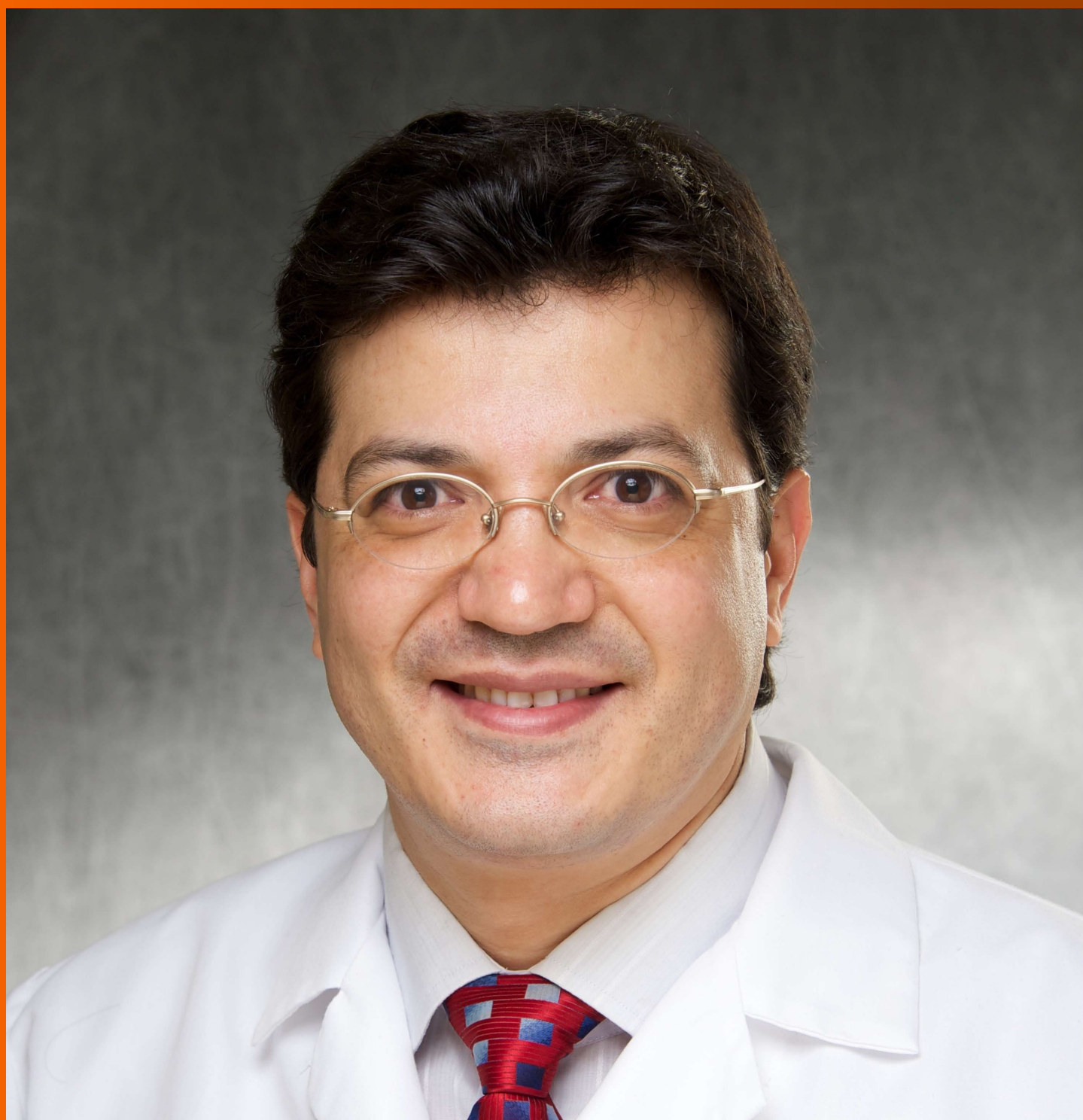


# World Journal of *Hepatology*

*World J Hepatol* 2017 May 18; 9(14): 645-688





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**ISSN**  
ISSN 1948-5182 (online)

**LAUNCH DATE**  
October 31, 2009

**FREQUENCY**  
36 Issues/Year (8<sup>th</sup>, 18<sup>th</sup>, and 28<sup>th</sup> of each month)

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**PUBLICATION DATE**  
May 18, 2017

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## Strategies to tackle the challenges of external beam radiotherapy for liver tumors

Michael I Lock, Jonathan Klein, Hans T Chung, Joseph M Herman, Edward Y Kim, William Small, Nina A Mayr, Simon S Lo

Michael I Lock, Department of Radiation Oncology, London Regional Cancer Program, University of Western Ontario, London, ON N6A 3K7, Canada

Jonathan Klein, Hans T Chung, Department of Radiation Oncology, Odette Cancer Centre, Sunnybrook Health Sciences Centre, Toronto, ON M4N 3M5, Canada

Joseph M Herman, Department of Radiation Oncology, the University of Texas, Houston, TX 77030, United States

Edward Y Kim, Nina A Mayr, Simon S Lo, Department of Radiation Oncology, University of Washington School of Medicine, Seattle, WA 98195, United States

William Small, Department of Radiation Oncology, Loyola University Medical Center, Maywood, IL 60153, United States

**Author contributions:** Lock MI and Lo SS completed the primary literature review and drafting of the manuscript; all authors contributed to this paper with literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

**Conflict-of-interest statement:** Lo SS has received research funding from Elekta AB through the International Oligometastasis Consortium; he has also received travel expenses and honorarium from Varian Medical Systems and travel expenses from Accuray Inc.; Lock MI has received fees as a consultant or research funding from AstraZeneca Limited, Accuray Incorporated, 3M Canada, Varian Medical Systems and Abbvie Corporation. No other potential conflicts of interest are declared. No financial support.

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**Manuscript source:** Invited manuscript

**Correspondence to:** Michael I Lock, MD, CCFP, FRCPC, FCFP, Department of Radiation Oncology, London Regional Cancer Program, University of Western Ontario, 790 Commissioners Rd East, London, ON N6A 3K7, Canada. [michael.lock@lhsc.on.ca](mailto:michael.lock@lhsc.on.ca)  
Telephone: +1-519-6858500-52833  
Fax: +1-519-6858627

**Received:** August 28, 2016

**Peer-review started:** August 29, 2016

**First decision:** November 21, 2016

**Revised:** April 3, 2017

**Accepted:** April 18, 2017

**Article in press:** April 20, 2017

**Published online:** May 18, 2017

### Abstract

Primary and metastatic liver cancer is an increasingly common and difficult to control disease entity. Radiation offers a non-invasive treatment alternative for these patients who often have few options and a poor prognosis. However, the anatomy and aggressiveness of liver cancer poses significant challenges such as accurate localization at simulation and treatment, management of motion and appropriate selection of dose regimen. This article aims to review the options available and provide information for the practical implementation and/or improvement of liver cancer radiation programs within the context of stereotactic body radiotherapy and image-guided radiotherapy guidelines. Specific patient inclusion and exclusion criteria are presented given the significant toxicity found in certain sub-populations treated with radiation. Indeed, certain sub-populations, such as those with tumor thrombosis or those with larger lesions treated with transarterial chemoembolization, have been shown to have significant improvements in outcome with the addition of radiation and merit special consideration. Implementing a liver radiation program

requires three primary challenges to be addressed: (1) immobilization and motion management; (2) localization; and (3) dose regimen and constraint selection. Strategies to deal with motion include simple internal target volume (ITV) expansions, non-gated ITV reduction strategies, breath hold methods, and surrogate marker methods to enable gating or tracking. Localization of the tumor and organs-at-risk are addressed using contrast infusion techniques to take advantage of different normal liver and cancer vascular anatomy, imaging modalities, and margin management. Finally, a dose response has been demonstrated and dose regimens appear to be converging. A more uniform approach to treatment in terms of technique, dose selection and patient selection will allow us to study liver radiation in larger and, hopefully, multicenter randomized studies.

**Key words:** Hepatocellular carcinoma; Liver metastases; 4DCT; Image-guided radiotherapy; Stereotactic body radiation therapy

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**Core tip:** Primary and metastatic liver cancer patients are a growing population seen in cancer centers. This population often has few options and a poor prognosis. Radiation offers a safe non-invasive treatment option, but those implementing a liver radiotherapy program must address specific challenges not always seen in other disease sites. A growing and large number of papers have investigated a wide range of strategies. Our objective is to consolidate this literature to provide a concise review of options to allow a pragmatic selection of management strategies.

Lock MI, Klein J, Chung HT, Herman JM, Kim EY, Small W, Mayr NA, Lo SS. Strategies to tackle the challenges of external beam radiotherapy for liver tumors. *World J Hepatol* 2017; 9(14): 645-656 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v9/i14/645.htm> DOI: <http://dx.doi.org/10.4254/wjgh.v9.i14.645>

## INTRODUCTION

Liver cancer is a major area of investigation as it is increasingly common and remains one of the deadliest diseases where clinicians have few options. According to Surveillance, Epidemiology, and End Results (SEER) statistics, the estimated numbers of cases of liver cancer (including intrahepatic bile duct cancers) will be 35660 in 2015 representing the second largest annual increase in incidence amongst all cancers in the United States<sup>[1]</sup>. Liver remains the most frequent site of metastatic disease for patients with colorectal cancer. Approximately 50%-60% of patients with colorectal cancer (CRC) will develop liver metastases and one third will die from liver failure from progressive disease<sup>[2]</sup>. In patients with only limited liver metastases, aggressive local treatment

with surgical extirpation could result in 5-year overall survival rates of 25%-40%<sup>[2]</sup>. Likewise, the mainstay of treatment for primary liver cancer is surgical resection or liver transplantation. Unfortunately, only 15%-25% of patients are eligible for curative resection or transplant at the time of diagnosis.

Traditionally, radiotherapy has not been routinely given to patients with liver tumors primarily due to the relatively low liver tolerance to radiation. With the advent of advanced radiation technology, it is now possible to deliver potentially curative radiation doses to liver tumors safely. Investigators from Sweden and Japan pioneered the use of stereotactic body radiotherapy (SBRT), a spin-off of intracranial stereotactic radiosurgery (SRS) for extracranial targets<sup>[3]</sup>. SBRT has also been applied for the treatment of liver tumors and the early results are promising. Following these results, advanced technologies such as protons have also been used to deliver radiotherapy to liver tumors with good results<sup>[4,5]</sup>.

Despite the availability of advanced radiotherapy technologies and evidence of efficacy, the use of radiotherapy for liver has not become standard<sup>[6]</sup>. This may be due to the fact that there are several difficult challenges for radiotherapy of liver lesions and a myriad of approaches to deal with these challenges. Clinicians must select an appropriate patient population, a safe and effective dose regimen, image guidance methods for tumor localization, methods to deal with respiratory motion, and methods to avoid radiation-induced complications. This overview will provide a practical review of the challenges and options for the treatment of primary and secondary liver tumors. This will assist the practical selection and implementation of options for a high-quality program that follows the guidelines on SBRT<sup>[7]</sup>.

## APPROPRIATE PATIENT SELECTION AND ACHIEVABLE CLINICAL OUTCOMES

### Hepatocellular carcinoma

There have been significant advances in the options available for hepatocellular carcinoma beyond surgery with level 1 evidence of an overall survival benefit for sorafenib, radiofrequency ablation and transarterial chemoembolization (TACE)<sup>[2]</sup>. Patient selection for sorafenib is limited to patients with hepatocellular carcinoma (HCC), that earlier treatments options are not suitable, or patients who have progressed on other treatments. This tends to be patients with extensive disease within or outside the liver, including patients with portal vein invasion based on two randomized controlled trials<sup>[8,9]</sup>. TACE has been shown in two randomized controlled trials and one metaanalysis to improve survival at two years<sup>[10-12]</sup>. Subsequent metaanalysis has added to the controversy indicating no improvement<sup>[13]</sup>. However, there are no prospective randomized studies to inform clinical practice beyond radiofrequency ablation, sorafenib, TACE and surgery. This makes selection of appropriate patients subject to interpretation of the evidence. For radiotherapy

there is no prospective randomized trial, and we must rely on interpretation of multiple studies reporting case series data with variable patient inclusion, treatment and length of follow-up (Table 1). However, the literature suggests that one subgroup can be identified: Unresectable, locally advanced disease without extrahepatic metastasis, Child-Pugh class A or B, and occupying less than 2/3 of the liver. Several guidelines already include radiation for this subgroup<sup>[14,15]</sup>. This is based on a growing body of level II evidence (retrospective and prospective case series data); this data indicates a 1-year overall survival of 48%-100%, a 1-year local control rate of 64%-100%, and a grade 3 or greater toxicity of 0%-36% (Table 1). Yet, the role of radiation for hepatocellular carcinoma can span early curative presentations to palliative treatment also based on retrospective data<sup>[16]</sup>. For tumors near luminal structures, conventional radiotherapy may be recommended over SBRT depending on the dose selection method and planning constraints applied. For the subgroup of recurrences or incomplete responses post chemotherapy or chemoembolizations, the data also suggest that radiation is a strong option. In the landmark trial by Shim, the 2-year overall survival for those receiving radiation was 37% compared to 14% for those who did not receive radiation<sup>[17]</sup>. This trial also highlighted the importance of tumor size in predicting success of TACE and the possible value of adding for certain patients. For tumors greater than 8 and 10 cm, no patients lived beyond 2-years with TACE. However, if external beam radiation was added, the survival was 50% and 17% for the 8 and 10 cm groups, respectively. Combination therapy, particularly in those with larger lesions where TACE is indicated, the addition of radiation may play a significant role.

Lastly, another group with a very poor outcome are those with portal vein thrombosis. Radiotherapy may be particularly useful for tumor thrombosis where current median survivals remain at 2-4 mo without radiation. To date there have been twelve retrospective<sup>[18-29]</sup> and 1 prospective case<sup>[30]</sup> series demonstrating a median survival improvement of two to five times historical cohorts. The larger studies used older radiotherapy techniques, including the largest from Yoon *et al.*<sup>[22]</sup>. Despite a relatively low median dose of 40 Gy in 2-5 fractions, the study achieved an impressive 43% one year overall survival and an acceptable 10% grade 3 or greater toxicity rate<sup>[22]</sup>. Randomized trials to address the value of radiation for patients with thrombosis are warranted given the possible survival benefit.

