journal.pone.0061279.t003

Discussion

Approximately 160 million people (2.35% of the worldwide population) are estimated to have HCV infection [27]. Since HCV carriers have an increased risk to develop liver cirrhosis and subsequent HCC [28,29], the prediction of cancer risk is especially important for CHC patients. In our previous study, we have identified that SNP rs2596542 located in the upstream of *MICA* gene was significantly associated with the risk of HCC development among CHC patients as well as the serum level of sMICA [6]. In this study, we found that the genetic variant at SNP rs2596538 strongly affected the binding affinity of SP1. Over-expression of SP1 remarkably induced *MICA* expression in cells carrying the G allele that has a higher affinity to the SP1 binding. These findings are concordant with higher serum sMICA level among HCC patients with the G allele at SNP rs2596538. SP1 is a

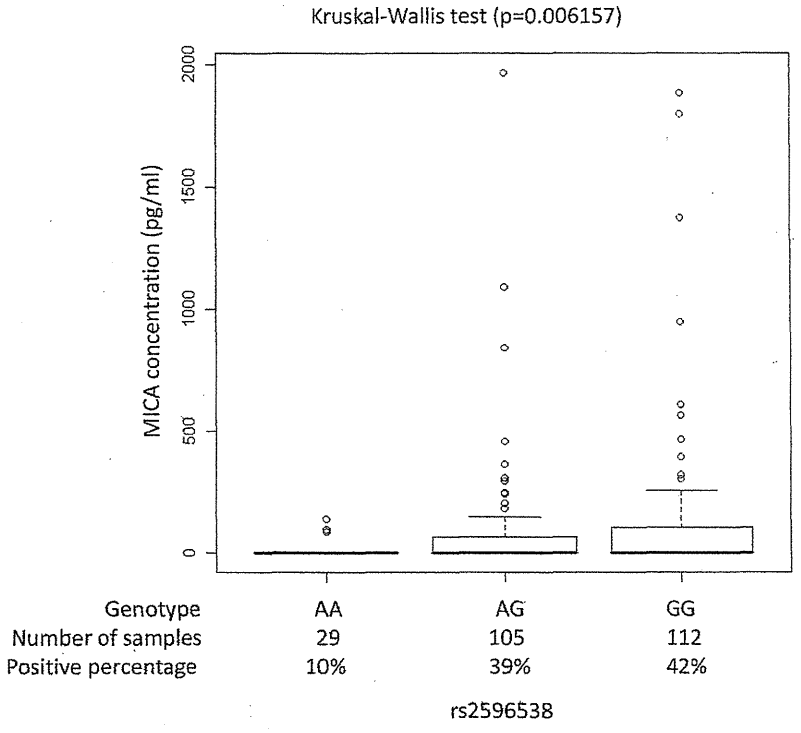

Figure 4. Association between the soluble MICA levels and SNP rs2596538 genotype. The samples were classified into 3 groups according to rs2596538 genotype. The sMICA levels measured by ELISA are indicated in y-axis. The numbers of samples and the proportion of sMICA positive subjects from each group are shown in x-axis. The percentage of the positive sMICA expression in each group are AA = 10%, AG = 39%, and GG = 42%. Statistical significance was determined by Kruskal-Wallis test.
doi:10.1371/journal.pone.0061279.g004

ubiquitously expressed transcription factor which binds to the GC-rich decanucleotide sequence (GC box) and activates the transcription of various viral and cellular genes [30,31]. Phosphorylation of SP1 was shown to be induced by HCV core protein and exhibited higher binding affinity to the promoter region of its downstream targets [32]. From our previous study, we showed a significant difference of sMICA expression between non-HCV individuals and CHC patients. This indicated that sMICA expression was induced after HCV infection [6]. Hence, we here propose the following hypothesis. After HCV infection, the virus core protein enhances the SP1 phosphorylation in hepatocytes, and the phosphorylated SP1 binds to the DNA segment corresponding to the G allele of SNP rs2596538 and then induces *MICA* expression. The membrane-bound MICA (mMICA) serves as a ligand for NKG2D to activate the immune system and results in the elimination of viral-infected cells by NK cells and CD8+ T cells [8,9]. Eventually, HCV-infected individuals with higher MICA level may cause stronger immune response to the infected cells and hence result in a reduced risk for HCC progression. Moreover, the mMICA is then shed by metalloproteinases that are often over-expressed in cancer tissues and convert mMICA to sMICA. This resulted in a significantly increase of sMICA level in the serum of HCV infected patients.

In contrast to HCV-induced HCC, our group had previously identified that higher sMICA level was associated with poor prognosis in HBV-induced HCC patients [33]. Such an opposite effect of *MICA* would be attributable to the difference in downstream pathway between HBV and HCV. HBV virus encodes hepatitis B virus X protein (HBx) that is pathogenic and promotes tumor formation. It had been reported that HBx protein was associated with an elevated expression of MT1-MMP, MMP2, and MMP3 [34,35]. HBx was also shown to transactivate MMP9 through ERKs and PI-3K-AKT/PKB pathway and suppress TIMP1 and TIMP3 activities [36,37]. The activation of metalloproteinases would induce the shedding of mMICA into sMICA, which promotes the tumor formation through the inhibitory effect of sMICA on NK cells. This can explain why high sMICA expression is a marker of poor prognosis for HBV-induced HCC. On the other hand, HCV infection was not associated with metalloproteinases activation, although the expression of sMICA was shown to be proportional to mMICA level. Therefore individuals with high MICA expression are likely to activate natural killer cells and CD8+ T cells to eliminate virus infected cells.

SP1 was previously identified as a transcriptional regulator of both *MICA* and *MICB* [7,9,38]. A polymorphism in the *MICB* promoter region was found to be associated with *MICB* transcription level [7]. To our knowledge, this is the first report showing that *MICA* transcription is directly influenced by functional variant. Moreover, this functional SNP is significantly associated with HCV-induced HCC. Our findings provide an insight that *MICA* genetic variation is a promising prognostic biomarker for CHC patients.

Supporting Information

Figure S1 Pairwise LD map between marker SNP and 11 candidates SNP. Black color boxes represent regions of high pairwise r^2 value. The LD was determined by direct DNA

sequencing of *MICA* promoter region from 50 randomly selected HCV-HCC patients.
(TIF)

Table S1 Characteristics of samples and methods used in this study.
(DOCX)

Table S2 The sequences of each oligo used in the EMSA and ChIP assay.
(DOCX)

Table S3 Copy number variation between HCV-HCC and control samples.
(DOCX)

Author Contributions

Conceived and designed the experiments: PHYL YN KM. Performed the experiments: PHYL YU VK. Analyzed the data: PHYL YU CT. Contributed reagents/materials/analysis tools: KK NK DM KC MK. Wrote the paper: PHYL KM.

References

1. Umemura T, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K (2009) Epidemiology of hepatocellular carcinoma in Japan. J Gastroenterol 44 Suppl 19: 102–107.
2. Fassio E (2010) Hepatitis C and hepatocellular carcinoma. Ann Hepatol 9 Suppl: 119–122.
3. Mbarek H, Ochi H, Urabe Y, Kumar V, Kubo M, et al. (2011) A genome-wide association study of chronic hepatitis B identified novel risk locus in a Japanese population. Hum Mol Genet 20: 3884–3892.
4. Kamatani Y, Wattanapokayakit S, Ochi H, Kawaguchi T, Takahashi A, et al. (2009) A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. Nat Genet 41: 591–595.
5. Zhang H, Zhai Y, Hu Z, Wu C, Qian J, et al. (2010) Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. Nat Genet 42: 755–758.
6. Kumar V, Kato N, Urabe Y, Takahashi A, Muroyama R, et al. (2011) Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. Nat Genet 43: 455–458.
7. Rodríguez-Rodero S, González S, Rodrigo L, Fernández-Morera JL, Martínez-Borra J, et al. (2007) Transcriptional regulation of MICA and MICB: a novel polymorphism in MICB promoter alters transcriptional regulation by Sp1. Eur J Immunol 37: 1938–1953.
8. Bauer S, Groh V, Wu J, Steinle A, Phillips JH, et al. (1999) Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 285: 727–729.
9. Zhang C, Wang Y, Zhou Z, Zhang J, Tian Z (2009) Sodium butyrate upregulates expression of NKG2D ligand MICA/B in HeLa and HepG2 cell lines and increases their susceptibility to NK lysis. Cancer Immunol Immunother 58: 1275–1285.
10. Sun D, Wang X, Zhang H, Deng L, Zhang Y (2011) MMP9 mediates MICA shedding in human osteosarcomas. Cell Biol Int 35: 569–574.
11. Waldhauer I, Goehlsdorf D, Gieseke F, Weinschenk T, Wittenbrink M, et al. (2008) Tumor-associated MICA is shed by ADAM proteases. Cancer Res 68: 6368–6376.
12. Jinushi M, Takehara T, Tatsumi T, Kanto T, Groh V, et al. (2003) Expression and role of MICA and MICB in human hepatocellular carcinomas and their regulation by retinoic acid. Int J Cancer 104: 354–361.
13. Kohga K, Takehara T, Tatsumi T, Ohkawa K, Miyagi T, et al. (2008) Serum levels of soluble major histocompatibility complex (MHC) class I-related chain A in patients with chronic liver diseases and changes during transcatheter arterial embolization for hepatocellular carcinoma. Cancer Sci 99: 1643–1649.
14. Ota M, Katsuyama Y, Mizuki N, Ando H, Furihata K, et al. (1997) Trinucleotide repeat polymorphism within exon 5 of the MICA gene (MHC class I chain-related gene A): allele frequency data in the nine population groups Japanese, Northern Han, Hui, Uygur, Kazakhstan, Iranian, Saudi Arabian, Greek and Italian. Tissue Antigens 49: 448–454.
15. Nakamura Y (2007) The BioBank Japan Project. Clin Adv Hematol Oncol 5: 696–697.
16. Tanikawa C, Urabe Y, Matsuo K, Kubo M, Takahashi A, et al. (2012) A genome-wide association study identifies two susceptibility loci for duodenal ulcer in the Japanese population. Nat Genet 44: 430–434, S431–432.
17. Miki D, Ochi H, Hayes CN, Abe H, Yoshima T, et al. (2011) Variation in the DEPDC5 locus is associated with progression to hepatocellular carcinoma in chronic hepatitis C virus carriers. Nat Genet 43: 797–800.
18. Urabe Y, Ochi H, Kato N, Kumar V, Takahashi A, et al. (2013) A genome-wide association study of HCV induced liver cirrhosis in the Japanese population identifies novel susceptibility loci at MHC region. J Hepatol.
19. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, et al. (2007) A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316: 1341–1345.
20. Consortium GP (2010) A map of human genome variation from population-scale sequencing. Nature 467: 1061–1073.
21. Venkataraman GM, Suciu D, Groh V, Boss JM, Spies T (2007) Promoter region architecture and transcriptional regulation of the genes for the MHC class I-related chain A and B ligands of NKG2D. J Immunol 178: 961–969.
22. Andrews NC, Faller DV (1991) A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. Nucleic Acids Res 19: 2499.
23. Hata J, Matsuda K, Ninomiya T, Yonemoto K, Matsushita T, et al. (2007) Functional SNP in an Sp1-binding site of AGTRL1 gene is associated with susceptibility to brain infarction. Hum Mol Genet 16: 630–639.
24. Barrett JC (2009) Haploview: Visualization and analysis of SNP genotype data. Cold Spring Harb Protoc 2009: pdb.ip71.
25. Komatsu-Wakui M, Tokunaga K, Ishikawa Y, Leelayuwat C, Kashiwase K, et al. (2001) Wide distribution of the MICA-MICB null haplotype in East Asians. Tissue Antigens 57: 1–8.
26. Tse KP, Su WH, Yang ML, Cheng HY, Tsang NM, et al. (2011) A gender-specific association of CNV at 6p21.3 with NPC susceptibility. Hum Mol Genet 20: 2889–2896.
27. Negro F, Alberti A (2011) The global health burden of hepatitis C virus infection. Liver Int 31 Suppl 2: 1–3.
28. Cabibbo G, Craxi A (2010) Epidemiology, risk factors and surveillance of hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 14: 352–355.
29. McGlynn KA, London WT (2011) The global epidemiology of hepatocellular carcinoma: present and future. Clin Liver Dis 15: 223–243.
30. Kadonaga JT, Tjian R (1986) Affinity purification of sequence-specific DNA binding proteins. Proc Natl Acad Sci U S A 83: 5889–5893.
31. Suske G (1999) The Sp-family of transcription factors. Gene 238: 291–300.
32. Lee S, Park U, Lee YI (2001) Hepatitis C virus core protein transactivates insulin-like growth factor II gene transcription through acting concurrently on Egr1 and Sp1 sites. Virology 283: 167–177.
33. Kumar V, Yi Lo PH, Sawai H, Kato N, Takahashi A, et al. (2012) Soluble MICA and a MICA Variation as Possible Prognostic Biomarkers for HBV-Induced Hepatocellular Carcinoma. PLoS One 7: e44743.
34. Ou DP, Tao YM, Tang FQ, Yang LY (2007) The hepatitis B virus X protein promotes hepatocellular carcinoma metastasis by upregulation of matrix metalloproteinases. Int J Cancer 120: 1208–1214.
35. Yu FL, Liu HJ, Lee JW, Liao MH, Shih WL (2005) Hepatitis B virus X protein promotes cell migration by inducing matrix metalloproteinase-3. J Hepatol 42: 520–527.
36. Chung TW, Lee YC, Kim CH (2004) Hepatitis B viral HBx induces matrix metalloproteinase-9 gene expression through activation of ERK and PI-3K/AKT pathways: involvement of invasive potential. FASEB J 18: 1123–1125.
37. Kim JR, Kim CH (2004) Association of a high activity of matrix metalloproteinase-9 to low levels of tissue inhibitors of metalloproteinase-1 and -3 in human hepatitis B-viral hepatoma cells. Int J Biochem Cell Biol 36: 2293–2306.
38. Andresen L, Jensen H, Pedersen MT, Hansen KA, Skov S (2007) Molecular regulation of MHC class I chain-related protein A expression after HDAC-inhibitor treatment of Jurkat T cells. J Immunol 179: 8235–8242.