### Liver metastases

There is growing interest in radiation for oligometastatic disease and palliation. Høyer *et al.*<sup>[31]</sup> reviewed five retrospective and seven prospective trials to determine which patients should be considered for liver SBRT. This review of the literature by a subcommittee of the American Society of Radiation Oncology (ASTRO) including members from the European Society for Therapeutic Radiology and Oncology (ESTRO), the Canadian Asso-

ciation of Radiation Oncology (CARO) and the Trans-Tasman Radiation Oncology Group (TROG), concluded that the ideal radiotherapy candidate would have an ECOG 0-1, possess adequate hepatic function, have no extrahepatic disease, and have an uninvolved liver volume of 700 mL or greater. This would result in local control rates ranging from 56%-100% at 2 years. Table 2 summarizes prospective trials of SBRT for liver metastases. For a large proportion, extra-hepatic progression develops after local treatment. Though no threshold dose has been found, this group recommended liver metastases receive 48 Gy in three fractions based on the available evidence.

## TECHNICAL IMPLEMENTATION CHALLENGES

### Immobilization and motion management

Surveys demonstrate that there is no universal standard for liver SBRT<sup>[32]</sup>, but there are recommendations from large SBRT groups. This includes the CARO Scope of Practice guidelines that were published to ensure safe practice in the major SBRT sites<sup>[31,33]</sup>. In conventional treatments, a larger margin for internal target volume (ITV) may be acceptable as multiple fractions averaged the dose errors caused by inaccurate organ localization, motion or set up error. SBRT relies on the delivery of accurate high doses to the target and errors in localization could result in increased toxicity, geometric tumor miss and cannot be easily "corrected" in later fractions. Therefore, the use of techniques or devices to localize the radiation to the tumor, minimize margins and optimize on-treatment quality assurance is critical. Furthermore, with the use of IMRT, improved motion management results in fewer unplanned hot and cold spots due to the interplay of the motion of anatomical structures and MLC leaf motion<sup>[34]</sup>.

The primary motion with liver SBRT is respiratory motion which can be controlled with fixed immobilization, breath hold and/or tracking. For immobilization, vacuum-bag systems or fixed body immobilizers are used where arms are kept up and out of field. A simple margin expansion to account for ITV is then applied based on a 4DCT scan, fluoroscopy and/or slow CT scanning to capture the full range of motion. These are categorized as ITV methods or motion encompassing methods. An additional margin for set-up motion is added for planning target volume (PTV) with recommendations ranging between 2 and 5 mm<sup>[35]</sup>. These methods in isolation necessitate larger treated volumes, greater normal tissue inclusion and a lower chance for dose escalation. Shallow breathing may be sufficient in many patients especially if patients are compliant and can maintain regular breathing motions. The American Association of Physicists in Medicine (AAPM) Task Group 76 emphasizes that some method of respiratory assessment be applied and a step-wise algorithm be applied to determine the amount of respiratory management required<sup>[36]</sup>. However, in a large proportion of patients, additional motion management techniques are necessary to achieve greater dose escalation and

**Table 1** Summary of hepatocellular carcinoma radiotherapy studies

Ref.	No. of patients	Percent of Child-Pugh B patients	Median tumor diameter (range), cm	Dose (range)/No. of fractions	Median follow-up interval, mo	1-yr OS	2-yr LC	Toxicity
Scorsetti <i>et al</i> <sup>[68]</sup> , 2015	43	36%	≤ 6 cm	48-75 Gy/3 (51%) and 36-60 Gy/6 (49%)	8	77.9%	64.4%	≥ gr3: 0
Yamashita <i>et al</i> <sup>[69]</sup> , 2015	79	11%	2.7 cm	48 Gy (40-60)/4-10	21	53% at 2 yr	40%	gr3-4: 4.6% gr2: 2.3%
Huertas <i>et al</i> <sup>[70]</sup> , 2015	77	14.3%	2.4 cm	45 Gy/3	12	81.8	99%	14.9%
Zhong <i>et al</i> <sup>[71]</sup> , 2014	72	26%	13.1 cm	35.6 Gy/12	18	56%	NR	gr1-2: 5.6% liver gr1-2: 9.8% gastrointestinal RILD 9.4%
Lo <i>et al</i> <sup>[72]</sup> , 2014	53	NR	4.3 cm	40 Gy/4-5	13.1	70.1%		0
Van de Voorde <i>et al</i> <sup>[73]</sup> , 2014	5	NR	NR	93.6 Gy (62.5-150)/3-10	21	85.4%	NR	0
Sanuki <i>et al</i> <sup>[74]</sup> , 2014	63	16%	2.6 cm	35-40 Gy/5	31.1	100%	95%	gr3: early: 16% late: 21% gr4-5: 0% gr3: 4% gr4-5: 0%
Park <i>et al</i> <sup>[75]</sup> , 2013	26	27%	2.8 cm	40-50 Gy; 4-5 Gy per fraction	20.2	88.5%	87.6%	gr3: 21% gr4: 2.9% gr5: 6.9%
Bujold <i>et al</i> <sup>[30]</sup> , 2013	102	0%	9.9 cm	24-54 Gy (36)/6	31.4	75%	74%	gr3: 4.3% gr4: 1.0% gr5: 1.0%
Yoon <i>et al</i> <sup>[76]</sup> , 2013	93	26%	2 cm	45 Gy (30-60)/3-4	25.6	86.0%	94.8% <sup>1</sup> (2 yr)	gr3: 6.5% gr4: 1.9% gr5: 0%
Jang <i>et al</i> <sup>[65]</sup> , 2013	108	10%	3.0 cm	51 Gy (33-60)/3	30	83% <sup>1</sup>	87%	gr ≥ 3: 7% gr3: 4% gr4: 1.3% gr5: 0%
Jung <i>et al</i> <sup>[77]</sup> , 2013	92	26%	Vol: 8.6 cc	45 Gy (30-60)/3-4	25.7	86.9%	92.1% (3 yr)	gr3: 10% gr4-5: 0% gr2: 31.8% gr5: 1.1%
Bibault <i>et al</i> <sup>[78]</sup> , 2013	75	11%	3.7 cm	40-45 Gy/3	10	78.5%	89.8%	gr3: 2.4% gr4-5: 0% gr2: 3%
Honda <i>et al</i> <sup>[79]</sup> , 2013	30	23%	16 cm	48 Gy/4	12.3	100%	95% <sup>1</sup>	gr3: 6.4% gr4: 4.3% gr5: 0%
Yuan <i>et al</i> <sup>[80]</sup> , 2013	22	45%	4.3 cm	45 Gy (39-54)/3-8	53.4	73%	92.9%	gr3: 4.8% gr4: 4.8% gr5: 0%
Sanuki <i>et al</i> <sup>[81]</sup> , 2013	185	15%	CP-A: 27 cm CP-B: 24 cm	CP-A: 40 Gy/5 CP-B: 35 Gy/5	24	95%	93% (2 yr)	NR
Xi <i>et al</i> <sup>[18]</sup> , 2013	41	0%	Mean GTV vol: 65.4 cc (SD: 47.9)	30-48 Gy (36)	10	50.3%	NR	gr3: 35% gr4: 1.7% gr5: 0%
Huang <i>et al</i> <sup>[82]</sup> , 2012	36	NR	1.1-12.3 cm	37 Gy (25-48)/4-5	14	64% at 2 yr	98%	gr ≥ 3 22% gr3: early 8% late 4%
Kang <i>et al</i> <sup>[83]</sup> , 2012	47	13%	2.9 cm	42-60 Gy/3	17	83% <sup>1</sup>	94.6%	gr3: 0% gr4: 2% gr5: 0%
Ibarra <i>et al</i> <sup>[84]</sup> , 2012	21	NR	GTV vol: 334.2 cc	30 Gy (18-50)/1-10	12.9	87%	57% <sup>1</sup> (2 yr)	gr3: 13 instances gr4: 11.8% gr5: 0%
Price <i>et al</i> <sup>[85]</sup> , 2012	26	46%	Median GTV vol: 33.9 cc	42 Gy (24-48)/3-5	13	77%	NR	gr2: 33% 0%
Andolino <i>et al</i> <sup>[86]</sup> , 2011	60	40%	3.1 cm	CP-A: 30-48 Gy/3 CP-B: 24-48 Gy/5	27	82% <sup>1</sup>	90% (2 yr)	gr3: 3% gr4-5: 0% gr3: 4.5% gr4-5: 0%
Chan <i>et al</i> <sup>[87]</sup> , 2011	11	25%	3 cm	45 Gy/10	24	62%	NR	
Louis <i>et al</i> <sup>[88]</sup> , 2010	25	12%	4.5 cm	45 Gy/3	12.7	79%	95%	
Kwon <i>et al</i> <sup>[89]</sup> , 2010	42	10%	Vol: 15.4 cc	30-39 Gy/3	28.7	92.9%	67.5%	
Cárdenes <i>et al</i> <sup>[66]</sup> , 2010	17	65%	≤ 6 cm	CP-A: 48 Gy/3 CP-B: 42 Gy/3 then 40/5	24	75%	100%	
Son <i>et al</i> <sup>[90]</sup> , 2010	47	8%	18.3 cm	36 Gy (30-39)/3	NR	NR	NR	
Goyal <i>et al</i> <sup>[91]</sup> , 2010	6	NR	9.3 cm	34 (24-45 Gy)/1-3	10	83%	100% at 9 mo	
Seo <i>et al</i> <sup>[92]</sup> , 2010	38	11%	Vol: 40.5 cc	33-57 Gy/3-4	15	68.4%	66.4% (local PFS)	
Choi <i>et al</i> <sup>[21]</sup> , 2008	22	14%	Vol: 23.5 cc	36 Gy (30-39)/3	11.5	88.1%	NR	

Tse <i>et al</i> <sup>[61]</sup> , 2008	31	0%	173 cc	36 Gy (24-54)/6	17.6	48%	NR	gr3: 29% gr4-5: 0%
Méndez Romero <i>et al</i> <sup>[93]</sup> , 2006	8	25%	3.2 cm	< 4 cm: 37.5 Gy/3 ≥ 4 cm: 25 Gy/5 or 30 Gy/3	12.9	75%	75% (22 mo)	gr5: 12.5% RILD

<sup>1</sup>Estimated from survival curve. CP-A: Child-Pugh class A; CP-B: Child-Pugh class B; gr: Grade; GTV: Gross tumor volume; LC: Local control; OS: Overall survival; NR: Not reported; PFS: Progression-free survival; RILD: Radiation induced liver disease; vol: Volume.

**Table 2 Prospective metastatic liver stereotactic body radiotherapy studies**

Ref.	No. of patients	Dose (Gy/fraction)	Median follow-up (mo)	2-yr local control (%)
Herfarth <i>et al</i> <sup>[94]</sup> , 2001	37	14-26 Gy/1	5.7	81 <sup>2</sup>
Hoyer <i>et al</i> <sup>[95]</sup> , 2006	44	45 Gy/3	51.6	79
Kavanagh <i>et al</i> <sup>[96]</sup> , 2006	36	60 Gy/3	19	93
Ambrosino <i>et al</i> <sup>[97]</sup> , 2009	27	Median 36 (25-60) Gy/3	13	74 crude <sup>2</sup>
Rusthoven <i>et al</i> <sup>[62]</sup> , 2009	47	36-60, 60 Gy/3	16	92
Lee <i>et al</i> <sup>[98]</sup> , 2009	68	Median 41.8 Gy/6	10.8	71 <sup>2</sup>
Méndez Romero <i>et al</i> <sup>[93]</sup> , 2006	17 <sup>1</sup>	30-37.5 Gy/2		86
Stintzing <i>et al</i> <sup>[99]</sup> , 2010	36 <sup>1</sup>	24 Gy/1	21.3	87 <sup>2</sup>
Goodman <i>et al</i> <sup>[100]</sup> , 2010	26 <sup>1</sup>	18-30 Gy/1	17	77 <sup>2</sup>
Rule <i>et al</i> <sup>[63]</sup> , 2011	27	30 Gy/5 50 Gy/5 60 Gy/5	20	56 89 100
Janoray <i>et al</i> <sup>[101]</sup> , 2014	56	45 Gy/3-60 Gy/3	12.5	64 <sup>2</sup>

<sup>1</sup>Included hepatocellular patients; <sup>2</sup>12-18 mo local control percentage.

safety. The AAPM suggests a cut-off of 5 mm after which respiratory management is recommended. The options can be categorized into three types: (1) non-gated ITV reduction strategies; (2) active or passive breath hold techniques; and/or (3) surrogate markers. These are applied uniformly based on institutional practice or after a trial assessment of patients who are then assigned to one or more additional motion control methods.

**Non-gated ITV reduction strategies:** Abdominal compression was one of the earliest motion management strategies and was first used in Karolinska Hospital for lung and liver lesions in the 1990s<sup>[37]</sup>. A compression plate was applied to the abdomen to reduce abdominal motion caused by respiration. Early data, primarily from lung cancer patients, has shown accuracy and reproducibility with median reductions of 7 mm<sup>[36,38]</sup>. Recent papers using fiducial markers to track motions have provided direct data on reproducibility and extent of motion reduction in liver patients using abdominal compression. Essentially, a motion minimization method, Wunderink and Méndez Romero<sup>[39]</sup> demonstrated reduced median excursion by 62% and essentially all residual excursions were reduced to less than 5 mm. Reproducibility was excellent between planning and treatment. Predating much of the 4D respiratory strategies, the appeal of this method includes better localization; this is due to more projection data from the entire breath cycle being available leading to better image quality than only a portion of the 4DCT data set. Another advantage of abdominal compression is the minimal technology requirements compared to more complicated strategies such as gating.