J Gastroenterol (2013) 48:254–268
DOI 10.1007/s00535-012-0631-y

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Meta-analysis: mortality and serious adverse events of peginterferon plus ribavirin therapy for chronic hepatitis C

Tatsuya Minami · Takahiro Kishikawa · Masaya Sato · Ryosuke Tateishi · Haruhiko Yoshida · Kazuhiko Koike

Received: 9 February 2012 / Accepted: 11 June 2012 / Published online: 12 July 2012

Abstract
Background Pegylated interferon (PEG-IFN) plus ribavirin (RBV) therapy is the current standard of care for patients with chronic hepatitis C. Determining precisely the risk of serious adverse events (SAEs) and mortality from a single study is rather difficult because of the infrequency of such events. The aim of this systematic review was to assess the rates of SAEs and the mortality of PEG-IFN/RBV therapy in a pooled large sample, and to assess the relationship between SAEs and mortality rates and therapeutic characteristics.
Methods A literature search was conducted using MEDLINE, EMBASE, and the Cochrane Library to identify randomized controlled trials evaluating the efficacy and safety of PEG-IFN/RBV therapy. We calculated the crude mortality and SAE rates with 95 % confidence intervals (CIs).
Results Eighty studies with 153 treatment arms that included 27569 patients were enrolled (14401 patients treated with Peg-IFN alpha-2a/RBV and 13168 with Peg-IFN alpha-2b/RBV). All-cause and treatment-related deaths were observed in 50 (0.18 %; 95 % confidence interval [CI] 0.13–0.24 %) and sixteen (0.058 %; 95 % CI 0.033–0.094 %) patients, respectively. The crude SAE rate was 7.08 % (95 % CI 6.75–7.41 %). Subgroup analysis revealed higher SAE rates in patients receiving PEG-IFN alpha-2a than in those with PEG-IFN alpha-2b (7.45 vs. 6.74 %), and higher SAE rates with higher doses than with the lower doses in PEG-IFN-2a and 2b (11.94 vs. 6.99 %, 7.10 vs. 5.05 %, respectively), and with extended duration (>48 weeks) than with standard duration (48 weeks) (15.5 vs. 6.67 %) in PEG-IFN alpha-2a.
Conclusion The mortality rate during PEG-IFN/RBV therapy was acceptably low, but the rate of SAEs was not negligible in a treatment for a benign disease, and the rate was affected by treatment regimens.

Keywords Peginterferon · Ribavirin · Hepatitis C · Mortality · Adverse event · Systematic review

Abbreviations

CI	Confidence interval
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
PEG-IFN	Pegylated interferon
RCT	Randomized controlled trial
RBV	Ribavirin
SAE	Serious adverse event
SVR	Sustained virological response
WHO	World Health Organization

Introduction

Chronic hepatitis C virus (HCV) infection affects more than 170 million people worldwide and is a major cause of cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. Currently the standard of care for patients with chronic hepatitis C is peginterferon (PEG-IFN) plus ribavirin (RBV) therapy,

Electronic supplementary material The online version of this article (doi:10.1007/s00535-012-0631-y) contains supplementary material, which is available to authorized users.

T. Minami · T. Kishikawa · M. Sato · R. Tateishi (✉) · H. Yoshida · K. Koike
Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: tateishi-tky@umin.ac.jp

which can induce a sustained virological response (SVR) in 40–50 % of treatment-naïve patients with genotype 1 and an SVR of approximately 80 % in treatment-naïve patients with genotypes 2 or 3 [3–8]. After an SVR is achieved, the risk of developing liver failure and HCC is greatly reduced [9]. However, this treatment is associated with various types of complications, some of which lead to fatal outcomes. Because death during treatment is a rare event, a large sample size is needed to accurately assess the mortality rate and risk factors. The aim of this systematic review was to assess the rates of serious adverse events (SAEs) and mortality during PEG-IFN/RBV therapy in a pooled large-sized sample and to assess the relationship between mortality and SAE rates and therapeutic characteristics.

Methods

Study search protocol

We searched MEDLINE, EMBASE, and the Cochrane Library to identify randomized controlled trials (RCTs) evaluating the efficacy and safety of PEG-IFN/RBV therapy published between December 1999 and October 2010. We used the following search terms: *chronic hepatitis C*, *interferon*, and *pegylated* or *peg* or *peginterferon* or *pegasys* or *pegintron*. The search was limited to the English language.

Inclusion criteria

Studies were included in the analysis if: (1) they were RCTs, (2) they included at least one PEG-IFN/RBV treatment group in patients with chronic hepatitis C, (3) they clearly specified adverse events, (4) patients were followed up until at least 24 weeks after the end of treatment, and (5) the studies had been published or accepted for publication as full-length articles. Studies were excluded if: (1) they dealt only with co-infection of HCV and HIV; (2) they dealt only with patients with a specific condition such as a comorbid disease (e.g., cryoglobulinemia), status after liver transplantation, or patients on dialysis; (3) they included patients under 18 years of age; or (4) they focused on specific adverse events or only on hemodynamic status. We restricted the included studies to RCTs on the premise that the quality of RCTs is superior to that of non-randomized or retrospective studies.

Data extraction

Two authors (T. M. and T. K.) independently screened titles and abstracts for potential eligibility and the full texts for final eligibility. Disagreements were resolved by consensus or by consulting a third author (R. T.). We extracted the data using a standardized data-collection form to record details of the study design, treatment doses and duration, number of patients in the arm, patient characteristics, and outcomes. A database using Microsoft Access 2010 (Microsoft, Redmond, WA, USA) was developed specifically for that purpose. Two authors independently entered data into the form, and the data were then compared. Any discrepancies were checked and resolved by consensus.

Statistical analysis

The primary and secondary outcome measures were mortality and SAE rates during PEG-IFN/RBV therapy, respectively. We recorded the number of SAEs and deaths observed in each arm of the included studies. We calculated crude mortality and SAE rates with 95 % confidence intervals (CI) by dividing the total number of deaths or SAEs observed by the total number of patients in the relevant group. Studies that did not discriminate serious adverse events from others were not included in the analyses of the corresponding outcome. We performed a subgroup analysis by comparing mortality and SAE rates between PEG-IFN alpha-2a and alpha-2b, high-dose and low-dose PEG-IFN, and shorter and longer treatment durations. We also performed a meta-regression analysis to investigate relationships between mortality and SAE rates and continuous variables (mean age; mean body weight; proportion of males; proportion of Caucasian, African, and Asian patients; and proportion of genotype 1 patients) using the random effects model. Heterogeneity was tested using the I^2 test to calculate the percentage of variation caused by heterogeneity rather than by chance alone [10]. The analyses were performed with S-plus Ver. 7.0 (Insightful, Seattle, WA, USA) and StatsDirect version 2.7.7 (StatsDirect, Chesire, UK). The threshold of the reported P value accepted as indicating significance was <0.05.

Results

Study characteristics

Figure 1 shows the results of the screening. Our initial database search retrieved 323 citations, of which 243 were excluded because they did not meet our inclusion criteria; therefore, a total of 80 RCTs with 153 arms that included 27569 patients were enrolled. Table 1 shows the characteristics of each enrolled study. All studies contained at least one treatment arm using PEG-IFN and RBV for chronic hepatitis C. There were 16797 male and 10254

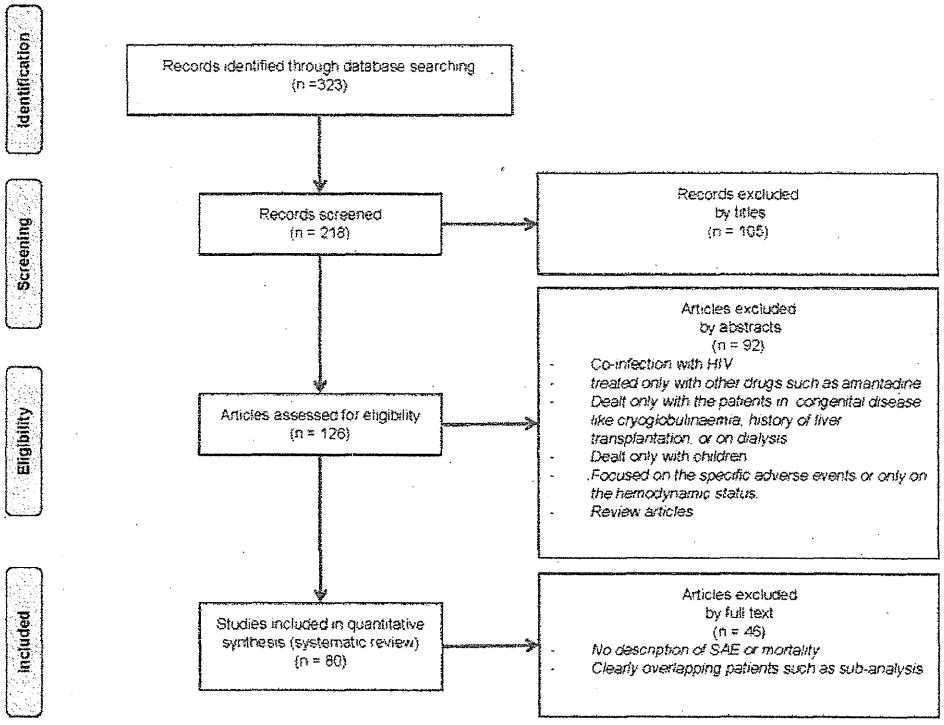

Fig. 1 The literature-search and study-selection process. *SAE* serious adverse events

females and the sex was not reported in the remaining 518 patients. The mean age was 45.9 years. A total of 14401 patients were treated with PEG-IFN alpha-2a/RBV, and 13168 with PEG-IFN alpha-2b/RBV. PEG-IFN alpha-2a was used at a fixed dose of 180–360 μg/body/week, and PEG-IFN alpha-2b at weight-based doses of 0.35–3.0 μg/kg/week. The RBV dose was fixed in 36 treatment arms and weight-based in 117 treatment arms. Treatment duration ranged from 12 to 72 weeks. The numbers of patients with HCV genotypes 1, 2, 3, and 4–6 were 18082, 3427, 3519, and 842, respectively. The genotype was not reported in the remaining 1699 patients. The treatment protocol is described in Supplementary Table 1.

Primary outcome

A total of 50 deaths from all causes were observed in the enrolled studies. Of these, sixteen were considered by the authors to be treatment-related. The crude overall and treatment-related mortality rates were 0.18 % (95 % CI 0.13–0.24 %) and 0.058 % (0.033–0.094 %), respectively. There was no evidence of heterogeneity among studies for mortality and treatment-related mortality ($I^2 = 0$ in both). The causes of mortality were suicide ($N = 6$), drug intoxication ($N = 6$), myocardial infarction ($N = 3$), sepsis ($N = 3$), aortic dissection ($N = 2$), traffic accident ($N = 2$), HCC ($N = 2$), rupture of a cerebral aneurysm ($N = 1$), bronchitis ($N = 1$), syncope ($N = 1$), pulmonary tuberculosis ($N = 1$), and unknown causes ($N = 22$). The six cases of suicide, the three of sepsis, two of drug intoxication, two of myocardial infarction, one of HCC, one of syncope, and one of pulmonary tuberculosis were considered by the investigators to be treatment-related mortality.

Subgroup analysis did not reveal any difference in mortality according to type and dose of PEG-IFN or in relation to duration of treatment (Fig. 2).

Secondary outcome

Seventy-two studies with 135 treatment arms including 23996 patients reported SAEs. They reported SAEs such as anemia requiring transfusion, neutropenia below 500/mm^3, hypothyroidism, psychosis, pneumonia, and cellulitis. SAEs were not discriminated from other adverse events in the remaining eighteen studies. The crude SAE rate was 7.08 % (95 % CI 6.75–7.41 %). Significant heterogeneity among studies was found for this outcome ($I^2 = 94.1$ %). SAE rates were higher in PEG-IFN alpha-2a than in PEG-IFN alpha-2b (7.45 vs. 6.74 %). In a subgroup analysis of the type and dose of PEG-IFN and duration of treatment, higher SAE rates were observed for intensive (270–360 μg) than for standard (180 μg) doses and for extended (>48 weeks) than for standard (48 weeks) treatment duration in patients treated with PEG-IFN alpha-2a, and for standard (1.5 μg/kg) than for lower (≤1.0 μg/kg) doses in patients treated with PEG-IFN alpha-2b (Fig. 3). However, heterogeneity remained evident in all subgroups.

Table 1 Characteristics of the enrolled studies

Study	Peginterferon type and dose	Ribavirin	Duration, weeks[a]	Patients, *N*	Male, *N*	Age, years (mean)	BW, kg (mean)	Ethnic group, *N*				HCV genotype, *N*			
								Caucasian	African	Asian	Other	G1	G2	G3	G4–6
Abergel [26]	2b 0.75 μg/kg	800 mg, fixed	48	102	70	51.1	NR	NR	NR	NR	NR	54	9	28	11
	2b 1.5 μg/kg	800 mg, fixed	48	101	65	49.3	NR	NR	NR	NR	NR	50	11	30	10
Alfaleh [27]	2b 100 μg	800 mg, fixed	48	48	22	48.4	NR	0	0	0	48	10	NR	NR	28
Andriulli [28]	2a 180 μg	1000–1200 mg	12[a]	61	30	52.5	70.6	NR	NR	NR	NR	0	52	9	0
	2a 180 μg	1000–1200 mg	12[a]	59	35	52.7	73	NR	NR	NR	NR	0	50	9	0
	2a 180 μg	1000–1200 mg	24[a]	24	18	47	71	NR	NR	NR	NR	0	10	14	0
Angelico [29]	2a 180 μg	800 mg, fixed	48[a]	42	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	2a 180 μg	800 mg, fixed	48[a]	57	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Ascione [14]	2b 1.5 μg/kg	1000–1200 mg	24–48[a]	160	94	48.9	69.9	160	0	0	0	92	50	17	1
	2a 180 μg	1000–1200 mg	24–48[a]	160	81	51.3	70.4	160	0	0	0	89	49	18	4
Benhamou [30]	2b 1.5 μg/kg	1000–1200 mg	24–48[a]	325	210	45.1	NR	271	12	14	28	226	NR	NR	10
Berg [31]	2a 180 μg	800 mg, fixed	48	12	5	44	70	11	0	1	0	10	0	2	0
	2a 180 μg	1000–1200 mg	48	52	46	44	83	47	2	3	0	35	6	6	5
Berg [32]	2a 180 μg	800 mg, fixed	48	230	128	42.7	76.3	222	2	6	0	230	0	0	0
	2a 180 μg	800 mg, fixed	72	225	122	42.7	75.3	213	3	6	3	225	0	0	0
Berg [33]	2b 1.5 μg/kg	800–1400 mg	48	225	128	42.8	NR	NR	NR	NR	NR	225	0	0	0
Bosques-Padilla [34]	2a 180 μg	1000–1200 mg	48	14	8	46	75	0	0	0	14	11	NR	NR	NR
Brady [35]	2b 1.5 μg/kg	800–1400 mg	48	311	156	45[b]	84.8	225	37	13	36	308	0	0	3
	2b 3.0 μg/kg	800–1400 mg	48[a]	299	149	45[b]	84.5	203	37	19	40	295	0	0	4
Brandao [36]	2a 180 μg	800 mg, fixed	48	31	19	40.8	76.4	26	0	2	3	31	0	0	0
	2a 180 μg	800 mg, fixed	24	54	46	42.3	80.7	50	2	0	2	0	NR	NR	NR
	2a 180 μg	800 mg, fixed	24	32	19	41.1	73.8	26	0	NR	6	32	0	0	0
Bressler [37]	2a 180 μg	1000–1200 mg	48	20	12	47.3	99	NR	NR	NR	NR	14	NR	NR	NR
	2a 270 μg	1000–1200 mg	48	20	14	44.3	98	NR	NR	NR	NR	14	NR	NR	NR
Bronowicki [38]	2a 180 μg	800 mg, fixed	24	516	306	46.2	70.8	NR	NR	NR	NR	NR	NR	NR	NR
Bruno [5]	2b 100 μg	1000–1200 mg	48[a]	163	101	49.9	69.4	NR	NR	NR	NR	163	0	0	0
Carr [39]	2b 1.5 μg/kg	1000–1200 mg	48	206	147	48	NR	160	26	4	16	166	NR	NR	NR
Ciancio [40]	2a 180 μg	1000–1200 mg	48	81	60	50	NR	NR	NR	NR	NR	66	9	3	3
Dalgard [41]	2b 1.5 μg/kg	800–1400 mg	24	150	97	38[b]	77	NR	NR	NR	NR	0	31	119	0
	2b 1.5 μg/kg	800–1400 mg	14	148	95	38[b]	79	NR	NR	NR	NR	0	29	119	0
Diago [42]	2a 180 μg	1000–1200 mg	48	28	21	40[b]	75.8	NR	NR	NR	NR	28	0	0	0
	2a 270 μg	1000–1200 mg	48[a]	20	15	44.5[b]	74.1	NR	NR	NR	NR	20	0	0	0
	2a 360 μg	1000–1200 mg	48[a]	24	20	41[b]	79	NR	NR	NR	NR	24	0	0	0

Table 1 continued

Study	Peginterferon type and dose	Ribavirin	Duration, weeks[a]	Patients, *N*	Male, *N*	Age, years (mean)	BW, kg (mean)	Ethnic group, *N*				HCV genotype, *N*			
								Caucasian	African	Asian	Other	G1	G2	G3	G4–6
Ferenci [43]	2a 180 μg	1000–1200 mg	48	95	65	44	NR	NR	NR	NR	NR	95	0	0	0
Ferenci [44]	2a 180 μg	400 mg, fixed	24	141	87	36.2	73.1	NR	NR	NR	NR	0	19	122	0
	2a 180 μg	800 mg, fixed	24	141	84	36.8	71.1	NR	NR	NR	NR	0	18	123	0
Ferenci [45]	2a 180 μg	1000–1200 mg	72[a]	150	98	44.3	76.9	NR	NR	NR	NR	134	0	0	16
	2a 180 μg	1000–1200 mg	48	139	90	45.1	78.5	NR	NR	NR	NR	127	0	0	12
Fried [4]	2a 180 μg	1000–1200 mg	48	453	324	42.8	79.8	372	27	28	26	298	54	86	13
Fried [46]	2a 180 μg	1200 mg, fixed	48	46	37	47.1	98.4	32	4	NR	10	46	0	0	0
	2a 180 μg	1600 mg, fixed	48	47	41	49.6	100.3	29	6	NR	12	47	0	0	0
	2a 270 μg	1200 mg, fixed	48	47	35	47.1	101	35	4	NR	8	47	0	0	0
	2a 270 μg	1600 mg, fixed	48	47	37	48.5	97	32	5	NR	10	47	0	0	0
Gish [47]	2a 180 μg	1000–1200 mg	24–48[a]	45	32	49	80	38	NR	NR	NR	NR	NR	NR	NR
Glue [48]	2b 0.35 μg/kg	600–800 mg	24	12	NR	39.8	65.6	NR	NR	NR	NR	9	NR	NR	NR
	2b 0.7 μg/kg	600–1200 mg	24	18	NR	39.8	65.6	NR	NR	NR	NR	5	NR	NR	NR
	2b 1.4 μg/kg	600–1200 mg	24	18	NR	39.8	65.6	NR	NR	NR	NR	4	NR	NR	NR
Hadziyannis [6]	2a 180 μg	1000–1200 mg	24	280	185	42	77.1	254	9	16	1	118	53	91	NR
	2a 180 μg	1000–1200 mg	48	436	287	43	77.3	394	11	26	5	271	66	87	NR
	2a 180 μg	800 mg, fixed	48	361	226	42.6	77	315	11	31	4	250	46	53	NR
	2a 180 μg	800 mg, fixed	24	207	140	41.2	78.3	183	7	14	3	101	39	57	NR
Hasan [49]	2b 1.5 μg/kg	1000–1200 mg	48	21	16	NR	NR	0	19	2	0	4	0	0	17
Helbling [50]	2a 180 μg	600–800 mg	48	60	36	47[b]	73	NR	NR	NR	NR	25	7	24	3
	2a 180 μg	1000–1200 mg	48	64	45	47[b]	74	NR	NR	NR	NR	30	11	18	4
Herrine [51]	2a 180 μg	800–1000 mg	48	32	24	48	NR	NR	NR	NR	NR	25	NR	NR	NR
Hezode [52]	2a 180 μg	1000–1200 mg	48	82	46	45[b]	NR	76	2	4	0	82	0	0	0
Ide [53]	2b 1.5 μg/kg	600–1000 mg	48	56	26	55.3	NR	0	0	56	0	56	0	0	0
	2b 1.5 μg/kg	600–1000 mg	48–68[a]	57	30	54.6	NR	0	0	57	0	57	0	0	0
Jacobson [54]	2b 1.0 μg/kg	1000–1200 mg	48	161	122	49.2	NR	118	23	NR	20	145	NR	NR	NR
	2b 1.5 μg/kg	800 mg, fixed	48	160	117	49.8	NR	106	27	NR	27	141	NR	NR	NR
Jacobson [55]	2b 1.5 μg/kg	800 mg, fixed	24–48[a]	2444	1560	45.8	83.8	1926	237	59	222	1506	526	386	23
	2b 1.5 μg/kg	800–1400 mg	24–48[a]	2469	1539	45.8	84	1993	208	59	209	1512	499	421	33
Jensen [56]	2a 180 μg	1000–1200 mg	48	313	212	48.5	80.9	282	25	NR	6	284	1	8	19
	2a 180 μg	1000–1200 mg	72	156	107	49.4	81.2	137	17	NR	2	142	1	5	8
	2a 360 μg	1000–1200 mg	48[a]	156	94	48.8	81.1	141	10	NR	5	142	3	4	5
	2a 360 μg	1000–1200 mg	72[a]	317	203	48.1	81.5	279	29	NR	9	288	2	7	19