However, the magnitude of improvement may be

smaller than initially reported. Updated data from Eccles reported in 2011 that the decrease in motion averaged 2.3 mm and 0.6 mm in the CC and AP direction; 28% saw an increase in motion with abdominal compression so this option does come with caveats<sup>[40]</sup>. Motion of other important structures such as the kidney do not appear to be improved with the use of this type of device<sup>[41]</sup>. Furthermore, not all patients require or can tolerate abdominal compression. Patients with abdominal aortic aneurysm also may not be suitable for abdominal compression. Therefore, other motion correction methods have been tested such as using the mean respiratory position for planning<sup>[42]</sup>. This strategy determines the diaphragm's mean cranio-caudal position in the respiratory cycle or selects a mid-ventilation CT data set. Velec was able to show that this simple method resulted in a 34% lower irradiated volume due to the significantly smaller PTV compared to standard full-motion ITV-based and dose probability PTVs. However, this group demonstrated that rigid motion correction still results in an 8% and 7% change in dose accumulation for the tumor and normal tissues, respectively<sup>[43]</sup>. These changes were found in a majority of patients and suggest the need for some additional form of respiratory control and further investigation of adaptive SBRT to deal with organ deformation.

**Breath hold methods:** The second category of motion management techniques are the breath hold methods. The simplest application is deep inspiration breath hold (DIBH). Initially pioneered at the Memorial Sloan-Kettering Cancer Center<sup>[44]</sup>, DIBH has shown reproducibility within a margin of  $2.2 \pm 2.0$  mm<sup>[45]</sup>. Voluntary DIBH can reduce internal

motion from 12.9 to 2.8 mm<sup>[46]</sup>. Additional margins for set-up error (typically 2-5 mm) and assessment of intra- and inter-fraction motion is required<sup>[26]</sup>. The addition of assisted or active breath hold, such as Active Breathing Control (ABC), reduces variability further. ABC was commercialized by Elekta, Inc. and uses a spirometer that monitors the phase of breathing. Usually after two preparatory breaths, a valve is closed at expiration thereby “holding the patient’s breath”. Issues with this strategy include concerns regarding reproducibility, cost of non-reusable components, time required, maintenance and patient tolerance. Reproducibility assessments have demonstrated good intrafraction absolute offsets of 3 mm or less. However, interfraction errors > 3 mm are found in 46% of cases further emphasizing the need for image guidance<sup>[47]</sup>. Alternatively, shallow breathing and voluntary breath hold can be monitored using the spirometry system; planning margins, treatment activation, and reproducibility of set up can be determined using the same equipment. This is useful as not all patients can tolerate active breath hold. While it has its own limitations, voluntary breath hold has many advantages compared to gating including no marker/tumor motion lag issues, about half the treatment time required, less specialized equipment, less training and software, plus more efficient simulation<sup>[29]</sup>.

### Surrogate markers to enable gating or tracking:

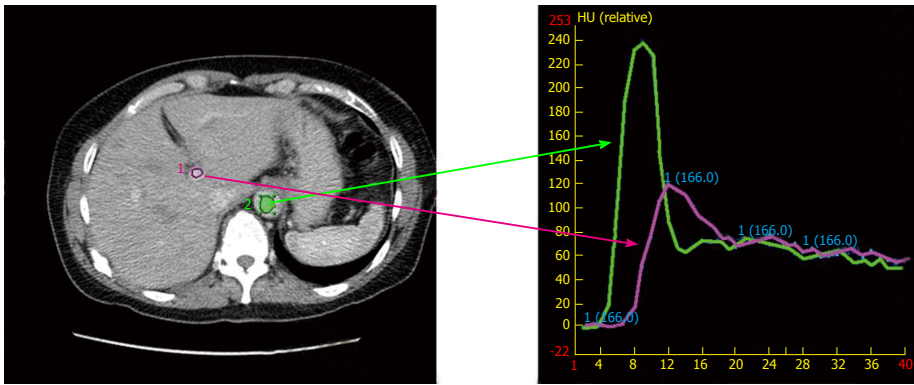
The third variation in motion management is the use of surrogate markers to enable gating. Depending on the surrogate, this overlaps with tumor localization strategies. Surrogates are used to assess the degree of motion which leads to individualization of motion management. Compared to breath hold techniques alone, this method is better tolerated and may represent a more accurate anatomical picture than deep inspiration or expiration. External respiratory surrogates include the Varian 3D infrared marker (Real-time Positioning Management or RPM) system, the Siemens respiratory strain gauge belt, and 3D laser surface respiratory assessment systems (C-RAD Sentinel). Internal surrogates include the diaphragm/lung interface, radiotransmitters (Calypso), and radio-opaque markers (including surgical clips, stents, lipiodol or anatomical calcifications). Internal markers provide the best surrogates, but usually are invasive to insert, risk complications and have a higher relative cost in time and money. Various gating options can be chosen based on the information from these surrogates. Respiratory gating can be grouped into three major categories: First, phase gating consists of treating the patient during a particular phase such as end-expiration. The advantage is that this portion of the breathing cycle is often the most stable with the least motion. Second, amplitude gating selects a certain portion of the respiratory cycle defined by a percentage of the amplitude of each cycle. As phase gating may result in binning errors due to breath to breath variations in slope, length of cycles and amplitudes, detractors suggest better sorting with fixed amplitude gating<sup>[48]</sup>. Third, gating may be based on the

surrogate marker at breath hold. In addition to motion assessment, these markers may be used during treatment for synchronizing treatment delivery. For example, simply gating the treatment beam when the surrogate indicates the tumor is in a certain position, or synchronizing the aperture *via* dynamic multileaf collimator (DMLC), or moving beam to the location of the lesion (Cyberknife) are valuable strategies<sup>[49]</sup>. A method to select an appropriate clinical target volume (CTV) to PTV margin has been developed by Keall and Vedam based on three challenges: (1) selection of amplitude vs phase gating; (2) accounting for phase shifts between markers and the lesion; and (3) the management of intrafraction motion vs increased delivery time<sup>[50]</sup>. Typical GTV to PTV margins are 5 mm axially and 10 mm craniocaudally<sup>[35]</sup>. Periodic monitoring during treatment is still necessary to confirm reproducibility of the motion compared to planning. Patient training plus visual or verbal prompting may allow better reproducibility and margins<sup>[50]</sup>.

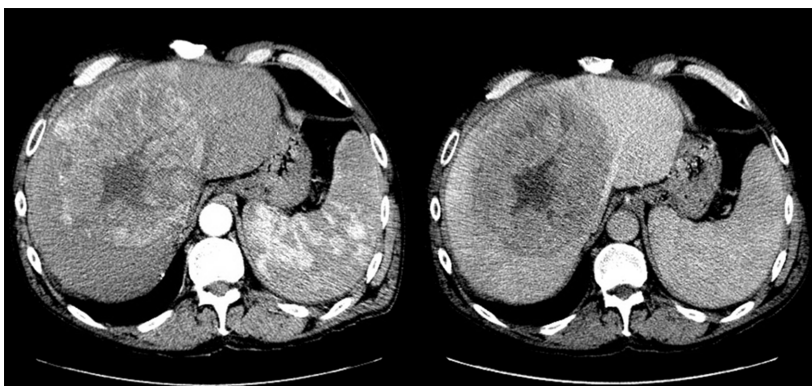
### Localization

At simulation, IV contrast is considered standard particularly for hepatocellular carcinoma. However, this does introduce fusion errors as the contrast infusion must be captured over several respiratory cycles. Various protocols are in place such as the MD Anderson standardized protocol<sup>[51]</sup> or those that individualize<sup>[52]</sup> binning by visualizing contrast in specific vessels. The later method accounts for patient differences in the time contrast reaches and leaves the lesion, anatomical location of the tumor relative to the start of the scan, body weight, time-density curves and cirrhosis (Figure 1). Figure 1 demonstrates the time-density intravenous contrast enhancement called Dynamic Contrast Enhanced CT (DCECT). Images are binned by location in respiratory cycle and when the contrast density within a vessel (such as the aorta or portal vein) signifies the arterial, portal venous, and delayed phase. Images are specific to each patient and individualized contrast enhanced images (Figure 2) offer the possibility of improved delineation without additional equipment or technology, but methods to eliminate motion during the long acquisitions are required<sup>[53,54]</sup>.

MRI and PET are becoming a standard part of management for liver lesions due to improved sensitivity and specificity<sup>[55]</sup>. MRI is particularly useful for small tumors, cirrhotic patients or those who are unable to tolerate IV contrast. MRI may play a greater role as experience with 4DCT, gated MRI and cine MRI accumulates<sup>[56,57]</sup>. Functional imaging assessments are useful for follow-up, and to determine the necessity to add additional treatments post radiation<sup>[58]</sup>. However, both MRI and PET have long acquisition times and require strategies to account for motion. Strategies such as multiple breath holds, parallel imaging for rapid acquisition and respiratory correlated PET are being investigated. Even if accurate localization is possible with the elimination of respiratory motion, strategies to register the MRI and/or PET to the CT image are then required. Additional margins will be required after deformable or rigid image registration in



**Figure 1** Image of a time-density intravenous contrast enhancement called Dynamic Contrast Enhanced computerized tomography. Images are binned by location in respiratory cycle and when the contrast density within a vessel (such as the aorta and portal vein) signifies the non-contrast, arterial and wash-out phase. Time is measured in seconds and density is measured in Hounsfield units (HU).



**Figure 2** Arterial and portal-venous phase images.

the range of 2.2 to 21.3 mm<sup>[59]</sup>. Therefore, the value of more accurate localization must be balanced against the additional margin, time, and cost.

Oral contrast is useful to localize luminal structures, which often represent the most critical organs at risk. The contrast is assigned a CT number for tissue equivalence prior to planning. Lastly, calcifications, vessels and other anatomical landmarks can be extremely useful. If possible, contouring these structures provides information to the therapists; communication with therapists to indicate which critical structures to localize, prioritize and/or avoid is a practical and valuable routine to incorporate.

At treatment, localization of the tumor in the liver is sometimes not possible in contrast to other sites such as lung cancer where the tumor location is often very clear. Internal and/or external surrogate markers or structures may be used as described earlier. At treatment the consistency of correlation with respiratory motion or breath hold ability at time of simulation must be verified. A commonly used structure is the diaphragm. Vedam has shown a strong linear correlation between the diaphragm and the external marker; a superior-inferior CTV-PTV margin of 0.8 cm provided sufficient coverage over multiple sessions with or without training<sup>[60]</sup>. Static images are acceptable, but real-time or near-real-time options exist. Some systems have the ability to acquire images in fluoroscopy or cine-mode and new systems now enable almost real-time dose accumulation to enable adaptive

treatment. However, with fewer fractions used in SBRT, the opportunity to correct dosing errors is limited and localization prior to and during each treatment remains the primary goal. Non-radiographic dependent internal tumor markers such as Calypso can track motion during treatment to provide a more accurate assessment of tumor motion. This real-time tracking has significant advantages over other motion control strategies including the ability to adjust beam delivery *via* synchronized aperture tracking methods or by directly following the lesion motion with the radiation beam.

#### **Dose selection and constraints**

An optimal dose for primary and secondary liver cancer has not been identified. Essentially there are two types of research approaches in the literature for dose finding: Radiobiologically-guided dose escalation and step-wise dose escalation. The first approach, such as the pioneering work of Tse *et al.*<sup>[61]</sup>, uses radiobiological calculation of risk to provide individualized dose recommendations. The second relies on maximally tolerated dose (MTD) techniques used successfully in drug trials. In many cases, the dose has been determined by normal tissue constraints. Furthermore, patient tolerance of radiation may vary due to underlying hepatic insufficiency, and previous or concurrent treatments (resections, chemotherapy). Despite the varied approaches and dose regimens, a convergence of dose recommendations may

be occurring (Table 1, summary of studies for HCC; and Table 2, summary of SBRT studies of metastatic cancer). For hepatic metastases, work published by Rusthoven demonstrated that 60 Gy in 3 fractions resulted in a local control rate of 92% at 2 years<sup>[62]</sup>. Similarly, Timmerman and Rule suggest that a 60 Gy in 5 fraction regimen is appropriate, particularly for tumors adjacent to critical structures<sup>[63]</sup>. Based on three dose escalation cohorts, the actuarial 24 mo local control was 100%. The authors state that a maximum tolerated dose was not reached; MTD was defined as the dose below which the dose limiting toxicity rate was  $\geq 33\%$ . Both groups used a critical volume model with at least 700 mL of normal liver receiving less than 15 Gy and 21 Gy for the 3 and 5 fraction regimen, respectively. However, in both studies tumors were highly selected with a median tumor size of less than 3 cm and few patients had centrally located lesions. Therefore, the excellent results may not be generalizable to a wider population especially those with larger lesions. However, for patients who can meet the trial constraints, the 100% local control rate is a strong argument that the optimal dose for hepatic metastases is 60 Gy in three or five fractions.

For primary liver cancer, a dose response relationship has been found<sup>[64]</sup>, but outcomes and regimens remain somewhat more varied than with metastatic disease (Table 1). HCC patient population is very heterogeneous with important parameters such as size of lesion, liver dysfunction, previous treatments received, presence of vascular invasion and number of lesions all influencing outcome. This heterogeneity increases the difficulty in generalizing data. Modeling suggests that a 90% probability of 6-mo control could be achieved with 84 Gy in 2 Gy equivalent doses<sup>[55]</sup>; much higher than the 53 Gy in 2 Gy equivalent required for metastatic disease. Review of trials reporting 2-year outcomes of greater than 90%, suggests that a dose of 45 Gy in 3-4 fractions or 35-40 Gy in 5 fractions need to be achieved (Table 1). A critical dose threshold likely exists for both local control and overall survival. In one of the larger SBRT studies, Jang *et al.*<sup>[65]</sup> demonstrated that above 54 Gy in 3 fractions, local control and survival was 100% and 71% at 2-years, respectively. However, if less than 45 Gy was achieved, the local control and overall survival dropped to 64% and 30%, respectively.

Unlike patients with liver metastases, a significant proportion of patients with HCC have underlying cirrhosis and/or other insufficiency. This factor has been a consistent parameter influencing dose selection, patient selection and outcome. Most commonly measured using the Child-Pugh score, groups have consistently found this issue to influence treatment and prognosis. Cárdenes *et al.*<sup>[66]</sup> from Indiana University conducted a phase I dose escalation trial of SBRT for HCC, where 17 Child-Pugh classes A or B patients with 25 tumors were included. The initial dose level was 36 Gy in 3 fractions and there was a 2-Gy per fraction increment. Patients received a maximum of two treatments per week. The protocol

required 700 cc of normal liver would receive  $< 15$  Gy. They were able to escalate the dose to 48 Gy in 3 fractions for Child-Pugh class A patients without causing dose-limiting toxicities. However, two Child-Pugh class B patients developed grade 3 liver toxicities when the dose was escalated to 42 Gy in 3 fractions. This observation has led these investigators to change the regimen for Child-Pugh class B patients, from 40 Gy in 3 fractions to 40 Gy in 5 fractions. This was considered the MTD and no further dose escalations are recommended. The most important factor associated with grade 3 or higher liver toxicities was a Child-Pugh score of  $\geq 8$ . Based on their experience, the group has recommended that the dose to one-third of the uninvolved liver should be restricted to  $\leq 10$  Gy (3.3 Gy/fraction) and  $\geq 500$  cc of uninvolved liver should receive  $< 7$  Gy (2.3 Gy/fraction) for Child-Pugh class A patients; for Child-Pugh class B patients, the dose to one-third of the uninvolved liver is restricted to  $\leq 18$  Gy, (3.6 Gy/fraction) and  $\geq 500$  cc of uninvolved liver should receive  $< 12$  Gy (2.4 Gy/fraction)<sup>[66]</sup>. A summary of suggested constraints based on recent randomized clinical trials with accepted fraction regimens is summarized in Table 3.

## FUTURE TRENDS

Patients with primary or secondary liver cancer are growing in incidence and have a rising mortality rate<sup>[1]</sup>. Current management with RFA, TACE, sorafenib and surgery often are not possible or result in moderate improvements<sup>[67]</sup>. Therefore, patients and their physicians must seek alternatives or combination of treatments. In addition to external beam radiation, work in the use of radionuclides, radiosensitizers (such as inhibitors of autophagy), epigenetic agents, liquid biopsies to better select patients, and immune modulation are exciting avenues of investigation. As for external beam radiotherapy, our review suggests that radiotherapy can be implemented safely and with high local control rates. In the future, we will continue to refine our technique and patient selection, but appropriate multidisciplinary randomized trials need to be completed before radiation can become a standard of care.

## CONCLUSION

Radiation plays an important role in the treatment of primary and metastatic liver cancer. Control rates can be high and toxicity is minimal in well-selected patients. Indeed it may play a primary role in subgroups such as large tumors and those with thrombosis. The wide ranging outcomes, differing techniques and varied dosing strategies make specific treatment recommendations difficult, but the literature is converging on a short list of important components of a high quality liver radiation program. This article aims to provide a practical review of options to provide the best care possible in this evolving field.

**Table 3** Summary of dose constraints by number of fractions

Organ at risk	3 fraction (RAS trial <sup>[102]</sup> )	5 fraction (RTOG 1112 <sup>[103]</sup> )	QUANTEC (1.8-2 Gy per fraction) <sup>[104]</sup>	Toxicity
Liver excluding CTV	700 mL < 15 Gy	V 10 < 70%	D mean < 30 Gy	Radiation induced liver dysfunction
Esophagus	D 1 mL < 21 Gy	D 0.5 mL < 32 Gy	V 35 < 50%	Esophagitis
Stomach	D 1 mL < 21 Gy	D 0.5 mL < 30 Gy	D 100 < 35 Gy	Ulceration
Kidney	D 35% < 15 Gy	D mean < 10 Gy	D mean < 28 Gy (1.8-2 Gy per fraction)	Renal dysfunction
Bowel and duodenum	D 1 mL < 21 Gy	D 0.5 mL < 30 Gy	D 45 < 195 cc	Enteritis/fistula
Spinal cord	Dmax < 18 Gy	D 0.5 mL < 25 Gy	Dmax = 45	Myelopathy
Heart	D 1 mL, 30 Gy	D 30 mL < 30 Gy	V 25 < 10%	Pericarditis

CTV: Clinical target volume.

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**P- Reviewer:** Cao GW, Cerwenka HR, Furka A, Tarazov PG

**S- Editor:** Kong JX **L- Editor:** A **E- Editor:** Li D



Retrospective Study

# Image quality and diagnostic performance of free-breathing diffusion-weighted imaging for hepatocellular carcinoma

Yukihisa Takayama, Akihiro Nishie, Yoshiki Asayama, Kousei Ishigami, Daisuke Kakihara, Yasuhiro Ushijima, Nobuhiro Fujita, Ken Shirabe, Atsushi Takemura, Hiroshi Honda

Yukihisa Takayama, Department of Radiology, Kitakyushu Municipal Medical Center, Kitakyushu, Fukuoka 802-0077, Japan

Akihiro Nishie, Yoshiki Asayama, Kousei Ishigami, Daisuke Kakihara, Yasuhiro Ushijima, Nobuhiro Fujita, Hiroshi Honda, Department of Clinical Radiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan

Ken Shirabe, Department of Hepatobiliary and Pancreatic Surgery, Gunma University, Graduate School of Medicine, Maebashi, Gunma 371-8511, Japan

Atsushi Takemura, Philips Electronics Japan, Minato-ku, Tokyo 108-8507, Japan

**Author contributions:** Takayama Y designed and performed the research and wrote the paper; Nishie A designed the research and supervised the report; Asayama Y, Ishigami K, Kakihara D, Ushijima Y and Fujita N designed the research and contributed to the analysis; Shirabe K and Takemura A provided clinical and technical advice; Honda H supervised the report.

**Institutional review board statement:** This study was reviewed and approved by the Ethics Committee of the Kyushu University Hospital.

**Informed consent statement:** This study was approved by the Institutional Review Board of our institute. The requirement for written informed consent was waived due to the retrospective nature of the study. For full disclosure, the details of the study are published on the home page of Kyushu University.

**Conflict-of-interest statement:** We have no financial relationships to disclose.

**Data sharing statement:** No additional data are available.

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**Manuscript source:** Invited manuscript

**Correspondence to:** Akihiro Nishie, MD, PhD, Associated Professor, Department of Clinical Radiology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. [anishie@radiol.med.kyushu-u.ac.jp](mailto:anishie@radiol.med.kyushu-u.ac.jp)  
Telephone: +81-92-6425695  
Fax: +81-92-6425708

**Received:** November 16, 2016

**Peer-review started:** November 17, 2016

**First decision:** February 4, 2017

**Revised:** February 11, 2017

**Accepted:** April 23, 2017

**Article in press:** April 24, 2017

**Published online:** May 18, 2017

## Abstract

### AIM

To retrospectively evaluate the diagnostic performance of free-breathing diffusion-weighted imaging (FB-DWI) with modified imaging parameter settings for detecting hepatocellular carcinomas (HCCs).

### METHODS

Fifty-one patients at risk for HCC were scanned with both FB-DWI and respiratory-triggered DWI with the navigator echo respiratory-triggering technique (RT-DWI). Qualitatively, the sharpness of the liver contour, the image noise and the chemical shift artifacts on each DWI with  $b$ -values of 1000 s/mm<sup>2</sup> were independently evaluated by three radiologists using 4-point scoring. We

compared the image quality scores of each observer between the two DWI methods, using the Wilcoxon signed-rank test. Quantitatively, we compared the signal-to-noise ratios (SNRs) of the liver parenchyma and lesion-to-nonlesion contrast-to-noise ratios (CNRs) after measuring the signal intensity on each DWI with a b-factor of 1000 s/mm<sup>2</sup>. The average SNRs and CNRs between the two DWI methods were compared by the paired t-test. The detectability of HCC on each DWI was also analyzed by three radiologists. The detectability provided by the two DWI methods was compared using McNemar's test.

## RESULTS

For all observers, the averaged image quality scores of FB-DWI were: Sharpness of the liver contour [observer (Obs)-1, 3.08 ± 0.81; Obs-2, 2.98 ± 0.73; Obs-3, 3.54 ± 0.75], those of the distortion (Obs-1, 2.94 ± 0.50; Obs-2, 2.71 ± 0.70; Obs-3, 3.27 ± 0.53), and the chemical shift artifacts (Obs-1, 3.38 ± 0.60; Obs-2, 3.15 ± 1.07; Obs-3, 3.21 ± 0.85). The averaged image quality scores of RT-DWI were: Sharpness of the liver contour (Obs-1, 2.33 ± 0.65; Obs-2, 2.37 ± 0.74; Obs-3, 2.75 ± 0.81), distortion (Obs-1, 2.81 ± 0.56; Obs-2, 2.25 ± 0.74; Obs-3, 2.96 ± 0.71), and the chemical shift artifacts (Obs-1, 2.92 ± 0.59; Obs-2, 2.21 ± 0.85; Obs-3, 2.77 ± 1.08). All image quality scores of FB-DWI were significantly higher than those of RT-DWI ( $P < 0.05$ ). The average SNR of the normal liver parenchyma by FB-DWI (11.0 ± 4.8) was not significantly different from that shown by RT-DWI (11.0 ± 5.0); nor were the lesion-to-nonlesion CNRs significantly different (FB-DWI, 21.4 ± 17.7; RT-DWI, 20.1 ± 15.1). For all three observers, the detectability of FB-DWI (Obs-1, 43.6%; Obs-2, 53.6%; and Obs-3, 45.0%) was significantly higher than that of RT-DWI (Obs-1, 29.1%; Obs-2, 43.6%; and Obs-3, 34.5%) ( $P < 0.05$ ).

## CONCLUSION

FB-DWI showed better image quality and higher detectability of HCC compared to RT-DWI, without significantly reducing the SNRs of the liver parenchyma and lesion-to-nonlesion CNRs.

**Key words:** Diffusion weighted-imaging; Liver; Magnetic resonance imaging; Hepatocellular carcinoma; Free-breathing technique

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**Core tip:** This retrospective study evaluated the image quality of free-breathing diffusion-weighted imaging (FB-DWI) of the liver and its diagnostic performance for hepatocellular carcinoma compared with respiratory-triggered DWI. The free-breathing technique is widely believed to be inappropriate for body DWI because motion artifact causes decreased image quality. However, after a modification of imaging parameters, FB-DWI showed better image quality without significantly reducing the signal-to-noise ratio of the normal liver parenchyma

and the lesion-to-nonlesion contrast-to-noise ratio compared to respiratory-triggering-DWI. As a result, the improvement of the image quality of FB-DWI contributed to an increased rate of detection of hepatocellular carcinoma.

Takayama Y, Nishie A, Asayama Y, Ishigami K, Kakihara D, Ushijima Y, Fujita N, Shirabe K, Takemura A, Honda H. Image quality and diagnostic performance of free-breathing diffusion-weighted imaging for hepatocellular carcinoma. *World J Hepatol* 2017; 9(14): 657-666 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v9/i14/657.htm> DOI: <http://dx.doi.org/10.4254/wjh.v9.i14.657>

## INTRODUCTION

Diffusion-weighted imaging (DWI) has been widely adopted as a magnetic resonance imaging (MRI) method in clinical practice<sup>[1,2]</sup>. DWI can be used for the detection and characterization of malignant and nonmalignant lesions<sup>[3-5]</sup>. Liver DWI has been applied to quantify the degrees of chronic liver disease and fibrosis, and to detect and characterize liver lesions<sup>[2,6-9]</sup>. DWI can provide additional information that can be used to differentiate malignant liver lesions from benign liver lesions and to estimate the histological grade of hepatocellular carcinomas (HCCs)<sup>[6,7,10]</sup>. The combination of DWI and dynamic contrast-enhanced (DCE)-MRI has shown higher diagnostic performance compared to DWI or DCE-MRI alone<sup>[11-13]</sup>. Although DWI has an essential role to play in the assessment of HCCs on liver MRI, its sensitivity for detecting HCCs is thought to be low compared to that of DCE-MRI<sup>[2,14]</sup>.

DWI suffers from image distortion and/or chemical shift artifacts related to the echo planar imaging (EPI) technique and to motion and susceptibility artifacts<sup>[15-17]</sup>. With the goal of overcoming these issues, a previous study investigated MR parameter settings to obtain high spatial resolution and less artifacts without losing a significant level of the signal-to-noise ratio on liver MRI<sup>[18]</sup>. With this method, DWI with MR parameter settings provides improved image quality and detections of malignant liver tumors such as HCCs and metastatic liver tumors<sup>[18]</sup>. Here we hypothesized that DWI with modified MR parameter settings for the improvement of image quality might result in further improvement in the detectability of HCCs.

The purpose of this retrospective study was to evaluate the image quality and the detectability of HCCs in patients with chronic liver disease on DWI with modified MR parameter settings.

## MATERIALS AND METHODS

### Subjects

This study was approved by the Institutional Review Board of our institute. The requirement for written informed consent was waived due to the retrospective nature of the study. From November 2010 to September 2011, 468 consecutive patients who underwent liver MRI at

**Table 1** Details of magnetic resonance parameters

Imaging technique	FB-DWI	RT-DWI
	Spin echo single-shot EPI	Spin echo single-shot EPI
SENSE factor	2	2
TR/TE (ms)	6250/56	1877/55
Flip angle (degree)	90°	90°
Field of view (mm <sup>2</sup> )	380 × 299	380 × 299
Matrix (frequency × phase)	112 × 176	112 × 68
Slice thickness (mm)	7	7
Slice gap (mm)	1	1
No. of slice	25	25
No. of excitations	2	2
<i>b</i> -value (s/mm <sup>2</sup> )	0.500 and 1.000	0.500 and 1.000
Respiratory compensation	Free-breathing without navigator echo	Respiratory-triggered with navigator echo
Fat-suppression	SPAIR	SPIR
SPAIR delay (ms)	100	
SPAIR TR (ms)	250	
Frequency offset (Hz)	250	180
EPI factor	75	25
Band width (Hz/pixel)	4050.4	4438.5
Scan time (min:s)	3:32	3:20 <sup>1</sup>

<sup>1</sup>The mean scan time of RT-DWI, because these values vary depending on the subjects' respiration condition. FB-DWI: Diffusion-weighted imaging with modified MR parameter settings for image improvement by referring to the literature<sup>[18]</sup>; RT-DWI: Respiratory-triggered diffusion-weighted imaging without modified MR parameter settings for the image improvement; EPI: Echo planar imaging; TR: Repetition time; TE: Echo time; SPAIR: Spectral attenuation with inversion recovery; SPIR: Spectral presaturation with inversion recovery; SPAIR delay: Inversion time from exposure of SPAIR pulse; SPAIR TR: TR between SPAIR pulses during the scan; Frequency offset: Bandwidth from the frequency of fat tissue; EPI factor: The number of k-space profiles collected per excitation.

our institute were enrolled. The inclusion criteria were: (1) patients who were admitted to the Department of Surgery and were suspected to have HCCs due to chronic liver disease; (2) patients who underwent gadoxetic acid-enhanced MRI (Gd-EOB-MRI) on the same MR scanners; and (3) patients who underwent treatments such as surgical resection, transcatheter arterial infusion chemotherapy (TAI) or transcatheter arterial chemoembolization (TACE). The exclusion criteria were: (1) patients with other malignant liver tumors, such as cholangiocellular carcinoma (ICC) and metastatic liver tumor; and (2) patients in whom follow-up computed tomography (CT) and/or MRI were not performed. Finally, 51 patients (age range: 26–82; mean: 63.8 years; male/female ratio: 33/18; Child-Pugh grades: A = 31, B = 16 and C = 4) were enrolled.