Table 1 continued

Study	Peginterferon type and dose	Ribavirin	Duration, weeks[a]	Patients, *N*	Male, *N*	Age, years (mean)	BW, kg (mean)	Ethnic group, *N*				HCV genotype, *N*			
								Caucasian	African	Asian	Other	G1	G2	G3	G4–6
Kamal [57]	2b 1.5 μg/kg	1000–1200 mg	24	95	49	41.6	NR	NR	NR	NR	NR	0	0	0	95
	2b 1.5 μg/kg	1000–1200 mg	36	96	51	43.9	NR	NR	NR	NR	NR	0	0	0	96
	2b 1.5 μg/kg	1000–1200 mg	48	96	50	41.2	NR	NR	NR	NR	NR	0	0	0	96
Kamal [58]	2b 1.5 μg/kg	10.6 mg/kg	24[a]	69	37	41[b]	NR	NR	NR	NR	NR	69	0	0	0
	2b 1.5 μg/kg	10.6 mg/kg	36[a]	79	32	40.5[b]	NR	NR	NR	NR	NR	79	0	0	0
	2b 1.5 μg/kg	10.6 mg/kg	48[a]	160	100	42.2	NR	NR	NR	NR	NR	160	0	0	0
	2b 1.5 μg/kg	10.6 mg/kg	48	50	26	43.2	NR	NR	NR	NR	NR	50	0	0	0
Kawaoka [59]	2b 1.0 μg/kg	600–1000 mg	24	26	9	57[b]	53	0	0	26	0	0	26	0	0
	2b 1.5 μg/kg	600–1000 mg	24	27	15	55[b]	61	0	0	27	0	0	27	0	0
Khattab [60]	2b 1.5 μg/kg	800–1400 mg	48	49	34	37	NR	0	49	0	0	0	0	0	49
Kuboki [61]	2a 180 μg	600–1000 mg	48	100	74	52[b]	66.7	0	0	100	0	85	15	0	0
	2a 180 μg	600–1000 mg	48	99	62	52	62.8	0	0	99	0	99	0	0	0
Lagging [62]	2a 180 μg	800 mg, fixed	12	194	123	41.5	79.8	NR	NR	NR	NR	0	55	137	0
	2a 180 μg	800 mg, fixed	24	188	105	42	76.5	NR	NR	NR	NR	0	49	139	0
Langlet [63]	2a 180 μg	1000–1200 mg	24–48[a]	314	173	45.4	73.1	278	24	7	5	166	NR	NR	49
Lee [64]	2b 1.5 μg/kg	1000–1200 mg	24	76	53	44.6	67.8	0	0	76	0	38	38	0	0
Liu [65]	2a 180 μg	1000–1200 mg	24	154	88	54	67.6	0	0	154	0	154	0	0	0
	2a 180 μg	1000–1200 mg	48	154	87	53	65.8	0	0	154	0	154	0	0	0
Lodato [66]	2b 1.0–1.5 μg/kg	10.6 mg/kg	24–48[a]	43	23	49.6	NR	NR	NR	NR	NR	NR	NR	NR	NR
	2b 1.5 μg/kg	10.6 mg/kg	24–48[a]	22	12	48.7	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mangia [7]	2b 1.0 μg/kg	1000–1200 mg	12	133	NR	NR	NR	NR	NR	NR	NR	0	102	31	0
	2b 1.0 μg/kg	1000–1200 mg	24	80	NR	NR	NR	NR	NR	NR	NR	0	58	22	0
	2b 1.0 μg/kg	1000–1200 mg	24	70	39	49.7	69.5	NR	NR	NR	NR	0	53	17	0
Manns [3]	2b 1.5 μg/kg	1000–1200 mg	48[a]	514	346	44	83	NR	NR	NR	NR	349	NR	NR	12
	2b 1.5 μg/kg	800 mg, fixed	48	511	321	43	82	NR	NR	NR	NR	348	NR	NR	16
Marcellin [67]	2b 1.5 μg/kg	800–1200 mg	24[a]	10	8	51.4	75.8	10	0	0	0	10	0	0	0
Marcellin [68]	2a 180 μg	1000–1200 mg	24–48[a]	318	201	45.1	NR	256	17	11	34	212	47	47	NR
McHutchison [15]	2a 180 μg [15]	1000–1200 mg	48	1035	613	47.6	82.8	733	200	20	82	1035	0	0	0
	2b 1.0 μg/kg	800–1400 mg	48	1016	607	47.5	83.4	724	187	21	84	1016	0	0	0
	2b 1.5 μg/kg	800–1400 mg	48	1019	613	47.5	84	732	183	10	94	1019	0	0	0
McHutchison [69]	2a 180 μg	1000–1200 mg	48	114	76	50[b]	NR	100	10	2	2	114	0	0	0
Mecenate [70]	2a 180 μg	1000–1200 mg	12[a]	72	NR	42[b]	NR	NR	NR	NR	NR	0	NR	NR	0
	2a 180 μg	1000–1200 mg	24[a]	67	54	45[b]	NR	NR	NR	NR	NR	0	37	30	0
	2a 180 μg	1000–1200 mg	24[a]	71	NR	42[b]	NR	NR	NR	NR	NR	0	NR	NR	0

Table 1 continued

Study	Peginterferon type and dose	Ribavirin	Duration, weeks[a]	Patients, *N*	Male, *N*	Age, years (mean)	BW, kg (mean)	Ethnic group, *N*				HCV genotype, *N*			
								Caucasian	African	Asian	Other	G1	G2	G3	G4–6
Mendez-Navarro [71]	2a 180 μg	1000–1200 mg	48	63	26	46.2	70.4	0	0	0	63	63	0	0	0
Meyer-Wyss [72]	2b 1.0 μg/kg	800 mg, fixed	24–48[a]	113	64	39[b]	72	NR	NR	NR	NR	49	14	41	9
	2b 1.5 μg/kg	800 mg, fixed	24–48[a]	106	76	42[b]	73	NR	NR	NR	NR	64	10	26	6
Napoli [73]	2b 1.5 μg/kg	800–1200 mg	48[a]	14	10	46.9	NR	NR	NR	NR	NR	14	0	0	0
	2b 1.5 μg/kg	800–1200 mg	48	17	11	47.3	NR	NR	NR	NR	NR	17	0	0	0
Pearlman [74]	2b 1.5 μg/kg	800–1400 mg	48	49	23	56[b]	NR	NR	23	NR	26	49	0	0	0
	2b 1.5 μg/kg	800–1400 mg	72	52	34	54[b]	NR	NR	25	NR	27	52	0	0	0
Roberts [75]	2a 180 μg	1000–1200 mg	48	438	285	43.3	78.7	365	55	0	1	436	0	0	0
	2a 360 μg	1000–1200 mg	48[a]	433	298	43.6	77.3	355	61	0	2	432	0	0	0
Roffi [76]	2b 1.0 μg/kg	1000–1200 mg	48	57	36	56[b]	75	NR	NR	NR	NR	33	15	9	0
Rossignol [77]	2a 180 μg	1000–1200 mg	48	40	36	39	NR	NR	NR	NR	NR	0	0	0	40
Rumi [16]	2a 180 μg	1000–1200 mg	24–48[a]	212	128	51.6	72.2	NR	NR	NR	NR	91	69	34	18
	2b 1.5 μg/kg	1000–1200 mg	24–48[a]	219	120	52.8	68.9	NR	NR	NR	NR	87	74	32	26
Rustgi [78]	2a 180 μg	1000–1200 mg	24–48[a]	117	81	50	89.7	79	26	0	12	117	0	0	0
Sanchez-Tapias [79]	2a 180 μg	800 mg, fixed	48	165	113	42.8	73.3	NR	NR	NR	NR	149	1	7	8
	2a 180 μg	800 mg, fixed	72	161	102	43.2	74.4	NR	NR	NR	NR	142	1	8	8
	2a 180 μg	800 mg, fixed	24	148	88	39.3	67.9	NR	NR	NR	NR	45	18	75	10
	2a 180 μg	800 mg, fixed	48	36	20	42.4	68.7	NR	NR	NR	NR	35	0	0	1
Scotto [17]	2a 180 μg	15 mg/kg	48	71	42	45.8	80.7	NR	NR	NR	NR	45	6	8	12
	2b 1.5 μg/kg	15 mg/kg	48	72	40	47.8	78.9	NR	NR	NR	NR	47	5	9	11
Shiffman [80]	2b 1.5 μg/kg	800–1400 mg	48	48	27	49[b]	82	NR	17	NR	31	48	0	0	0
Shiffman [81]	2a 180 μg	800 mg, fixed	16	732	448	46	81.5	635	22	21	54	NR	372	358	NR
	2a 180 μg	800 mg, fixed	24	731	461	45.6	81.6	638	21	18	54	NR	356	369	NR
Shiffman [82]	2a 180 μg	1000–1200 mg	48	936	673	50	NR	693	157	NR	NR	936	0	0	0
Sjogren [83]	2b 1.5 μg/kg	1000–1200 mg	48	29	19	46[b]	82	17	9	2	1	29	0	0	0
Sood [84]	2b 1.0 μg/kg	1000–1200 mg	24	76	67	43.1	NR	0	0	76	0	0	0	76	0
	2b 1.5 μg/kg	1000–1200 mg	24	27	21	37.3	NR	0	0	27	0	0	0	27	0
Tang [85]	2a 180 μg	1000–1200 mg	20[a]	11	4	42[b]	70	8	NR	NR	3	11	0	0	0
	2a 180 μg	1000–1200 mg	32[a]	10	5	38[b]	69	10	0	0	0	10	0	0	0
	2a 180 μg	1000–1200 mg	44[a]	11	8	41[b]	79	10	NR	NR	1	11	0	0	0
	2a 180 μg	1000–1200 mg	48[a]	13	9	41[b]	71	11	NR	NR	2	13	0	0	0
Toyoda [86]	2b 1.5 μg/kg	600–1000 mg	8[a]	15	5	53.6	NR	0	0	15	0	0	15	0	0
	2b 1.5 μg/kg	600–1000 mg	24[a]	28	16	57.8	62.2	0	0	28	0	0	28	0	0
	2b 1.5 μg/kg	600–1000 mg	24[a]	17	7	NR	NR	0	0	17	0	0	17	0	0

Table 1 continued

Study	Peginterferon type and dose	Ribavirin	Duration, weeks[a]	Patients, *N*	Male, *N*	Age, years (mean)	BW, kg (mean)	Ethnic group, *N*				HCV genotype, *N*			
								Caucasian	African	Asian	Other	G1	G2	G3	G4–6
Wagner [87]	2a 180 μg	800–1200 mg	16	71	52	38	75.3	NR	NR	NR	NR	0	19	51	0
	2a 180 μg	800–1200 mg	24	71	41	39	74.6	NR	NR	NR	NR	0	19	52	0
	2a 180 μg	800–1200 mg	24	11	4	42	80.1	NR	NR	NR	NR	0	1	10	0
Wagner [88]	2a 180 μg	1000–1200 mg	48	352	183	45.4	74	344	0	0	8	352	0	0	0
Yenice [18]	2a 180 μg	800–1200 mg	48	37	13	49.9	NR	NR	NR	NR	NR	37	0	0	0
	2b 1.5 μg/kg	800–1200 mg	48	37	10	50.8	NR	NR	NR	NR	NR	37	0	0	0
Yu [89]	2b 80–100 μg	1000–1200 mg	24[a]	45	28	45.4	68.3	0	0	45	0	45	0	0	0
	2b 80–100 μg	1000–1200 mg	48[a]	15	11	45.1	68.6	0	0	15	0	15	0	0	0
Yu [90]	2a 180 μg	1000–1200 mg	16	50	32	50.8	67.7	0	0	50	0	0	50	0	0
	2a 180 μg	1000–1200 mg	24	100	58	49.9	65.8	0	0	100	0	0	100	0	0
Yu [91]	2a 180 μg	1000–1200 mg	24	100	57	49.7	65.5	0	0	100	0	100	0	0	0
	2a 180 μg	1000–1200 mg	48	100	58	49.1	67.5	0	0	100	0	100	0	0	0
Zeuzem [92]	2a 180 μg	800 mg, fixed	24	212	90	43.8	73.9	183	17	5	7	144	38	20	10
	2a 180 μg	800 mg, fixed	48	210	82	43.9	73.7	180	20	4	6	141	41	18	10
Zeuzem [93]	2a 180 μg	1000–1200 mg	24[a]	43	33	39.1	74.7	NR	NR	NR	NR	23	NR	NR	1
	2a 180 μg	1000–1200 mg	48	134	83	43.2	73.8	NR	NR	NR	NR	90	NR	NR	6
	2a 360 μg	1000–1200 mg	48	11	6	42.6	79	NR	NR	NR	NR	9	NR	NR	2
Zeuzem [94]	2b 1.5 μg/kg	800–1200 mg	24	237	127	42.2	71.3	225	NR	NR	NR	237	0	0	0
Zeuzem [95]	2a 180 μg	1000–1200 mg	48	114	66	41.9	73.4	105	NR	NR	9	114	0	0	0

HCV hepatitis C virus, *BW* body weight, *NR* not reported

[a] Details of pegylated (Peg) interferon dose and duration of treatment are described in Supplementary Table 1