### Imaging protocol

**MR protocol:** MR examinations were performed on a clinical whole-body 3.0 Tesla MR system (Achieva 3.0 T TX; Philips Healthcare, Best, the Netherlands) using a 32-channel cardiac phased-array coil. For the comparison of imaging modalities, each patient was scanned with two different types of DWI. One type was DWI with modified MR parameter settings for the improvement of images referring to the literature<sup>[18]</sup>; we called this type of DWI “free-breathing (FB)-DWI” in this study because the FB technique was applied. The other type was DWI without modified MR parameter settings using a navigator-echo-based, real-time respiratory-gating and respiratory-triggering technique which we refer to as RT-DWI in this study. A navigator-echo-based technique was not applied

for respiratory-triggering (RT)-DWI.

The details of the MR parameters of the two DWI methods are summarized in Table 1. An apparent diffusion coefficient (ADC) map was developed for each DW image, by referring to the signal intensity decay on the DW image with *b*-values of 0, 500 and 1000 s/mm<sup>2</sup>.

Other imaging sequences included an axial T2-weighted single-shot turbo spin echo, axial dual-echo T1-weighted fast field echo, and a gadoxetic acid-enhanced dynamic study. For the gadoxetic acid-enhanced dynamic study, a multiphase dynamic study including arterial, portal, late and hepatobiliary phases was performed using axial enhanced T1 high-resolution isotropic volume excitation (eTHRIVE). First, pre-contrast images were scanned. Gadoxetic acid (Primovist; Bayer, Osaka, Japan) at 0.1 mL/kg was injected through the antecubital vein for 5 s at a variable injection rate using a power injector, followed by a bolus administration of 20 mL of saline at the same injection rate. The timing of the arterial dominant phase was determined with a test injection of 0.5 mL of gadoxetic acid. The scanning of the portal, late and hepatobiliary phases began at the arterial phase +30 s, 180 s and 20 min after the injection of the contrast agent, respectively.

**CT protocol:** On the follow-up CT examination, the scanning was performed before and after 100 mL of iodinated contrast medium (Iopamiron 370: Bayer Schering Pharma, Osaka, Japan; or Omnipaque 350: Daiichi-Sankyo, Tokyo) was administered, using a 64-MDCT scanner (Aquilion 64, Toshiba Medical, Tokyo). The contrast was intravenously administered at a rate of 3 mL/s. Contrast-enhanced

**Table 2** Image quality scores of sharpness of the liver contour, distortion and chemical shift artifacts

Score	Sharpness of the liver contour	Distortion and chemical shift artifacts
1	Unclear liver contour	Severe distortion or artifacts compromise the diagnostic capability of DWI in the whole liver
2	The liver contour is partially unclear	Distortion or artifacts are moderate, and they compromise the diagnostic capability of DWI in 50% or more of the liver
3	The liver contour is mostly clear	Distortion or artifacts are mild, and they compromise the diagnostic capability of DWI in less than 50% of the liver
4	The entire liver contour is clear	No distortion or artifacts; the diagnostic capability of DWI is not compromised

DWI: Diffusion-weighted imaging.

images were obtained during the arterial phase (43 s after the initiation of the injection), the portal venous phase (70 s), and the delayed phase (240 s). The imaging acquisition parameters were as follows: Voltage, 120 kV; electric current, automatic; collimation, 0.5 mm; image reconstruction thickness, 5 mm; and helical pitch, 53.

### Image quality assessment

Qualitatively, the sharpness of the liver contour, the image noise and the chemical shift artifacts on FB-DWI and RT-DWI with  $b$ -values of 1000 s/mm<sup>2</sup> were independently evaluated by three radiologists (Daisuke Kakihara, Yasuhiro Ushijima, and Nobuhiro Fujita, with 17, 17 and 12 years of experience in interpreting liver MRI, respectively) who were blinded to the imaging information and clinical data, using 4-point scoring. The details of the 4-point scoring are shown in Table 2. Quantitatively, the SNRs of the liver parenchyma and the lesion-to-nonlesion CNRs between the liver parenchyma and HCCs were calculated after drawing polygonal regions of interest (ROIs) on each DWI with a  $b$ -factor of 1000 s/mm<sup>2</sup>; this procedure was performed by one radiologist using a commercially available PACS workstation (SYNAPSE; Fujifilm Medical, Tokyo). The SNRs and CNRs were calculated using the following equations, as described in detail elsewhere<sup>[19,20]</sup>:

SNR of the liver parenchyma =  $S_{\text{liver}}/SD_{\text{liver}}$ , Lesion to nonlesion CNR =  $|S_{\text{liver}} - S_{\text{tumor}}|/SD_{\text{liver}}$

Where  $S_{\text{liver}}$  is the signal intensity of the liver parenchyma,  $S_{\text{tumor}}$  the signal intensity of the tumor, and  $SD_{\text{liver}}$  the standard deviation of the SI of the liver parenchyma. The SD of the liver parenchyma was taken as the estimated local noise for the calculation. In parallel imaging, noise is not distributed homogeneously throughout the image, and thus it is better to estimate noise in close proximity to the site of SI measurement<sup>[21]</sup>. The SNR cannot be calculated as a characteristic of the entire image but rather is calculated as a local property that characterizes the signal quality with respect to local noise levels<sup>[21]</sup>.

Three ROIs were made as large as possible on the normal liver parenchyma to avoid major vessels, tumors, and artifacts for each patient. The same ROIs were duplicated on each DW image. The range and averaged areas of ROIs of the normal liver parenchyma were 102.1–1414.0 mm<sup>2</sup> and 385.4 mm<sup>2</sup>, and the corresponding values for the hepatic lesions were 146.7–1237.4 mm<sup>2</sup> and 502.7 mm<sup>2</sup>. The measurements of the SNR of normal liver parenchyma and a lesion-to-nonlesion CNR were repeated

three times for each subject. The same ROIs were duplicated at the same slice and position for the two DWI methods.

### Confirmation of HCC

A final total of 105 HCCs (size range: 5–140 mm; mean: 17.1 mm; location, left lobe/right lobe: 52/53) was used for the assessment of the detectability of HCCs by the two different types of DWI. The number and location of HCCs were determined by one coordinator (Yukihisa Takayama, with 15 years of experience in interpreting liver MRI) who was a coordinator of this study and had knowledge of the clinical data of each patient. In the 38 patients who underwent surgery, 38 HCCs were identified using pathological reports after surgical resection. Of the other 67 HCCs in the 13 patients who underwent TAI or TACE, the HCCs were clinically defined using the enhancement in the early phase and hypointensity in the hepatobiliary phase of Gd-EOB-MRI and the nodular accumulation of emulsion of iodized oil (Lipiodol Ultrafluid; Terumo, Tokyo) on follow-up CT performed 2 wk after the TAI or TACE<sup>[22–24]</sup>.

The 3-mo follow-up Gd-EOB-MRI or DCE-CT confirmed the absence of HCCs in other liver parenchyma. The treated HCCs and hepatic hemangiomas were diagnosed based on the lack of early enhancement on Gd-EOB-MRI and DCE-CT and high signal intensity on T2-weighted imaging at an least 6-mo follow-up Gd-EOB-MRI or DCE-CT<sup>[24–26]</sup>. Liver tumors other than hemangiomas were not identified in 51 patients.

### Detectability of HCC

The detectability of HCC by each type of DWI was analyzed by three radiologists (Daisuke Kakihara, Yasuhiro Ushijima, and Nobuhiro Fujita, with 17, 17 and 12 years of experience in interpreting liver MRI, respectively) who independently interpreted the sets of DWI with  $b$ -values of 0, 500 and 1000 s/mm<sup>2</sup> and the ADC map at a 1-mo interval in random order. They were blinded to the clinical data and other imaging results such as those obtained by T1- and T2-weighted imaging, Gd-EOB-MRI, and DCE-CT.

On each type of DWI, HCC was diagnosed as a lesion showing mild-to-moderate hyperintensity compared to the liver parenchyma on DW images at a  $b$ -value of 0 s/mm<sup>2</sup> and restricted diffusion (*i.e.*, the lesion remained hyperintense) at a  $b$ -value of 500 and/or 1000 s/mm<sup>2</sup>, with an ADC value visually lower or equal to that of the surrounding liver parenchyma. Liver hemangiomas were

**Table 3** Results of qualitative assessment of the free-breathing diffusion-weighted imaging and respiratory-triggering-diffusion-weighted imaging results

	Sharpness of the liver contour		Distortion		Chemical shift artifacts	
	FB-DWI	RT-DWI	FB-DWI	RT-DWI	FB-DWI	RT-DWI
Observer 1	3.08 ± 0.81	2.33 ± 0.65 <sup>a</sup>	2.94 ± 0.50	2.81 ± 0.56 <sup>a</sup>	3.38 ± 0.60	2.92 ± 0.59 <sup>a</sup>
Observer 2	2.98 ± 0.73	2.37 ± 0.74 <sup>a</sup>	2.71 ± 0.70	2.25 ± 0.74 <sup>a</sup>	3.15 ± 1.07	2.21 ± 0.85 <sup>a</sup>
Observer 3	3.54 ± 0.75	2.75 ± 0.81 <sup>a</sup>	3.27 ± 0.53	2.96 ± 0.71 <sup>a</sup>	3.21 ± 0.85	2.77 ± 1.08 <sup>a</sup>

Data are the average ± SD. <sup>a</sup> $P < 0.05$  by the Wilcoxon signed-rank test. FB-DWI: Diffusion-weighted imaging with modified MR parameter settings for image improvement by referring to the literature<sup>[18]</sup>; RT-DWI: Navigator-echo-based, real-time respiratory-gating and respiratory-triggered diffusion-weighted imaging without modified MR parameter settings.

diagnosed by referring to the hyperintensity on DWI with a  $b$ -value of 0 s/mm<sup>2</sup> and an ADC value visually higher than that of the surrounding liver parenchyma. Each observer recorded the location of each HCC by placing an arrow on the image. The detectability of HCC was calculated on a tumor-by-tumor basis, and the detectability provided by the two types of DWI were compared. If liver hemangiomas were interpreted by each observer as HCC, those lesions were considered false-positives and were excluded from the assessment of detectability by the coordinator. In addition, the relationship between detectability and the sizes of the HCCs was analyzed.

### Statistical analysis

Image quality scores of the sharpness of the liver contour, the distortion, and the chemical shift artifacts between FB-DWI and RT-DWI were compared with the Wilcoxon signed-rank test. The SNRs of normal liver parenchyma and lesion-to-nonlesion CNRs between the two types of DWI were compared with a paired  $t$ -test. The detectability provided by the two DWI methods was compared using McNemar's test. A  $P$ -value  $< 0.05$  was considered to indicate a significant difference for each analysis. Statistical analyses were performed using IBM SPSS statistics 18.0 software (IBM Japan, Tokyo). The statistical methods of this study were reviewed by Akihiro Nishie from the Department of Clinical Radiology, Graduate School of Medical Sciences, Kyushu University and Junji Kishimoto from the Center for Clinical and Translational Research, Kyushu University.

## RESULTS

### Image quality assessments

The results of the qualitative assessment by the three observers are shown in Table 3. For all three observers, the average image quality scores of the sharpness of the liver contour, the distortion, and the chemical shift artifacts of FB-DWI were significantly higher than those of RT-DWI ( $P < 0.05$ ). There were no significant differences between FB-DWI and RT-DWI in the average or SDs of the SNR of the normal liver parenchyma (FB-DWI,  $11.0 \pm 4.8$ ; RT-DWI,  $11.0 \pm 5.0$ ) or the lesion-to-nonlesion CNR (FB-DWI,  $21.4 \pm 17.7$ ; RT-DWI,  $20.1 \pm 15.1$ ). Three representative cases are shown in Figures 1-3.

In Figure 1, the HCC is more clearly described as hyperintensity on FB-DWI than on RT-DWI. In Figure

2, the HCC was detected by FB-DWI, whereas it was concealed by a chemical shift artifact on RT-DWI. A pseudolesion caused by a chemical shift artifact from fat tissue between the liver parenchyma and diaphragm is shown in Figure 3.