[b] Data values are expressed as medians

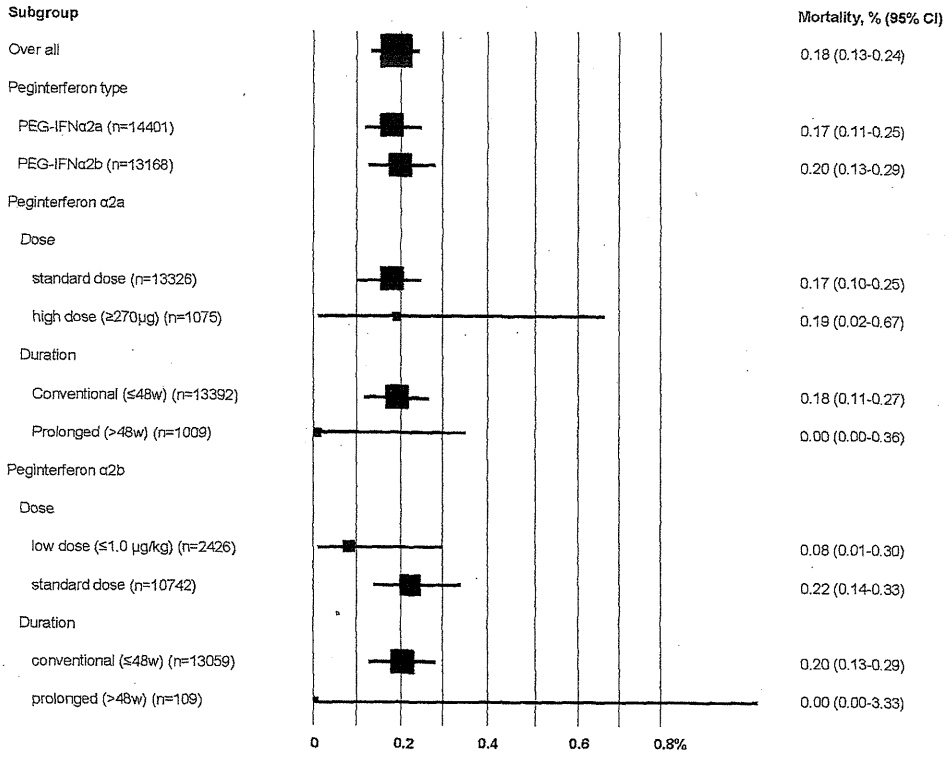

Fig. 2 Forest plot of mortality, comparing treatment regimens. Sizes of the *boxes* reflect sample sizes, with the *bars* showing the 95 % confidence interval (*CI*)

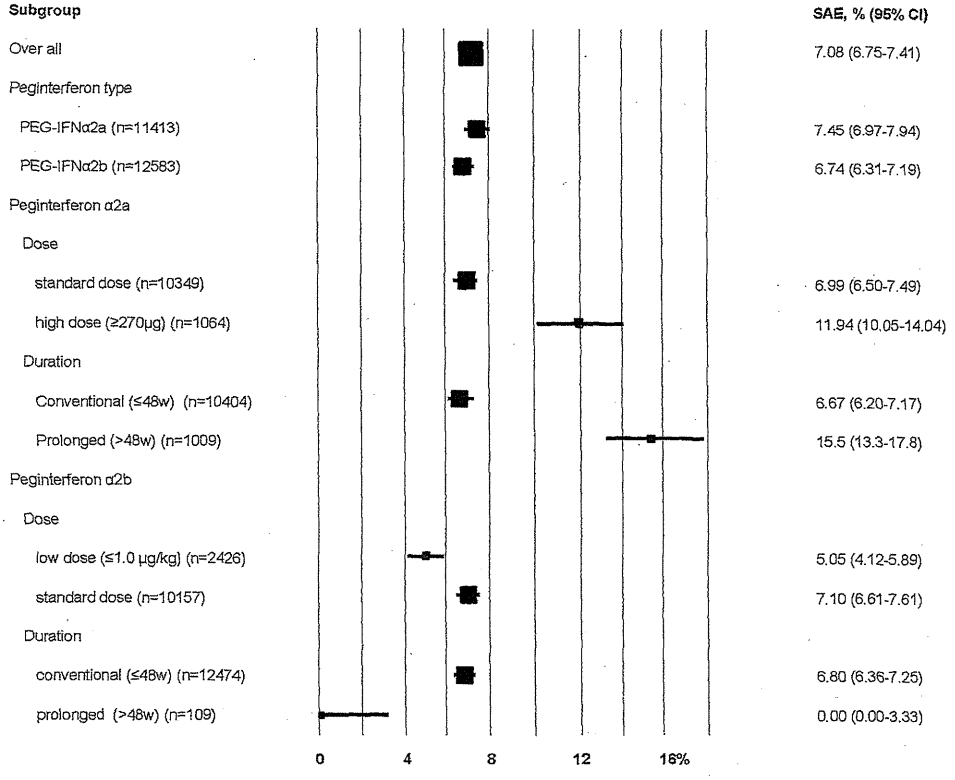

Fig. 3 Forest plot of SAEs, comparing treatment regimens. Sizes of the *boxes* reflect sample sizes, with the *bars* showing the 95 % confidence interval

Meta-regression analysis showed that greater body weight, an increased proportion of male patients, an increased proportion of HCV genotype 1, and an increased proportion of Caucasian patients and decreased proportion of Asian patients were significantly associated with increased SAE rates (Table 2). There was no significant

association between increased SAE rates and the mean patient age or the proportion of African patients.

Discussion

According to a report by the World Health Organization (WHO), age-specific annual death rates from all causes in individuals aged 45–49 years in the United States, Italy, and Japan (locations of the majority of our enrolled studies) were 409, 216, and 248 per 100000, respectively [11]. The crude mortality of 0.18 % (180 per 100000) found in the present study is low by comparison, even allowing for the biased population tolerable to PEG-IFN/RBV. Furthermore, the annual mortality rate could have been lower than the crude mortality rate, considering that the study period was longer than 1 year (including the follow-up period) in most enrolled studies. The annual treatment-related mortality rate could have been lower than our finding of a treatment-related mortality of 0.06 %. However, the treatment-related mortality rate may be an underestimate, as assessment of the causal relationship between treatment and mortality can be subjective and/or biased. Nonetheless, these PEG-IFN/RBV-related mortality rates would be acceptable considering the high SVR rates and considering that SVR drastically reduces adverse events related to chronic hepatitis C infection. In the present study, the most common cause of mortality was suicide, and all of the suicides were considered as treatment-related. This finding should alert treating physicians when they are treating patients with a history of psychiatric illness.

Two types of PEG-IFNs (i.e., PEG-IFN alpha-2a and 2b) are approved for the treatment of chronic hepatitis C. PEG-IFN alpha-2a has a molecular mass of 40 kDa and PEG-IFN alpha-2b a mass of 12 kDa. In comparison with PEG-IFN alpha-2b, PEG-IFN alpha-2a is less effectively

Table 2 Meta-regression analysis for continuous variables

Variables	Slope[a]	Standard error	*P* value
Mean age, per year increase			
All studies ($N = 100$)	−0.00244	0.00380	0.52
Alpha-2a ($N = 60$)	−0.00049	0.00513	0.93
Alpha-2b ($N = 40$)	−0.00203	0.00488	0.68
Mean body weight, per 1 kg increase			
All studies ($N = 95$)	0.00343	0.00147	0.02
Alpha-2a ($N = 64$)	0.00584	0.00242	0.02
Alpha-2b ($N = 31$)	0.00067	0.00178	0.71
Proportion of male patients, per 1 % increase			
All studies ($N = 125$)	0.00305	0.00130	0.02
Alpha-2a ($N = 73$)	0.00218	0.00182	0.23
Alpha-2b ($N = 52$)	0.00257	0.00166	0.13
Proportion of Caucasian patients, per 1 % increase			
All studies ($N = 75$)	0.00167	0.00043	<0.001
Alpha-2a ($N = 46$)	0.00102	0.00062	0.11
Alpha-2b ($N = 29$)	0.00201	0.00061	0.003
Proportion of African patients, per 1 % increase			
All studies ($N = 72$)	−0.00092	0.00113	0.42
Alpha-2a ($N = 41$)	0.00781	0.00394	0.55
Alpha-2b ($N = 31$)	−0.00030	0.00109	0.79
Proportion of Asian patients, per 1 % increase			
All studies ($N = 58$)	−0.00092	0.00042	0.03
Alpha-2a ($N = 32$)	−0.00042	0.00061	0.50
Alpha-2b ($N = 26$)	−0.00106	0.00053	0.06
Proportion of genotype 1 patients, per 1 % increase			
All studies ($N = 129$)	0.00143	0.00036	<0.001
Alpha-2a ($N = 73$)	0.00179	0.00048	<0.001
Alpha-2b ($N = 56$)	0.00075	0.00046	0.104

[a] Slope values indicate increases (decreases) in the rates of serious adverse events (SAEs) per unit. For example, a 1-year increase in mean age in a study results in 0.00464 (0.464 %) decrease in the SAE rate

cleared by the kidneys and therefore has a longer half-life. In fact, pharmacokinetic analysis in 22 patients showed that PEG-IFN alpha-2a was still detectable in 10 patients 168 h after the administration of 180 μg/week, whereas the administration of 1.0 μg/kg/week of PEG-IFN alpha-2b was undetectable in 11 of 12 patients at the same time point [12]. PEG-IFN alpha-2a is thought to be more effective than PEG-IFN alpha-2b because of its longer half-life. A recent meta-analysis showed a higher SVR rate after treatment with PEG-IFN alpha-2a than after treatment with PEG-IFN alpha-2b [13]. On the other hand, the half-life of each PEG-IFN may be related to its safety profile. However, among studies that have directly compared the safety of the two PEG-IFNs, only one reported a significant difference between SAE rates for PEG-IFN alpha-2a and PEG-IFN alpha-2b (11.7 vs. 8.6 %, $P = 0.02$) [14–18]. The inability of the other studies to detect such a difference may have been due to small sample sizes. In fact, a difference in SAE rates between the two PEG-IFNs was observed in pooled samples in our study.

Increasing the dose intensity of PEG-IFN and prolonging treatment duration have been attempted to achieve higher IFN levels in blood for longer periods, eventually resulting in a higher SVR rate. Treatment dose and duration are also expected to be related to the safety profile. The higher SAE rates in regimens with more intensive dosing observed for PEG-IFN alpha-2a and 2b and longer treatment duration observed for PEG-IFN alpha-2a support this hypothesis. The higher SAE rates in regimens with longer treatment duration were not observed for PEG-IFN alpha-2b, probably due to small sample sizes in regimens with longer treatment duration of PEG-IFN alpha-2b.

As mortality and SAE during PEG-IFN/RBV treatment are rare, most studies reported no such events. Therefore, the proportion calculated using the DerSimonian and Laird weight for the random-effect model showed considerable discrepancies between crude and pooled rates. In fact, pooled and treatment-related mortalities calculated using the random-effects (DerSimonian and Laird) model were 0.30 % (0.24–0.37 %) and 0.17 % (0.12–0.22 %), respectively, which were considerably different from the crude rates of each outcome (data not shown). Thus, we adopted crude instead of pooled rates for mortality and SAE.

Our meta-regression analysis showed a significant association between increased SAEs and HCV genotype 1. It is plausible that patients with genotype 1, which is difficult to treat, received a higher dose and longer duration of treatment. This is consistent with the results of the subgroup analysis.

A significant positive association between the SAE rate and the proportion of Caucasian patients, and an inverse relationship between SAEs and the proportion of Asian patients were also observed. This result may suggest a role of genetic diversity in the mechanisms underlying the adverse effects of PEG-IFN/RBV. Indeed, inosine triphosphate pyrophosphatase (*ITPA*) gene variants are associated with RBV-induced hemolytic anemia, and genetic polymorphisms near the interleukin-28B (*IL-28B*) gene were reported to be associated with response to HCV treatment with PEG-IFN and RBV, and the frequency of the variants differed between ethnic groups [19, 20].

We found that greater body weight was associated with a higher SAE rate. Of note, in the PEG-IFN alpha-2a-based regimen, the starting dose was fixed regardless of body weight; thus, with the PEG-IFN alpha-2a regimen, there might have been an overdose for patients of lower weight, leading to SAEs. However, whether such overdosing occurred was not clear in this study because there was a positive correlation between body weight and the SAE rate in patients receiving the PEG-IFN alpha-2a regimen. The reason for this positive relationship remains unclear; however, it may be because obesity is itself associated with various medical comorbidities.

We also found that an increased proportion of male patients in a study was associated with a higher SAE rate. It has been reported that female gender was an independent factor contributing to severe anemia [21], so the reason for the present finding of the increased proportion of male patients remains unclear; it may be correlated with increased body weight which caused a higher SAE rate. However, whether the proportions of individuals with obesity differed between male and female patients is not clear, because data on body mass index was often lacking.

In the present study increased mean age was not associated with a higher SAE rate, whereas discontinuation, dose reduction, and grade 3 adverse events were more frequent in older patients in previous studies [22, 23]. The lack of an association between mean age and the SAE rate in the present study could be due in part to the patients' mean age of 45.9 years, and the proportion of patients over 60 being small. Low-risk patients tend to be included in RCTs. This is one of the limitations of this study.

Recently, the use of HCV nonstructural 3/4A serine protease inhibitors combined with PEG-IFN and RBV were reported to achieve higher SVR rates in genotype 1 patients compared with conventional PEG-IFN/RBV. These triple therapies are considered to be the next standard of care for chronic hepatitis C [24, 25]. Adverse events during triple therapies could include those related to PEG-IFN/RBV, as these regimens include PEG-IFN/RBV.

We extracted only RCTs for our analysis in order to obtain highly reliable data and minimize the influence of recall bias because RCTs are prospectively designed, and SAEs should be defined a priori. However, several limitations are still worth noting. The latent limitation of this study is inter-study variability in the definition of SAE. The

precise meaning of 'serious' has not been determined, and some discrepancies between studies exist. These discrepancies may diminish the accuracy of the pooled SAE rate in this study. Second, even by choosing only RCTs, we could not completely exclude the influence of publication bias.

Overall, PEG-IFN/RBV treatment is relatively safe, with low mortality, considering the fact that chronic hepatitis C patients carry a high risk of cirrhosis and HCC. Nevertheless, the SAE rate with this treatment is not negligible and the development of safer regimens should be, and is, encouraged.

Conflict of interest Kazuhiko Koike has served as a speaker for MSD and Chugai Pharmaceutical Co., Ltd., and has received research funding from MSD and Chugai Pharmaceutical Co., Ltd.

References

1. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol. 2006;45(4):529–38.
2. Yvan H, Mary EK, Gregory JD, Joseph FP, Gregory LA, Geoffrey D, et al. Global burden of disease (GBD) for hepatitis C. J Clin Pharmacol. 2004;44(1):20–9.
3. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alpha-2b plus ribavirin compared with interferon alpha-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001;358(9286):958–65.
4. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alpha-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002; 347(13):975–82.
5. Bruno S, Camma C, Di Marco V, Rumi M, Vinci M, Camozzi M, et al. Peginterferon alpha-2b plus ribavirin for naive patients with genotype 1 chronic hepatitis C: a randomized controlled trial. J Hepatol. 2004;41(3):474–81.
6. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med. 2004;140(5): 346–55.
7. Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, et al. Peginterferon alpha-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. N Engl J Med. 2005;352(25):2609–17.
8. Brok J, Gluud LL, Gluud C. Meta-analysis: ribavirin plus interferon vs. interferon monotherapy for chronic hepatitic C—an updated Cochrane review. Aliment Pharmacol Ther. 2010;32(7): 840–50.
9. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med. 1999;131(3):174–81.
10. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557–60.
11. World Health Organization. Available at: http://apps.who.int/ghodata/?vid=720 (2011). Accessed 24 Oct 2011.
12. Bruno R, Sacchi P, Ciappina V, Zochetti C, Patruno S, Maiocchi L, et al. Viral dynamics and pharmacokinetics of peginterferon alpha-2a and peginterferon alpha-2b in naive patients with chronic hepatitis C: a randomized, controlled study. Antivir Ther. 2004;9(4):491–7.
13. Awad T, Thorlund K, Hauser G, Stimac D, Mabrouk M, Gluud C. Peginterferon alpha-2a is associated with higher sustained virological response than peginterferon alpha-2b in chronic hepatitis C: systematic review of randomized trials. Hepatology. 2010;51(4):1176–84.
14. Ascione A, De Luca M, Tartaglione MT, Lampasi F, Di Costanzo GG, Lanza AG, et al. Peginterferon alpha-2a plus ribavirin is more effective than peginterferon alpha-2b plus ribavirin for treating chronic hepatitis C virus infection. Gastroenterology. 2010;138(1):116–22.
15. McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, et al. Peginterferon alpha-2b or alpha-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med. 2009;361(6):580–93.
16. 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(1):108–15.
17. Scotto G, Fazio V, Fornabaio C, Tartaglia A, Di Tullio R, Saracino A, et al. Peg-interferon alpha-2a versus Peg-interferon alpha-2b in nonresponders with HCV active chronic hepatitis: a pilot study. J Interferon Cytokine Res. 2008;28(10):623–9.
18. Yenice N, Mehtap O, Gumrah M, Arican N. The efficacy of pegylated interferon alpha 2a or 2b plus ribavirin in chronic hepatitis C patients. Turk J Gastroenterol. 2006;17(2):94–8.
19. Fellay J, Thompson AJ, Ge D, Gumbs CE, Urban TJ, Shianna KV, et al. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. Nature. 2010;464(7287):405–8.
20. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461(7262):399–401.
21. Hung CH, Lee CM, Lu SN, Wang JH, Chen CH, Hu TH, et al. Anemia associated with antiviral therapy in chronic hepatitis C: incidence, risk factors, and impact on treatment response. Liver Int. 2006;26(9):1079–86.
22. Iwasaki Y, Ikeda H, Araki Y, Osawa T, Kita K, Ando M, et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology. 2006;43(1): 54–63.
23. Oze T, Hiramatsu N, Yakushijin T, Mochizuki K, Oshita M, Hagiwara H, et al. Indications and limitations for aged patients with chronic hepatitis C in pegylated interferon alpha-2b plus ribavirin combination therapy. J Hepatol. 2011;54(4):604–11.
24. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med. 2009;360(18):1827–38.
25. Poordad F, McCone J Jr, Bacon BR, Bruno S, Manns MP, Sulkowski MS, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med. 2011;364(13):1195–206.
26. Abergel A, Hezode C, Leroy V, Barange K, Bronowicki JP, Tran A, et al. Peginterferon alpha-2b plus ribavirin for treatment of chronic hepatitis C with severe fibrosis: a multicentre randomized controlled trial comparing two doses of peginterferon alpha-2b. J Viral Hepat. 2006;13(12):811–20.
27. Alfaleh FZ, Hadad Q, Khuroo MS, Aljumah A, Algamedi A, Alashgar H, et al. Peginterferon alpha-2b plus ribavirin compared with interferon alpha-2b plus ribavirin for initial treatment of chronic hepatitis C in Saudi patients commonly infected with genotype 4. Liver Int. 2004;24(6):568–74.

28. Andriulli A, Cursaro C, Cozzolongo R, Iacobellis A, Valvano MR, Mangia A, et al. Early discontinuation of ribavirin in HCV-2 and HCV-3 patients responding to Peg-interferon alpha-2a and ribavirin. J Viral Hepat. 2009;16(1):28–35.
29. Angelico M, Koehler-Horst B, Piccolo P, Angelico F, Gentile S, Francioso S, et al. Peginterferon alpha-2a and ribavirin versus peginterferon alpha-2a monotherapy in early virological responders and peginterferon alpha-2a and ribavirin versus peginterferon alpha-2a, ribavirin and amantadine triple therapy in early virological nonresponders: the SMIEC II trial in naive patients with chronic hepatitis C. Eur J Gastroenterol Hepatol. 2008;20(7):680–7.
30. Benhamou Y, Afdhal NH, Nelson DR, Shiffman ML, Halliman DG, Heise J, et al. A phase III study of the safety and efficacy of viramidine versus ribavirin in treatment-naive patients with chronic hepatitis C: ViSER1 results. Hepatology. 2009;50(3): 717–26.
31. Berg C, Goncales FL Jr, Bernstein DE, Sette H Jr, Rasenack J, Diago M, et al. Re-treatment of chronic hepatitis C patients after relapse: efficacy of peginterferon-alpha-2a (40 kDa) and ribavirin. J Viral Hepat. 2006;13(7):435–40.
32. Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, et al. Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alpha-2a plus ribavirin. Gastroenterology. 2006;130(4):1086–97.
33. Berg T, Weich V, Teuber G, Klinker H, Moller B, Rasenack J, et al. Individualized treatment strategy according to early viral kinetics in hepatitis C virus type 1-infected patients. Hepatology. 2009;50(2):369–77.
34. Bosques-Padilla F, Trejo-Estrada R, Campollo-Rivas O, Cortez-Hernandez C, Dehesa-Violante M, Maldonado-Garza H, et al. Peginterferon alpha-2a plus ribavirin for treating chronic hepatitis C virus infection: analysis of Mexican patients included in a multicenter international clinical trial. Ann Hepatol. 2003;2(3): 135–9.
35. Brady DE, Torres DM, An JW, Ward JA, Lawitz E,Harrison SA. Induction pegylated interferon alpha-2b in combination with ribavirin in patients with genotypes 1 and 4 chronic hepatitis C: a prospective, randomized, multicenter, open-label study. Clin Gastroenterol Hepatol. 2010;8(1):66–71e1.
36. Brandao C, Barone A, Carrilho F, Silva A, Patelli M, Caramori C, et al. The results of a randomized trial looking at 24 weeks vs. 48 weeks of treatment with peginterferon alpha-2a (40 kDa) and ribavirin combination therapy in patients with chronic hepatitis C genotype 1. J Viral Hepat. 2006;13(8):552–9.
37. Bressler B, Wang K, Grippo JF, Heathcote EJ. Pharmacokinetics and response of obese patients with chronic hepatitis C treated with different doses of PEG-IFN alpha-2a (40 kD) (PEGASYS). Br J Clin Pharmacol. 2009;67(3):280–7.
38. Bronowicki JP, Ouzan D, Asselah T, Desmorat H, Zarski JP, Foucher J, et al. Effect of ribavirin in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2a plus ribavirin. Gastroenterology. 2006;131(4):1040–8.
39. Carr C, Hollinger FB, Yoffe B, Wakil A, Phillips J, Bzowej N, et al. Efficacy of interferon alpha-2b induction therapy before retreatment for chronic hepatitis C. Liver Int. 2007;27(8): 1111–8.
40. Ciancio A, Picciotto A, Giordanino C, Smedile A, Tabone M, Manca A, et al. A randomized trial of pegylated-interferon-alpha2a plus ribavirin with or without amantadine in the retreatment of patients with chronic hepatitis C not responding to standard interferon and ribavirin. Aliment Pharmacol Ther. 2006;24(7):1079–86.
41. Dalgard O, Bjoro K, Ring-Larsen H, Bjornsson E, Holberg-Petersen M, Skovlund E, et al. Pegylated interferon alpha and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. Hepatology. 2008;47(1):35–42.
42. Diago M, Crespo J, Olveira A, Perez R, Barcena R, Sanchez-Tapias JM, et al. Clinical trial: pharmacodynamics and pharmacokinetics of re-treatment with fixed-dose induction of peginterferon alpha-2a in hepatitis C virus genotype 1 true non-responder patients. Aliment Pharmacol Ther. 2007;26(8):1131–8.
43. Ferenci P, Formann E, Laferl H, Gschwantler M, Hackl F, Brunner H, et al. Randomized, double-blind, placebo-controlled study of peginterferon alpha-2a (40 kD) plus ribavirin with or without amantadine in treatment-naive patients with chronic hepatitis C genotype 1 infection. J Hepatol. 2006;44(2):275–82.
44. Ferenci P, Brunner H, Laferl H, Scherzer TM, Maieron A, Strasser M, et al. A randomized, prospective trial of ribavirin 400 mg/day versus 800 mg/day in combination with peginterferon alpha-2a in hepatitis C virus genotypes 2 and 3. Hepatology. 2008;47(6):1816–23.
45. Ferenci P, Laferl H, Scherzer TM, Maieron A, Hofer H, Stauber R, et al. Peginterferon alpha-2a/ribavirin for 48 or 72 weeks in hepatitis C genotypes 1 and 4 patients with slow virologic response. Gastroenterology. 2010;138(2):503–12e1.
46. Fried MW, Jensen DM, Rodriguez-Torres M, Nyberg LM, Di Bisceglie AM, Morgan TR, et al. Improved outcomes in patients with hepatitis C with difficult-to-treat characteristics: randomized study of higher doses of peginterferon alpha-2a and ribavirin. Hepatology. 2008;48(4):1033–43.
47. Gish RG, Arora S, Rajender Reddy K, Nelson DR, O'Brien C, Xu Y, et al. Virological response and safety outcomes in therapy-naive patients treated for chronic hepatitis C with taribavirin or ribavirin in combination with pegylated interferon alpha-2a: a randomized, phase 2 study. J Hepatol. 2007;47(1):51–9.
48. Glue P, Rouzier-Panis R, Raffanel C, Sabo R, Gupta SK, Salfi M, et al. A dose-ranging study of pegylated interferon alpha-2b and ribavirin in chronic hepatitis C. The Hepatitis C Intervention Therapy Group. Hepatology. 2000;32(3):647–53.
49. Hasan F, Al-Khaldi J, Asker H, Al-Ajmi M, Owayed S, Varghese R, et al. Peginterferon alpha-2b plus ribavirin with or without amantadine [correction of amantidine] for the treatment of non-responders to standard interferon and ribavirin. Antivir Ther. 2004;9(4):499–503.
50. Helbling B, Jochum W, Stamenic I, Knopfli M, Cerny A, Borovicka J, et al. HCV-related advanced fibrosis/cirrhosis: randomized controlled trial of pegylated interferon alpha-2a and ribavirin. J Viral Hepat. 2006;13(11):762–9.
51. Herrine SK, Brown RS Jr, Bernstein DE, Ondovik MS, Lentz E, Te H. Peginterferon alpha-2a combination therapies in chronic hepatitis C patients who relapsed after or had a viral breakthrough on therapy with standard interferon alpha-2b plus ribavirin: a pilot study of efficacy and safety. Dig Dis Sci. 2005;50(4): 719–26.
52. Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med. 2009;360(18):1839–50.
53. Ide T, Hino T, Ogata K, Miyajima I, Kuwahara R, Kuhara K, et al. A randomized study of extended treatment with peginterferon alpha-2b plus ribavirin based on time to HCV RNA negative-status in patients with genotype 1b chronic hepatitis C. Am J Gastroenterol. 2009;104(1):70–5.
54. Jacobson IM, Gonzalez SA, Ahmed F, Lebovics E, Min AD, Bodenheimer HC Jr, et al. A randomized trial of pegylated interferon alpha-2b plus ribavirin in the retreatment of chronic hepatitis C. Am J Gastroenterol. 2005;100(11):2453–62.
55. Jacobson IM, Brown RS Jr, Freilich B, Afdhal N, Kwo PY, Santoro J, et al. Peginterferon alpha-2b and weight-based or flat-dose ribavirin in chronic hepatitis C patients: a randomized trial. Hepatology. 2007;46(4):971–81.