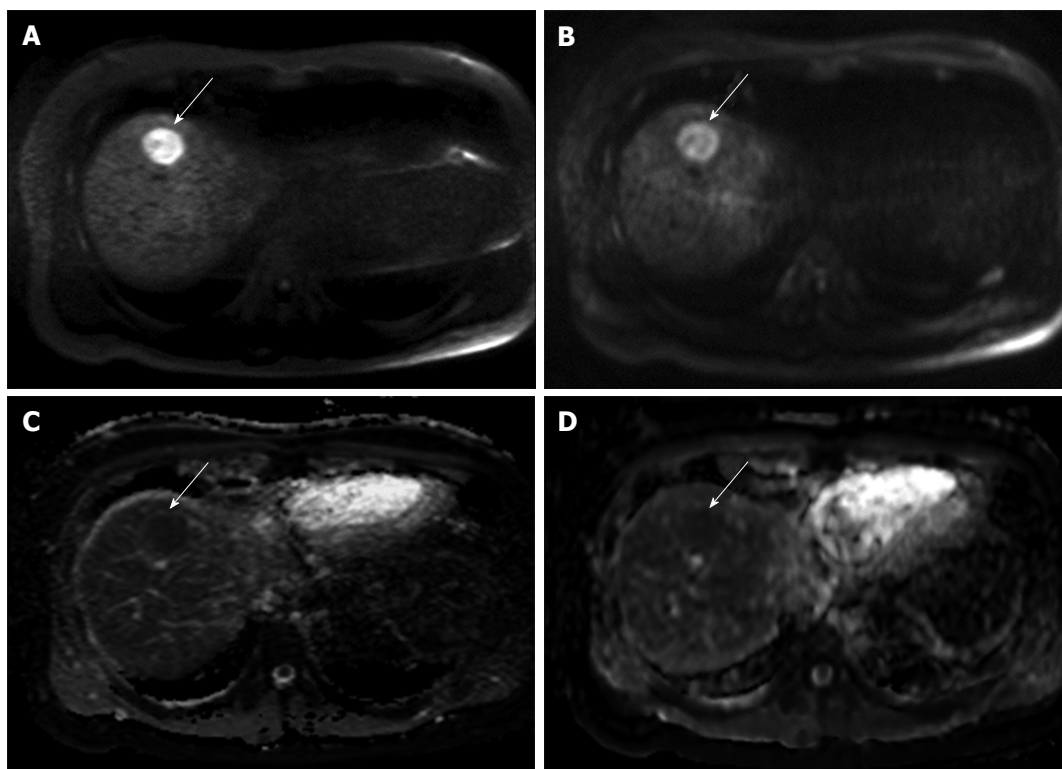
### Detectability of HCC

For all three observers, the sensitivity of FB-DWI [observer (Obs)-1, 43.6%; Obs-2, 53.6%; and Obs-3, 45.0%] was significantly higher than that of RT-DWI (Obs-1, 29.1%; Obs-2, 43.6%; and Obs-3, 34.5%) ( $P < 0.05$ ). Regarding the relationship between detectability and the size of the HCCs, the detectability of the two types of DWI was significantly different when the tumor size was 5-22 mm. FB-DWI showed significantly higher detectability of these HCCs (Obs-1, 36.0%; Obs-2, 48.3%; and Obs-3, 33.7%) compared to RT-DWI (Obs-1, 18.0%; Obs-2, 36.0%; and Obs-3, 24.7%) ( $P < 0.05$ ). There was no significant difference in the detectability of HCCs between the two types of DWI when the tumor size was  $> 22$  mm.

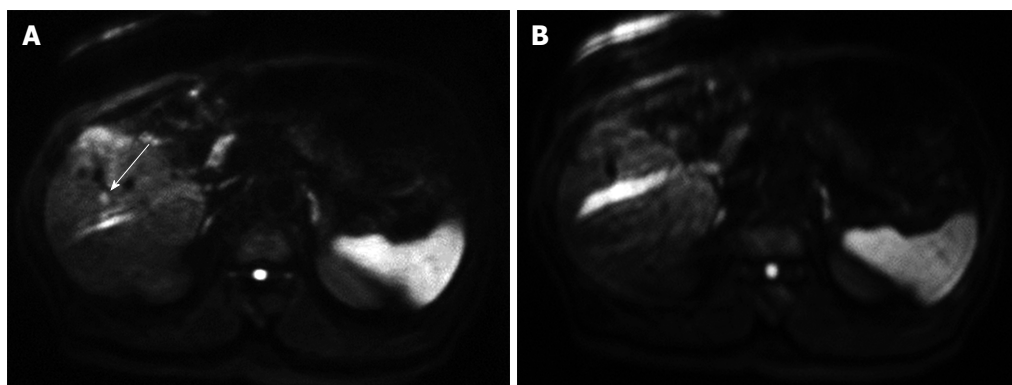
## DISCUSSION

The results of the present study revealed that, compared to RT-DWI, FB-DWI showed better image quality without significantly reducing the SNR of the normal liver parenchyma and the lesion-to-nonlesion CNR. The improvement of the image quality of FB-DWI might contribute to an increased detection rate of HCCs. The free-breathing technique was applied to FB-DWI of liver MRI even though it is widely believed that this technique is inappropriate due to its susceptibility to sensitive motion artifacts, which results in decreased SNRs and CNRs and image blurring<sup>[3,27]</sup>. However, our present findings demonstrated that FB-DWI had better image quality and equivalent SNRs of the liver parenchyma and lesion-to-nonlesion CNRs compared to RT-DWI.

Other studies have obtained similar results using DWI with a free-breathing technique, such as good image quality, good reproducibility of ADC values of liver tumors, and good diagnostic performance for liver lesions<sup>[28-31]</sup>. There are several possible reasons for the good image quality provided by DWI with a free-breathing technique; one is that an increased number of excitations contributes to a reduction in motion artifacts. However, FB-DWI used



**Figure 1** A 36-year-old male. The HCC in segment 8 of the liver showing hyperintensity on FB-DWI (A) is more clearly described than on RT-DWI (B). The ADC values of HCC were  $1.02 \times 10^{-3} \text{ mm}^2/\text{s}$  on the ADC map of FB-DWI (C) and  $1.16 \times 10^{-3} \text{ mm}^2/\text{s}$  on the ADC map of RT-DWI (D). HCC: Hepatocellular carcinoma; FB-DWI: Free-breathing diffusion-weighted imaging; RT-DWI: Respiratory-triggering diffusion-weighted imaging; ADC: Apparent diffusion coefficient.



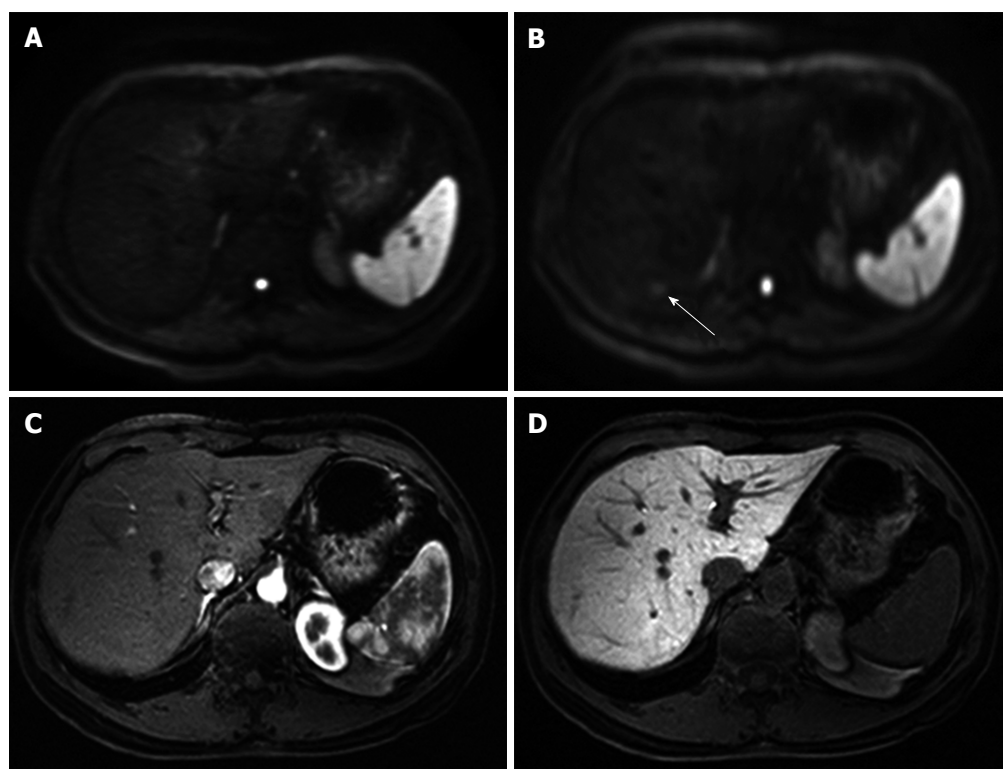
**Figure 2** A 78-year-old female. The HCC in segment 5 of the liver was detected by FB-DWI (A), whereas on RT-DWI, it was concealed by a chemical shift artifact (B). HCC: Hepatocellular carcinoma; FB-DWI: Free-breathing diffusion-weighted imaging; RT-DWI: Respiratory-triggering diffusion-weighted imaging.

only two excitations, and thus the results showing good image quality, SNR and CNR cannot be attributed to a multiplicity of excitations.

Another possible reason is the advances in MR technology in recent years (such as statistic and gradient magnetic fields, a 32-channel torso-cardiac phased-array coil, and dual-source parallel radiofrequency excitation and transmission technology) that address problems related to the EPI technique<sup>[9,18,32]</sup>. In particular, the rapid image acquisition of the EPI sequence allows minimization of the blurring from T2\* signal intensity decay during the gradient-echo train<sup>[9,18,32]</sup>, and it is insensitive to the effects of macroscopic patient motion because of the very fast

readout of the complete image data (within approximately 100 ms)<sup>[9]</sup>. This may account for the good image quality of FB-DWI in the present study.

Homogeneous fat suppression is essential for DWI, to avoid the image degradation caused by chemical shifts when using EPI<sup>[27]</sup>. For the FB-DWI in the present series, the SPAIR technique was applied because this technique is minimally affected by B1 inhomogeneity and is effective for obtaining homogeneous fat-suppression<sup>[33]</sup>. Chemical shift artifacts on DWI can be reduced by providing homogeneous fat-suppression as well. Fewer chemical artifacts on DWI are advantageous for the depiction of liver lesions. The present study's findings related to the



**Figure 3** A 53-year-old female. There was no detectable lesion on FB-DWI (A) but the nodular hyperintensity in segment 7 of the liver was seen on RT-DWI (B). It was diagnosed as a pseudolesion caused by a chemical shift artifact from fat tissue between the liver parenchyma and diaphragm, by referring to precontrast-enhanced imaging (C) and hepatobiliary phase imaging (D) of Gd-EOB-MRI. A follow-up MR examination also showed no progressive lesion (not shown). FB-DWI: Free-breathing diffusion-weighted imaging; RT-DWI: Respiratory-triggering diffusion-weighted imaging; Gd-EOB-MRI: Gadoxetic acid-enhanced magnetic resonance imaging.

reduction of chemical shift artifacts occasionally mimicked a hepatic tumor. We thus conclude that FB-DWI will be advantageous for the depiction of HCCs.

In this study, we found that the detectability of HCC by FB-DWI was superior to that by RT-DWI. In fact, the detection of HCC by FB-DWI was equivalent to the reported results using breath-hold or respiratory-triggered techniques (45%–55%)<sup>[2,34,35]</sup>. That is, FB-DWI provides better spatial resolution, fewer chemical shift artifacts, and a comparable SNR and lesion-to-nonlesion CNR compared to RT-DWI<sup>[18]</sup>. This improvement in the detectability of HCCs, especially that of small-sized HCCs, is also probably due to the better spatial resolution and fewer chemical shift artifacts of FB-DWI.

The lower detectability of HCCs on DWI alone is a limitation because of the difficulty of differentiating a tumor from surrounding cirrhotic liver due to their similar diffusion properties and ADC values, and to the tumor grades of HCCs<sup>[2,7,14]</sup>. In contrast, two earlier meta-analyses showed that DWI had high sensitivity (81% and 93%) for detecting HCCs<sup>[13,36]</sup>. We speculate that the reasons for the difference in the rate of sensitivity between our results and those of the two prior meta-analyses might be related to patient selection bias, the background of liver parenchyma in patients with chronic liver disease (cirrhotic or not), and/or the tumor characteristics (*e.g.*, tumor size and malignant grade)<sup>[13,36]</sup>.

Generally, DCE-MRI is the first choice for the evalua-

tion of liver tumors, and DWI is not used alone. However, it is known that the combination of DCE-MRI and DWI improves the detectability and provides additional information to characterize liver lesions<sup>[10–12,37,38]</sup>. DWI thus plays an important role in the assessment of liver tumors for patients with contrast-agent contraindications, such as renal failure or a history of adverse reaction to a contrast agent<sup>[38,39]</sup>. DWI has also been applied for other evaluations of HCCs, such as for assessment of the treatment effects of TACE and molecular target therapy<sup>[40,41]</sup>. FB-DWI can also contribute to the detection or treatment assessment of HCCs in combination with DCE-MRI.

There are several limitations of this study. First, most of the HCCs examined in this study were confirmed by imaging findings, although some HCCs were confirmed by histological results after surgical resection. Therefore, we did not check the histological subtypes of the HCCs, such as whether they were well-, moderately or poorly differentiated. The more aggressive HCCs (*i.e.*, poorly differentiated HCCs) are known to show more water molecule restriction within the tumor compared to the well-differentiated HCCs. In other words, it was difficult to detect well-differentiated HCCs on DWI. In addition, there was a risk to include small ICCs which showed hypervascular tumor like HCCs. However, in this study, we did not focus on the differential diagnosis or characterization of liver tumors. The results of our study were not influenced even if a few ICCs were included in the subjects.

Second, several cases showed severe artifacts at the lateral segment of the liver because of cardiac and respiratory motions. In such cases, the artifacts hampered the visualization of the HCCs. The reduction of motion artifact at the lateral segment of the liver remains a problem to be solved.

In conclusion, FB-DWI provided better image quality and showed higher detectability of HCCs in patients with chronic liver disease compared to RT-DWI, without significantly reducing the SNR of the normal liver parenchyma or the lesion-to-nonlesion CNR. FB-DWI was better at detecting HCCs in patients with chronic liver disease compared to RT-DWI. Free-breathing diffusion-weighted imaging with modified MR parameter settings is advantageous in the diagnosis of HCCs.

## ACKNOWLEDGMENTS

We thank Dr. Junji Kishimoto, Associate Professor, Center for Clinical and Translational Research, Kyushu University for providing guidance for the statistical methods of this study.

## COMMENTS

### Background

Diffusion-weighted imaging (DWI) is widely adopted as a magnetic resonance imaging (MRI) method in clinical practice because it is useful for the detection and characterization of benign and malignant lesions. DWI is also important for liver MRI to evaluate hepatocellular carcinomas (HCCs) in patients with chronic liver disease. Although dynamic contrast-enhanced MRI has shown higher diagnostic performance for HCCs, DWI can be used as a substitute for the detection and characterization of HCCs for patients who have a contraindication for contrast agents. However, liver DWI occasionally suffers from image distortion and/or chemical shift artifacts related to the echo planar imaging technique and to motion and susceptibility artifacts. To overcome these issues, a previous study investigated the ideal MR parameter settings for obtaining high spatial resolution and fewer artifacts without losing a significant portion of the signal-to-noise ratio or contrast-to-noise ratio on liver MRI. Although DWI with modified MR parameter settings for the improvement of image quality might result in further improvement in the detectability of HCCs, no previous study has investigated this, to our knowledge. In this study, the authors evaluated the image quality and the detectability of HCCs in patients with chronic liver disease on DWI with modified MR parameter settings.