56. Jensen DM, Marcellin P, Freilich B, Andreone P, Di Bisceglie A, Brandao-Mello CE, et al. Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha2b: a randomized trial. Ann Intern Med. 2009;150(8):528–40.
57. Kamal SM, El Tawil AA, Nakano T, He Q, Rasenack J, Hakam SA, et al. Peginterferon {alpha}-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral kinetics on sustained virological response. Gut. 2005;54(6): 858–66.
58. Kamal SM, El Kamary SS, Shardell MD, Hashem M, Ahmed IN, Muhammadi M, et al. Pegylated interferon alpha-2b plus ribavirin in patients with genotype 4 chronic hepatitis C: the role of rapid and early virologic response. Hepatology. 2007;46(6): 1732–40.
59. Kawaoka T, Kawakami Y, Tsuji K, Ito H, Kitamoto M, Aimitsu S, et al. Dose comparison study of pegylated interferon-alpha-2b plus ribavirin in naive Japanese patients with hepatitis C virus genotype 2: a randomized clinical trial. J Gastroenterol Hepatol. 2009;24(3):366–71.
60. Khattab M, Emad M, Abdelaleem A, Eslam M, Atef R, Shaker Y, et al. Pioglitazone improves virological response to peginterferon alpha-2b/ribavirin combination therapy in hepatitis C genotype 4 patients with insulin resistance. Liver Int. 2010;30(3): 447–54.
61. Kuboki M, Iino S, Okuno T, Omata M, Kiyosawa K, Kumada H, et al. Peginterferon alpha-2a (40 kD) plus ribavirin for the treatment of chronic hepatitis C in Japanese patients. J Gastroenterol Hepatol. 2007;22(5):645–52.
62. Lagging M, Langeland N, Pedersen C, Farkkila M, Buhl MR, Morch K, et al. 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(6):1837–45.
63. Langlet P, D'Heygere F, Henrion J, Adler M, Delwaide J, Van Vlierberghe H, et al. Clinical trial: a randomized trial of pegylated-interferon-alpha-2a plus ribavirin with or without amantadine in treatment-naive or relapsing chronic hepatitis C patients. Aliment Pharmacol Ther. 2009;30(4):352–63.
64. Lee SD, Yu ML, Cheng PN, Lai MY, Chao YC, Hwang SJ, et al. Comparison of a 6-month course peginterferon alpha-2b plus ribavirin and interferon alpha-2b plus ribavirin in treating Chinese patients with chronic hepatitis C in Taiwan. J Viral Hepat. 2005;12(3):283–91.
65. Liu CH, Liu CJ, Lin CL, Liang CC, Hsu SJ, Yang SS, et al. Pegylated interferon-alpha-2a plus ribavirin for treatment-naive Asian patients with hepatitis C virus genotype 1 infection: a multicenter, randomized controlled trial. Clin Infect Dis. 2008;47(10):1260–9.
66. Lodato F, Azzaroli F, Brillanti S, Colecchia A, Tame MR, Montagnani M, et al. Higher doses of peginterferon alpha-2b administered twice weekly improve sustained virological response in difficult-to-treat patients with chronic hepatitis C: results of a pilot randomized study. J Viral Hepat. 2005;12(5):536–42.
67. Marcellin P, Horsmans Y, Nevens F, Grange JD, Bronowicki JP, Vetter D, et al. Phase 2 study of the combination of merimepodib with peginterferon-alpha2b, and ribavirin in nonresponders to previous therapy for chronic hepatitis C. J Hepatol. 2007;47(4): 476–83.
68. Marcellin P, Gish RG, Gitlin N, Heise J, Halliman DG, Chun E, et al. Safety and efficacy of viramidine versus ribavirin in ViSER2: randomized, double-blind study in therapy-naive hepatitis C patients. J Hepatol. 2010;52(1):32–8.
69. McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, et al. Telaprevir for previously treated chronic HCV infection. N Engl J Med. 2010;362(14):1292–303.
70. Mecenate F, Pellicelli AM, Barbaro G, Romano M, Barlattani A, Mazzoni E, et al. Short versus standard treatment with pegylated interferon alpha-2A plus ribavirin in patients with hepatitis C virus genotype 2 or 3: the cleo trial. BMC Gastroenterol. 2010;10:21.
71. Mendez-Navarro J, Chirino RA, Corey KE, Gorospe EC, Zheng H, Moran S, et al. A randomized controlled trial of double versus triple therapy with amantadine for genotype 1 chronic hepatitis C in Latino patients. Dig Dis Sci. 2010;55(9):2629–35.
72. Meyer-Wyss B, Rich P, Egger H, Helbling B, Mullhaupt B, Rammert C, et al. Comparison of two PEG-interferon alpha-2b doses (1.0 or 1.5 μg/kg) combined with ribavirin in interferon-naive patients with chronic hepatitis C and up to moderate fibrosis. J Viral Hepat. 2006;13(7):457–65.
73. Napoli N, Giannelli G, Antonaci A, Antonaci S. The use of different peg-interferon alpha-2b regimens plus ribavirin in HCV-1b-infected patients after rapid virological response does not affect the achievement of sustained virological response. J Viral Hepat. 2008;15(4):300–4.
74. Pearlman BL, Ehleben C, Saifee S. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis c genotype 1-infected slow responders. Hepatology. 2007;46(6):1688–94.
75. Roberts SK, Weltman MD, Crawford DH, McCaughan GW, Sievert W, Cheng WS, et al. Impact of high-dose peginterferon alpha-2A on virological response rates in patients with hepatitis C genotype 1: a randomized controlled trial. Hepatology. 2009;50(4):1045–55.
76. Roffi L, Colloredo G, Pioltelli P, Bellati G, Pozzpi M, Parravicini P, et al. Pegylated interferon-alpha2b plus ribavirin: an efficacious and well-tolerated treatment regimen for patients with hepatitis C virus related histologically proven cirrhosis. Antivir Ther. 2008;13(5):663–73.
77. Rossignol JF, Elfert A, El-Gohary Y, Keeffe EB. Improved virologic response in chronic hepatitis C genotype 4 treated with nitazoxanide, peginterferon, and ribavirin. Gastroenterology. 2009;136(3):856–62.
78. Rustgi VK, Lee WM, Lawitz E, Gordon SC, Afdhal N, Poordad F, et al. Merimepodib, pegylated interferon, and ribavirin in genotype 1 chronic hepatitis C pegylated interferon and ribavirin nonresponders. Hepatology. 2009;50(6):1719–26.
79. Sanchez-Tapias JM, Diago M, Escartin P, Enriquez J, Romero-Gomez M, Barcena R, et al. Peginterferon-alpha2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. Gastroenterology. 2006;131 (2):451–60.
80. Shiffman ML, Salvatore J, Hubbard S, Price A, Sterling RK, Stravitz RT, et al. Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. Hepatology. 2007;46(2):371–9.
81. Shiffman ML, Suter F, Bacon BR, Nelson D, Harley H, Sola R, et al. Peginterferon alpha-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. N Engl J Med. 2007;357(2):124–34.
82. Shiffman ML, Ghany MG, Morgan TR, Wright EC, Everson GT, Lindsay KL, et al. Impact of reducing peginterferon alpha-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. Gastroenterology. 2007;132(1):103–12.
83. Sjogren MH, Sjogren R Jr, Lyons MF, Ryan M, Santoro J, Smith C, et al. Antiviral response of HCV genotype 1 to consensus interferon and ribavirin versus pegylated interferon and ribavirin. Dig Dis Sci. 2007;52(6):1540–7.
84. Sood A, Midha V, Hissar S, Kumar M, Suneetha PV, Bansal M, et al. Comparison of low-dose pegylated interferon versus standard high-dose pegylated interferon in combination with ribavirin in patients with chronic hepatitis C with genotype 3: an Indian experience. J Gastroenterol Hepatol. 2008;23(2):203–7.

85. Tang KH, Herrmann E, Pachiadakis I, Paulon E, Tatman N, Zeuzem S, et al. Clinical trial: individualized treatment duration for hepatitis C virus genotype 1 with peginterferon-alpha 2a plus ribavirin. Aliment Pharmacol Ther. 2008;27(9):810–9.
86. Toyoda H, Kumada T, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Eight-week regimen of antiviral combination therapy with peginterferon and ribavirin for patients with chronic hepatitis C with hepatitis C virus genotype 2 and a rapid virological response. Liver Int. 2009;29(1):120–5.
87. von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, et al. Peginterferon-alpha-2a (40 kD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology. 2005;129(2):522–7.
88. von Wagner M, Hofmann WP, Teuber G, Berg T, Goeser T, Spengler U, et al. Placebo-controlled trial of 400 mg amantadine combined with peginterferon alpha-2a and ribavirin for 48 weeks in chronic hepatitis C virus-1 infection. Hepatology. 2008;48(5):1404–11.
89. Yu ML, Dai CY, Lin ZY, Lee LP, Hou NJ, Hsieh MY, et al. A randomized trial of 24- vs. 48-week courses of PEG interferon alpha-2b plus ribavirin for genotype-1b-infected chronic hepatitis C patients: a pilot study in Taiwan. Liver Int. 2006;26(1):73–81.
90. Yu ML, Dai CY, Huang JF, Hou NJ, Lee LP, Hsieh MY, et al. A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. Gut. 2007;56(4):553–9.
91. Yu ML, Dai CY, Huang JF, Chiu CF, Yang YH, Hou NJ, et al. Rapid virological response and treatment duration for chronic hepatitis C genotype 1 patients: a randomized trial. Hepatology. 2008;47(6):1884–93.
92. Zeuzem S, Diago M, Gane E, Reddy KR, Pockros P, Prati D, et al. Peginterferon alpha-2a (40 kD) and ribavirin in patients with chronic hepatitis C and normal aminotransferase levels. Gastroenterology. 2004;127(6):1724–32.
93. Zeuzem S, Pawlotsky JM, Lukasiewicz E, von Wagner M, Goulis I, Lurie Y, et al. International, multicenter, randomized, controlled study comparing dynamically individualized versus standard treatment in patients with chronic hepatitis C. J Hepatol. 2005;43(2):250–7.
94. Zeuzem S, Buti M, Ferenci P, Sperl J, Horsmans Y, Cianciara J, et al. Efficacy of 24 weeks treatment with peginterferon alpha-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. J Hepatol. 2006;44(1):97–103.
95. Zeuzem S, Yoshida EM, Benhamou Y, Pianko S, Bain VG, Shouval D, et al. Albinterferon alpha-2b dosed every two or four weeks in interferon-naive patients with genotype 1 chronic hepatitis C. Hepatology. 2008;48(2):407–17.