### Research frontiers

DWI is an important diagnostic imaging tool for the detection and characterization of liver tumors, including HCC in patients with chronic liver disease. Especially in the case of patients who are contraindicated for a contrast agent, DWI plays an important role for the evaluation of HCCs. Nonetheless, few prior reports have analyzed free-breathing DWI for the liver. The results of the present study may help clarify the clinical utility of free-breathing DWI for the diagnosis of HCC in patients with chronic liver disease.

### Innovations and breakthroughs

In this study, the authors report the clinical utility of FB-DWI with modified MR parameter settings for the improvement of image quality for diagnosing HCC in patients with chronic liver disease. The free-breathing technique is generally avoided in liver DWI because it is hampered by image distortion and/or chemical shift artifacts related to the echo planar imaging technique and to motion and susceptibility artifacts. They evaluated the clinical impacts of previously reported modified MR parameter settings to overcome these issues with FB-DWI. FB-DWI with modified MR parameter settings provided better image quality without reducing the SNR of the normal liver parenchyma and the

lesion-to-nonlesion CNR. In addition, the improvement of the image quality of FB-DWI might help increase the detection of HCCs.

## Applications

These findings indicate that FB-DWI with modified MR parameter settings is especially useful for patients who are contraindicated for contrast agents and have difficulty holding their breath during the MRI scan. The improvement of image quality helps increase the detection of HCCs without reducing the SNR of the normal liver parenchyma and the lesion-to-nonlesion CNR.

## Terminology

FB-DWI: Diffusion-weighted imaging using free-breathing technique during the scan; RT-DWI: Diffusion-weighted imaging using a navigator-echo-based, real-time respiratory-gating and respiratory-triggering technique during the scan.

## Peer-review

This paper aims to show modified FB-DWI be to detect HCC than conventional MR sequence and patients who are contraindicated for contrast agents.

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**P- Reviewer:** Jia NY **S- Editor:** Gong ZM **L- Editor:** A  
**E- Editor:** Li D



## Protein tolerance to standard and high protein meals in patients with liver cirrhosis

Octavio Campollo, Dirk Sprengers, Gitte Dam, Hendrik Vilstrup, Neil McIntyre

Octavio Campollo, Center of Studies on Alcohol and Addictions, Antiguo Hospital Civil de Guadalajara, Universidad de Guadalajara, Guadalajara, Jal CP 44280, Mexico

Dirk Sprengers, Department of Gastroenterology and Hepatology, Gasthuis Zusters Antwerpen, B 2610 Wilrijk-Antwerpen, Belgium

Gitte Dam, Hendrik Vilstrup, Department of Medicine V (Gastroenterology and Hepatology), Aarhus University Hospital, DK-8200 Aarhus, Denmark

Neil McIntyre, University College Royal Free School of Medicine, London NW32QG, United Kingdom

**Author contributions:** Sprengers D and Campollo O planned, designed, and selected the patients, and conducted the clinical experiment; McIntyre N planned and designed the experiment; McIntyre N, Dam G and Campollo O analyzed the data; McIntyre N, Dam G, Vilstrup H and Campollo O wrote the manuscript; Sprengers D, Dam G and Vilstrup H reviewed the manuscript; Vilstrup H also edited the manuscript.

**Conflict-of-interest statement:** There are no conflicts of interest arising from this work.

**Data sharing statement:** No additional data are available.

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**Manuscript source:** Invited manuscript

**Correspondence to:** Dr. Octavio Campollo, Professor and Researcher, Center of studies on Alcohol and Addictions, Antiguo Hospital Civil de Guadalajara, Universidad de Guadalajara, Calle Hospital 278, Col. El Retiro, Guadalajara, Jal CP 44280, Mexico. [calcohol@hotmail.com](mailto:calcohol@hotmail.com)  
Telephone: +52-33-36142179

Received: October 27, 2016

Peer-review started: October 31, 2016

First decision: December 1, 2016

Revised: February 21, 2017

Accepted: April 23, 2017

Article in press: April 24, 2017

Published online: May 18, 2017

### Abstract

#### AIM

To investigate the plasma amino acid response and tolerance to normal or high protein meals in patients with cirrhosis.

#### METHODS

The plasma amino acid response to a 20 g mixed protein meal was compared in 8 biopsy-proven compensated cirrhotic patients and 6 healthy subjects. In addition the response to a high protein meal (1 g/kg body weight) was studied in 6 decompensated biopsy-proven cirrhotics in order to evaluate their protein tolerance and the likelihood of developing hepatic encephalopathy (HE) following a porto-caval shunt procedure. To test for covert HE, the "number connection test" (NCT) was done on all patients, and an electroencephalogram was recorded in patients considered to be at Child-Pugh C stage.

#### RESULTS

The changes in plasma amino acids after a 20 g protein meal were similar in healthy subjects and in cirrhotics except for a significantly greater increase ( $P < 0.05$ ) in isoleucine, leucine and tyrosine concentrations in the cirrhotics. The baseline branched chain amino acids/aromatic amino acids (BCAA/AAA) ratio was higher in the healthy persons and remained stable-but it decreased significantly after the meal in the cirrhotic group. After the high protein meal there was a marked increase in the levels of most amino acids, but only small changes occurred in the levels of taurine, citrulline, cysteine and

histidine. The BCAA/AAA ratio was significantly higher 180 and 240 min after the meal. Slightly elevated basal plasma ammonia levels showed no particular pattern. Overt HE was not observed in any patients.

### CONCLUSION

Patients with stable liver disease tolerate natural mixed meals with a standard protein content. The response to a high protein meal in decompensated cirrhotics suggests accumulation of some amino acids but it did not precipitate HE. These results support current nutritional guidelines that recommend a protein intake of 1.2-1.5 g/kg body weight/day for patients with cirrhosis.

**Key words:** Branched chain amino acids; Fischer's ratio; Liver; Protein; Cirrhosis; Tolerance; Nutrition; Amino acids; Diet

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**Core tip:** In this study we investigated the plasma amino acid response to standard and high protein meals in patients with liver cirrhosis and looked for evidence of protein intolerance by testing for the presence of either covert or overt hepatic encephalopathy. We sought to improve on previous methodology by selecting a more homogeneous group of patients with biopsy proven cirrhosis, and by using natural mixed protein meals at two protein levels: A standard (20 g) meal and a high (1 g/kg per body weight) protein meal. We found small differences in the plasma amino acid changes after the standard protein meal but there were marked increments in most amino acids after the high protein meal. Noteworthy no patient showed overt clinical signs of encephalopathy and minor electroencephalograph changes were seen in only one patient after the high protein meal. These results present experimental evidence to support current nutritional guidelines for patients with cirrhosis.

Campollo O, Sprengers D, Dam G, Vilstrup H, McIntyre N. Protein tolerance to standard and high protein meals in patients with liver cirrhosis. *World J Hepatol* 2017; 9(14): 667-676 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v9/i14/667.htm> DOI: <http://dx.doi.org/10.4254/wjh.v9.i14.667>

## INTRODUCTION

The liver plays a key role in the metabolism of amino acids and controls, to a great extent, their homeostasis in the plasma free amino acid pool; it removes them from the plasma, interconverts them and may incorporate them into new protein molecules. Consequently, patients with liver disease show abnormalities in their plasma amino-acid profile<sup>[1-4]</sup> and the fact that some patients with decompensated liver cirrhosis develop protein intolerance<sup>[5]</sup> has been a matter of major clinical concern

over the years<sup>[6]</sup>. The plasma amino acid increase after ingestion of amino acids or protein tends to be associated with an increase in plasma ammonia, which in turn has been implicated in the development of hepatic encephalopathy (HE)<sup>[7-9]</sup>. Under normal circumstances ammonia is detoxified in the liver.

Several studies have investigated the effect of protein ingestion on circulating amino acid levels in patients with liver cirrhosis. The findings have been used to plan therapeutic interventions involving the use of different mixtures of amino acids either to improve nutritional status or as an adjunct in the treatment of HE<sup>[10-13]</sup>.

However, both the type and dosage of protein feed or formula and/or the routes of administration have been varied<sup>[3,14-17]</sup>. Nevertheless, even though most nutritional guidelines recommend high protein diets for liver cirrhosis protein restriction is still considered appropriate in some clinics<sup>[18,19]</sup>. The aim of this study was therefore to investigate the plasma amino acid response to a natural meal with normal protein content in compensated cirrhotic patients compared to a group of healthy subjects in accordance with current guidelines<sup>[18,20-22]</sup>. Furthermore, a group of patients with decompensated cirrhosis were studied in a protocol where they received a meal with high protein content. All the patients were tested for both covert and overt HE to examine the concept of "protein tolerance".

## MATERIALS AND METHODS

### Study subjects

We administered a 20 g mixed protein meal to 8 male patients with biopsy-proven compensated cirrhosis who were Child-Pugh class A (*i.e.*, without complications of cirrhosis)<sup>[19]</sup>. Patients were recruited from the liver clinic at the Royal Free Hospital. A control group comprising 6 healthy age matched volunteers also received the 20 g protein meal. A group of 6 patients (5 male and 1 female) with biopsy-proven decompensated cirrhosis Child-Pugh class C (*i.e.*, with ascites and esophageal varices but not HE) were also studied. They were being assessed for a porto-caval shunt procedure for the treatment of portal hypertension and so were studied before and after ingestion of a high protein meal (1 g protein/kg body weight). Exclusion criteria in this group were present or former HE and variceal bleeding within one week before the study. The high protein meal was used to predict the likelihood of HE developing following a shunt procedure<sup>[23]</sup>. To test for covert HE, the "number connection test" (NCT)<sup>[24]</sup> was performed in all patients and an electroencephalogram<sup>[25]</sup> was recorded in Child-Pugh stage C patients. This protocol was approved by the Ethics Committee of the Royal Free Hospital and all patients agreed to participate in the study.

### Test meals

**Twenty grams protein meal:** We recorded the self-selected meals of 10 in-patients with compensated

**Table 1** Amino acid content of the 20 g protein meal

Amino acid	Content (mg)
Isoleucine	934
Leucine	1500
Lysine	1588
Methionine	563
Cysteine	240
Phenylalanine	867
Tyrosine	723
Threonine	872
Tryptophan	255
Arginine	1161
Histidine	637
Alanine	1104
Asparagine	2109
Glutamic acid	3229
Glycine	935
Proline	999
Serine	901

cirrhosis (5 males and 5 females) in order to design a test meal. The lunch of the 10 patients contained on average  $16.2 \pm 1.6$  g of protein,  $44.6 \pm 6.4$  g of carbohydrates and  $18.2 \pm 3.2$  g of fat. Afterwards we created a test meal consisting of beef, green beans, peach slices, ice cream and butter providing 546 kcal (2312.34 kJ), 19.4 g protein, 20.5 g fat and 76.5 g carbohydrate. The amino acid composition of the meal is presented in Table 1. To test for covert HE NCT were performed before and during the study at the same time as blood sampling and patients were checked for clinical signs of overt HE.

#### One gram per kilogram of body weight protein meal:

The Child-Pugh class C cirrhotics were allowed to select the source of protein from a variety of foods. Meat and chicken were the main sources of animal protein in the 1 g/kg of body weight protein dose. The proportions of fat and carbohydrate varied widely. The composition of the individual meals is shown in Table 2. Patients were also checked for clinical signs of overt HE, NCT were performed as mentioned previously, and an EEG was recorded before and 3 h after the meal.

#### Blood samples

Samples were taken before the meal and at 30, 60, 120, 180 and 240 min and were processed as described previously<sup>[26]</sup>. In the healthy persons the 240 min sample was not taken.

#### Laboratory analysis

Plasma ammonia was measured by an enzymatic method (No. 170-UV Sigma Diagnostics, St Louis, MO, United States)<sup>[27]</sup> and amino acid levels on an LKB 4151 Alpha plus amino acid analyzer with a 200 mm  $\times$  4.6 mm high performance analytical column filled with Ultropac 8 cation-exchange resin<sup>[26]</sup>. Values for glutamine and glutamic acid were inaccurate as their apparent concentration depends on the time period between sampling and analysis. Tryptophan was not measured. Because of

**Table 2** Protein content of the 1 g/kg protein meals

Patient	Energy (kcal)	Protein (g)	%kcal
1	914	94	40
2	973	78	32
3	590	56	36
4	653	38	23
5	1545	78	20
6	1507	65	17

technical problems citrulline was not reported in the healthy control group. Total alpha-amino nitrogen was determined by the fluorodinitrobenzene (DNFB) method<sup>[28]</sup>.

#### Statistical analysis

Differences in amino acid concentration between groups were compared using the Student's "t" test and differences within a group by the paired "t" test.

## RESULTS

#### Response to a 20 g protein meal

The plasma baseline levels of asparagine, cysteine, tyrosine and ornithine were significantly higher in the patients with stable cirrhosis compared to the healthy subjects ( $P < 0.05$ ). The total alpha-amino-N response to the meal in the cirrhotic subjects did not differ from that of healthy subjects (Figure 1 and Table 3). However, as to individual amino acids, isoleucine, tyrosine and leucine increased significantly more in the cirrhotic patients (Figure 2). The baseline branched chain amino acids/aromatic amino acids (BCAA/AAA) ratio was higher in the healthy persons and remained stable after the meal while there was a further significant decrease after two hours in the cirrhotic group (Table 3). At 60 and 120 min cirrhotic patients showed a significant increase in plasma ammonia concentration after the meal than normal subjects ( $P < 0.01$ , Figure 3). The NCT remained normal and there were no clinical signs of HE.