HEPATOLOGY

HEPATOLOGY, Vol. 57, No. 1, 2013

Table 1. Characteristics of the Randomized Cohorts and SVR Rates of Heterozygous Genotype rs12979860CT With Additional Genotyping of rs8099917

Random Sample Size	Sample Number	Mean Age ±SD	Male	HCV RNA ≥400.000 IU/mL	Severe Fibrosis	SVR rs12979680CT/ rs8099917TT	SVR rs12979680CT/ rs8099917TG	P-value
10%	96	47±11	58%	69%	55%	48%	36%	0.408
20%	192	48±11	59%	80%	43%	43%	32%	0.379
30%	295	48±11	60%	72%	48%	50%	38%	0.154
40%	396	47±11	63%	66%	55%	57%	39%	**0.012**
50%	474	47±11	60%	68%	53%	56%	37%	**0.003**
60%	588	48±11	58%	71%	52%	57%	35%	**0.0001**
70%	654	47±11	58%	72%	52%	56%	39%	**0.002**
80%	754	48±11	58%	70%	51%	55%	39%	**0.002**
90%	835	48±11	59%	71%	52%	56%	40%	**0.001**
100%	942	48±11	59%	70%	52%	55%	40%	**0.001**

SD, standard deviation; IU, international units; SVR, sustained virological response; $P < 0.05$ considered to be statistically significant.

fibrosis stage on the SVR rates of genotype rs12979860CT/rs8099917TT and rs12979860CT/rs8099917TG (Supporting Table 1). Again, it becomes obvious that the impact of additional genotyping of rs8099917 on the prediction of SVR is improved in patients with heterozygous genotype of rs12979860 who have high baseline HCV RNA levels ($P = 3.7 \times 10^{-5}$), HCV subtype 1a ($P = 3.3 \times 10^{-5}$), or severe fibrosis stages ($P = 0.001$), being female ($P = 0.023$), or of younger age ($P = 0.029$). Thus, the different patient characteristics most likely explain the differences in the SVR rates.

From that, one possibly may conclude that two SNPs are good in large cohorts but not relevant for clinical practice. However, the idea of large studies is to inform individual clinical practice. Our results derived from a large cohort suggest that algorithms and models that include both rs12979860 and rs809917 as well as baseline parameters and viral factors are informative to guide therapeutic decision making.[3]

JANETT FISCHER, PH.D.[1]
STEPHAN BÖHM[1]
JACOB GEORGE[2]
CHRISTOPH SARRAZIN[3]
THOMAS BERG, M.D.[1]
[1]*Department of Hepatology, Clinic of Gastroenterology and Rheumatology, Universitätsklinikum Leipzig, Leipzig, Germany*
[2]*Storr Liver Unit, Westmead Hospital and Westmead Millennium Institute, University of Sydney, Sydney, Australia*
[3]*Department of Internal Medicine I J. W. Goethe-University Hospital, Frankfurt, Germany*

References

1. Galmozzi E, De Nicola S, Aghema A, Colombo M. Is there a need for more than one IL28B SNP in hepatitis C clinical practice? HEPATOLOGY 2013;58;416.
2. Fischer J, Böhm S, Scholz M, Müller T, Witt H, George J, et al. Combined effects of different interleukin-28B gene variants on the outcome of dual combination therapy in chronic hepatitis C virus type 1 infection. HEPATOLOGY 2012;55:1700-1710.
3. Ladero JM, Martin EG, Fernández C, Carballo M, Devesa MJ, Martínez C, et al. Predicting response to therapy in chronic hepatitis C: an approach combining interleukin-28B gene polymorphisms and clinical data. J Gastroenterol Hepatol 2012;27:279-285.

DOI 10.1002/hep.25923
Supported by the German Competence Network for Viral Hepatitis (Hep-Net), funded by the German Ministry of Education and Research (BMBF, Grant No. 01 KI 0437, Project No. 10.1.3 and Core Project No. 10.1 Genetic host factors in viral hepatitis and Genetic Epidemiology Group in viral hepatitis), by the EU-Vigilance network of excellence combating viral resistance (VIRGIL, Project No. LSHM-CT-2004-503359), and by the BMBF Project: Host and viral determinants for susceptibility and resistance to hepatitis C virus infection (Grant No. 01KI0787). Parts of the work were supported by an Australian Research Council Linkage Project Grant (LPO0990067), a National Health and Medical Research Council Grant (1006759) and the Robert W. Storr Bequest to the Sydney Medical Foundation, University of Sydney.

Plasma Lysophosphatidic Acid Levels and Hepatocellular Carcinoma

To the Editor:

We read with interest the article by Mazzocca et al.,[1] showing that serum lysophosphatidic acid (LPA) levels are increased in hepatocellular carcinoma (HCC) patients correlated with tumor burden, while not enhanced in cirrhosis patients. However, we think that their LPA values in serum samples need to be carefully evaluated, because of some technical issues in the measurement of LPA levels in blood samples. First, because LPA is released from platelets, LPA has been measured in plasma but not in serum when evaluating its clinical significance.[2,3] Second, as we previously demonstrated,[4] LPA levels in plasma samples are markedly increased after sample preparation unless the temperature is kept under strict control, potentially because the synthetic enzyme autotaxin (ATX) and the substrate lysophosphatidyl choline coexist in plasma samples to abundantly produce LPA. LPA was once reported as a biomarker of ovarian cancer,[2] but contrary data were later demonstrated, in which a distinct sampling of plasma may explain this discrepancy.[3] Indeed, LPA levels in serum reported by Mazzocca et al. were approximately 10 times higher than the previously reported LPA levels in plasma.[2,3] If their LPA values in serum were increased after sampling similarly in each sample, plasma LPA levels might be correlated with HCC burden as reported. To clarify this, we have newly measured plasma LPA levels in HCC patients,

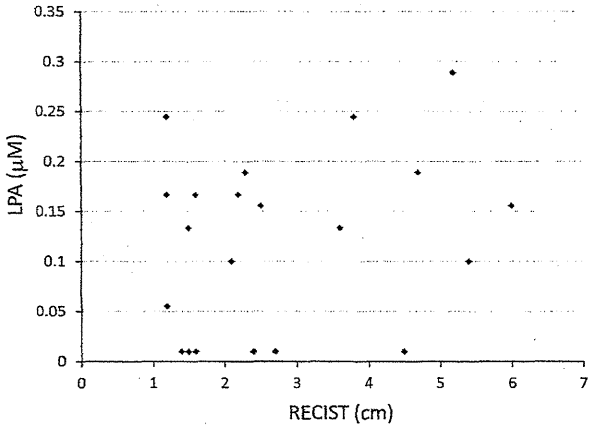

Fig. 1. Plasma LPA levels and HCC burden. Plasma LPA levels, measured in 21 HCC patients (13 males and 8 females; 2 patients with chronic hepatitis B, 15 with chronic hepatitis C, and 4 with non-B non-C chronic liver disease), were not significantly correlated with HCC burden as evaluated by RECIST (Response Evaluation Criteria in Solid Tumors; Spearman rank, $r = 0.158$, $P = 0.4937$). This study was approved by the Institutional Research Ethics Committee and informed consent was obtained for the use of the samples.

and found that they were not correlated with tumor burden, as shown in Fig. 1. Moreover, plasma LPA levels in HCC patients (0.12 ± 0.09 mM, mean $\pm$ SD, $n = 21$), were not different from the previously reported levels in non-HCC patients with chronic hepatitis C (0.10 ± 0.05 mM).[5] Although Mazzocca et al. reported no enhancement of serum LPA levels in cirrhosis patients, we[5] and others[6] previously showed that plasma LPA levels and serum ATX activity were increased in chronic liver diseases in association with fibrosis and cholestatic pruritus, from which HCC frequently arises. Collectively, a role of LPA in HCC should be cautiously analyzed.

HITOSHI IKEDA, M.D., PH.D.[1,2]
KENICHIRO ENOOKU, M.D. PH.D.[1,2]
RYUNOSUKE OHKAWA, PH.D.[1]
KAZUHIKO KOIKE, M.D., PH.D.[2]
YUTAKA YATOMI, M.D., PH.D.[1]
[1]*Department of Clinical Laboratory Medicine*
Graduate School of Medicine
University of Tokyo
Tokyo, Japan
[2]*Department of Gastroenterology*
Graduate School of Medicine
University of Tokyo
Tokyo, Japan

References

1. Mazzocca A, Dituri F, Lupo L, Quaranta M, Antonaci S, Giannelli G. Tumor-secreted lysophostatidic acid accelerates hepatocellular carcinoma progression by promoting differentiation of peritumoral fibroblasts in myofibroblasts. HEPATOLOGY 2011;54:920-930.
2. Xu Y, Shen Z, Wiper DW, Wu M, Morton RE, Elson P, et al. Lysophosphatidic acid as a potential biomarker for ovarian and other gynecologic cancers. JAMA 1998;280:719-723.
3. Baker DL, Morrison P, Miller B, Riely CA, Tolley B, Westermann AM, et al. Plasma lysophosphatidic acid concentration and ovarian cancer. JAMA 2002;287:3081-3082.
4. Nakamura K, Ohkawa R, Okubo S, Tozuka M, Okada M, Aoki S, et al. Measurement of lysophospholipase D/autotaxin activity in human serum samples. Clin Biochem 2007;40:274-277.
5. Watanabe N, Ikeda H, Nakamura K, Ohkawa R, Kume Y, Aoki J, et al. Both plasma lysophosphatidic acid and serum autotaxin levels are increased in chronic hepatitis C. J Clin Gastroenterol 2007;41:616-623.
6. Kremer AE, Martens JJ, Kulik W, Rueff F, Kuiper EM, van Buuren HR, et al. Lysophosphatidic acid is a potential mediator of cholestatic pruritus. Gastroenterology 2010;139:1008-1018.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.25886
Potential conflict of interest: Nothing to report.

Reply:

Ikeda et al. remark that platelets are a main source of lysophosphatidic acid (LPA) and therefore the interpretation of LPA serum concentrations deserves careful attention. However, the same authors previously reported[1] an inverse correlation between plasma LPA concentrations and the number of platelets in patients with chronic C hepatitis. Therefore, it is possible that in physiologic conditions platelets remain the main source of LPA, while in chronic inflammation such as hepatitis C, liver cirrhosis, or hepatocellular carcinoma (HCC), the platelet contribution to LPA production may likely become less relevant. In our study we analyzed sera for LPA detection in healthy donors, liver cirrhosis, and HCC patients, performing well-standardized procedures of collection for each sample. Thus, the contribution of platelets to the LPA concentration was, in reality, normalized. On the contrary, the authors should consider that even in plasma or whole blood, platelet activation is an extremely difficult problem to deal with and control. For example, prolonged tourniquet application, or twisting of the needle in the vein, are major factors interfering with the function of platelets during blood withdrawal, as reviewed by Ruggeri.[2] Unfortunately, these limitations are common for a number of molecules involved both in cancer and in blood cell biology.[3]

Moreover, Ikeda et al. investigated patients with chronic hepatitis C, in whom the inflammatory response is a key component of the tissue microenvironment. In their study, the fibrotic status was also questionable, due to their choice of statistical method (comparison among groups should be done with Kruskal-Wallis tests), and because of the very limited number of patients (14), further stratified into four different groups, which means the conclusions were affected by low power.[1] In our study,[4] we compared liver cirrhosis versus HCC. In the former case, the inflammation is reduced while the fibrotic response is increased, consequently inducing a different microenvironment response.[5] This could explain why patients with liver cirrhosis display relatively low levels of LPA. In addition, it is conceivable that when HCC develops in cirrhotic liver, LPA levels rise once more, as in cases of active inflammatory states (i.e., viral hepatitis). Another key point is patient selection. Ikeda et al. do not provide any information with regard to the clinical features of the patients, i.e., etiology, BCLC stage, previous therapy, etc., as well as how they calculated the size of the tumor in patients with multifocal disease, for instance. Finally, some differences between Caucasian and Asian patients with HCC are to be expected, since the natural history is completely different in Western and Southeast Asian countries.[6] In our study,[4] we demonstrated that LPA has a role in promoting tumor progression and we did not attempt to speculate about the use of LPA as a clinical biomarker. To validate LPA as a potential biomarker for HCC a different study design is required, as well as first considering the power of the study. The enhancement of serum LPA levels reported by Watanabe et al.[1] referred to a relatively small number of patients with chronic hepatitis C. In addition, the