#### Response to a 1 g/kg body weight protein meal

Decompensated cirrhotics had different basal concentration of some amino acids compared to those with stable cirrhosis (elevated: Alanine, tyrosine, decreased: isoleucine, leucine) (Figure 4 and Table 4). Hence, the BCAA/AAA ratio was significantly lower in the patients with unstable cirrhosis. After the meal, the concentration of most plasma amino acids (except for taurine, proline, citrulline, cysteine and histidine) had increased significantly at 120 min (Figure 4 and Table 4). Those increments were significantly larger than those observed in the 20 g protein group (Figure 1). The largest increases were observed in the cases of isoleucine (148%), leucine (119%) and methionine (88%) (Figures 1 and 3). The BCAA/AAA ratio was significantly higher 180 and 240 min after the meal (Table 4). Slightly elevated basal plasma ammonia levels increased in two patients, decreased in one and showed no change in two (Figure 3). After the protein meal only

**Table 3** Plasma amino acid response to a 20-g protein mixed meal in cirrhotic patients and controls

Amino acid	Group	Basal ( $\pm$ SE)	30 min ( $\pm$ SE)	60 min ( $\pm$ SE)	120 min ( $\pm$ SE)	180 min ( $\pm$ SE)	240 min ( $\pm$ SE)
Tau	Control	69 $\pm$ 17	65 $\pm$ 10	74 $\pm$ 10	74 $\pm$ 11	69 $\pm$ 8	
	Cirrhotic	48 $\pm$ 5	46 $\pm$ 7	42 $\pm$ 5 <sup>a</sup>	47 $\pm$ 6	44 $\pm$ 4	42 $\pm$ 7
Thr	Control	101 $\pm$ 5	117 $\pm$ 10 <sup>c</sup>	110 $\pm$ 3	115 $\pm$ 8	114 $\pm$ 12	
	Cirrhotic	113 $\pm$ 10	124 $\pm$ 8	125 $\pm$ 11	118 $\pm$ 8	101 $\pm$ 8	111 $\pm$ 15
Ser	Control	94 $\pm$ 8	105 $\pm$ 8 <sup>f</sup>	104 $\pm$ 9 <sup>d</sup>	99 $\pm$ 11	92 $\pm$ 13	
	Cirrhotic	105 $\pm$ 7	117 $\pm$ 8 <sup>c</sup>	114 $\pm$ 10	108 $\pm$ 9	96 $\pm$ 7	103 $\pm$ 12
Asn	Control	23 $\pm$ 2	40 $\pm$ 2 <sup>d</sup>	41 $\pm$ 4 <sup>d</sup>	40 $\pm$ 4 <sup>d</sup>	35 $\pm$ 5 <sup>c</sup>	
	Cirrhotic	35 $\pm$ 3 <sup>a</sup>	43 $\pm$ 3 <sup>f</sup>	44 $\pm$ 5 <sup>d</sup>	44 $\pm$ 4 <sup>d</sup>	37 $\pm$ 4	38 $\pm$ 5
Glu	Control	246 $\pm$ 33	299 $\pm$ 35	263 $\pm$ 17	276 $\pm$ 24	237 $\pm$ 26	
	Cirrhotic	375 $\pm$ 30	388 $\pm$ 20	380 $\pm$ 34	375 $\pm$ 23	354 $\pm$ 22	378 $\pm$ 45
Gln	Control	163 $\pm$ 11	163 $\pm$ 14	154 $\pm$ 13	142 $\pm$ 11	144 $\pm$ 26	
	Cirrhotic	226 $\pm$ 21 <sup>a</sup>	217 $\pm$ 18	213 $\pm$ 14 <sup>a</sup>	213 $\pm$ 20	190 $\pm$ 12	180 $\pm$ 29
Pro	Control	145 $\pm$ 11	152 $\pm$ 13	151 $\pm$ 5	155 $\pm$ 12	135 $\pm$ 7	
	Cirrhotic	152 $\pm$ 15	147 $\pm$ 17	173 $\pm$ 19	185 $\pm$ 17 <sup>c</sup>	155 $\pm$ 16	150 $\pm$ 15
Gly	Control	178 $\pm$ 8	187 $\pm$ 11	190 $\pm$ 14	193 $\pm$ 16	176 $\pm$ 21	
	Cirrhotic	174 $\pm$ 14	177 $\pm$ 14	166 $\pm$ 13	176 $\pm$ 11	170 $\pm$ 12	165 $\pm$ 16
Ala	Control	271 $\pm$ 18	323 $\pm$ 21 <sup>d</sup>	339 $\pm$ 29 <sup>d</sup>	354 $\pm$ 31 <sup>d</sup>	309 $\pm$ 34	
	Cirrhotic	262 $\pm$ 17	310 $\pm$ 24 <sup>c</sup>	334 $\pm$ 34	345 $\pm$ 25 <sup>d</sup>	307 $\pm$ 26	284 $\pm$ 38
Cit	Cirrhotic	27 $\pm$ 3	23 $\pm$ 2	20 $\pm$ 2	27 $\pm$ 2	28 $\pm$ 2	33 $\pm$ 4
Val	Control	167 $\pm$ 13	186 $\pm$ 14 <sup>d</sup>	182 $\pm$ 16 <sup>c</sup>	188 $\pm$ 11 <sup>d</sup>	173 $\pm$ 16	
	Cirrhotic	180 $\pm$ 4	198 $\pm$ 10 <sup>c</sup>	203 $\pm$ 9 <sup>c</sup>	193 $\pm$ 10	170 $\pm$ 8	175 $\pm$ 18
Cys	Control	42 $\pm$ 1	41 $\pm$ 2	40 $\pm$ 2	42 $\pm$ 3	39 $\pm$ 4	
	Cirrhotic	58 $\pm$ 3 <sup>b</sup>	59 $\pm$ 2 <sup>e</sup>	60 $\pm$ 3 <sup>e</sup>	62 $\pm$ 2	58 $\pm$ 4 <sup>b</sup>	56 $\pm$ 5
Met	Control	25 $\pm$ 2	24 $\pm$ 1	25 $\pm$ 1	29 $\pm$ 4	27 $\pm$ 4	
	Cirrhotic	32 $\pm$ 3	30 $\pm$ 2 <sup>a</sup>	33 $\pm$ 3	31 $\pm$ 2	25 $\pm$ 3	25 $\pm$ 3
Iso	Control	49 $\pm$ 3	55 $\pm$ 4	54 $\pm$ 5	62 $\pm$ 5	57 $\pm$ 7	
	Cirrhotic	55 $\pm$ 2	68 $\pm$ 3 <sup>a,d</sup>	71 $\pm$ 4 <sup>a,d</sup>	59 $\pm$ 2	50 $\pm$ 3	55 $\pm$ 7
Leu	Control	91 $\pm$ 6	101 $\pm$ 8 <sup>c</sup>	97 $\pm$ 10	103 $\pm$ 8	95 $\pm$ 11	
	Cirrhotic	101 $\pm$ 2	126 $\pm$ 7 <sup>a,d</sup>	128 $\pm$ 7 <sup>a,d</sup>	106 $\pm$ 5	87 $\pm$ 5	95 $\pm$ 12
Tyr	Control	53 $\pm$ 3	56 $\pm$ 2 <sup>c</sup>	55 $\pm$ 3	56 $\pm$ 4	54 $\pm$ 6	
	Cirrhotic	73 $\pm$ 7 <sup>a</sup>	80 $\pm$ 7	83 $\pm$ 8 <sup>b</sup>	82 $\pm$ 8 <sup>a</sup>	73 $\pm$ 6	76 $\pm$ 10
Phe	Control	37 $\pm$ 0.8	39 $\pm$ 1	40 $\pm$ 2	42 $\pm$ 4	40 $\pm$ 5	
	Cirrhotic	48 $\pm$ 5	58 $\pm$ 7	61 $\pm$ 9	62 $\pm$ 9	57 $\pm$ 7	56 $\pm$ 7
Orn	Control	41 $\pm$ 2	44 $\pm$ 2	44 $\pm$ 3	47 $\pm$ 3	45 $\pm$ 5	
	Cirrhotic	61 $\pm$ 5 <sup>b</sup>	67 $\pm$ 4	69 $\pm$ 6	71 $\pm$ 7 <sup>d</sup>	68 $\pm$ 6	71 $\pm$ 11
Lys	Control	145 $\pm$ 10	169 $\pm$ 13 <sup>d</sup>	175 $\pm$ 13 <sup>d</sup>	194 $\pm$ 13 <sup>f</sup>	169 $\pm$ 17 <sup>c</sup>	
	Cirrhotic	144 $\pm$ 6	167 $\pm$ 9 <sup>c</sup>	171 $\pm$ 11 <sup>c</sup>	164 $\pm$ 11	153 $\pm$ 9	150 $\pm$ 15
His	Control	64 $\pm$ 3	73 $\pm$ 3 <sup>d</sup>	72 $\pm$ 4 <sup>c</sup>	72 $\pm$ 4 <sup>c</sup>	67 $\pm$ 8	
	Cirrhotic	63 $\pm$ 4	70 $\pm$ 3	73 $\pm$ 4	72 $\pm$ 4	69 $\pm$ 4	65 $\pm$ 5
Arg	Control	70 $\pm$ 4	83 $\pm$ 5 <sup>d</sup>	85 $\pm$ 6 <sup>c</sup>	88 $\pm$ 6 <sup>d</sup>	77 $\pm$ 8	
	Cirrhotic	73 $\pm$ 5	81 $\pm$ 6	85 $\pm$ 7	86 $\pm$ 6	80 $\pm$ 7	71 $\pm$ 8
BCAA/AAA	Control	3.44 $\pm$ 0.2	3.62 $\pm$ 0.2	3.49 $\pm$ 0.2	3.65 $\pm$ 0.2	3.54 $\pm$ 0.2	
	Cirrhotic	2.89 $\pm$ 0.2	2.97 $\pm$ 0.2	2.92 $\pm$ 0.2	2.62 $\pm$ 0.2 <sup>c</sup>	2.45 $\pm$ 0.2 <sup>d</sup>	2.53 $\pm$ 0.2 <sup>f</sup>

Plasma amino acid response to a 20 g protein mixed meal in cirrhotic patients and controls. The results are expressed as means  $\pm$  SEM. Healthy subjects  $n = 6$ , cirrhotics  $n = 8$ . Plasma amino acids are given in nmol/mL; significantly different from the corresponding value of control subject: <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$ ; Significantly different from basal value within the same group <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ , <sup>f</sup> $P < 0.001$ .

one patient presented mild electroencephalographic features of covert encephalopathy but there were no clinical manifestations.

## DISCUSSION

A characteristic pattern of plasma amino acids has been described in cirrhotic subjects<sup>[3,4,29-31]</sup> and metabolic and biochemical differences have been shown between stable and unstable cirrhotics<sup>[3,4,32]</sup>. In advanced liver disease there is usually an increased concentration of the AAA tyrosine, phenylalanine and tryptophan, and decreased concentration of the BCAA leucine, isoleucine and valine<sup>[3,4,6,9,14]</sup>. We have previously reported differences

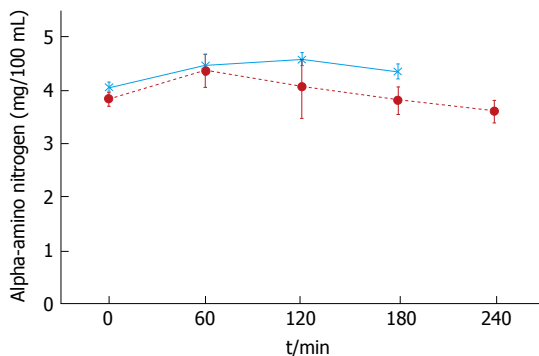
between different stages of liver disease and small or no significant differences between patients with stable liver disease and normal subjects<sup>[26]</sup>. Plasma amino acid concentrations change in the postabsorptive state reflects the balance between uptake by the liver and release by extrahepatic tissue, primarily muscle<sup>[2,3,29,33,34]</sup>. Following a mixed meal, the BCAA are transferred from the gut through the liver to peripheral tissues<sup>[35]</sup>. All other amino acids, including the AAA and methionine, are retained to a greater extent by splanchnic tissues and particularly by the liver<sup>[35,36]</sup>. The BCAA are then primarily metabolized in extrahepatic tissues i.e. skeletal muscle<sup>[28,36]</sup>.

In most previous studies the methods for patient selection (*i.e.*, disease severity) and nutritional intervention

**Table 4** Plasma amino acid response to a high (1 g protein/kg body weight) meal in cirrhotic patients

Amino acid	Group	Basal ( $\pm$ SE)	30 min ( $\pm$ SE)	60 min ( $\pm$ SE)	120 min ( $\pm$ SE)	180 min ( $\pm$ SE)	240 min ( $\pm$ SE)
Taurine	Cirrhotic	58 $\pm$ 1	54 $\pm$ 4	59 $\pm$ 5	58 $\pm$ 3	57 $\pm$ 4	54 $\pm$ 3
Threonine	Cirrhotic	150 $\pm$ 25	152 $\pm$ 22	169 $\pm$ 23	195 $\pm$ 29 <sup>c</sup>	216 $\pm$ 43	216 $\pm$ 31
Serine	Cirrhotic	114 $\pm$ 11	131 $\pm$ 17	151 $\pm$ 20	153 $\pm$ 16 <sup>f</sup>	156 $\pm$ 21	158 $\pm$ 15
Asparagine	Cirrhotic	46 $\pm$ 3	60 $\pm$ 5	67 $\pm$ 6 <sup>f</sup>	76 $\pm$ 3	76 $\pm$ 7	73 $\pm$ 8
Glutamic ac.	Cirrhotic	110 $\pm$ 30	94 $\pm$ 25	88 $\pm$ 36	109 $\pm$ 29	130 $\pm$ 26	153 $\pm$ 79
Glutamine	Cirrhotic	449 $\pm$ 29	462 $\pm$ 64	514 $\pm$ 56	560 $\pm$ 43	578 $\pm$ 39	520 $\pm$ 82
Proline	Cirrhotic	182 $\pm$ 35	212 $\pm$ 30	220 $\pm$ 26	278 $\pm$ 30	266 $\pm$ 32	245 $\pm$ 34
Glycine	Cirrhotic	199 $\pm$ 13	225 $\pm$ 20	245 $\pm$ 25	272 $\pm$ 12 <sup>d</sup>	276 $\pm$ 25	273 $\pm$ 21
Alanine	Cirrhotic	326 $\pm$ 23	408 $\pm$ 41	465 $\pm$ 48 <sup>c</sup>	462 $\pm$ 12	456 $\pm$ 29	475 $\pm$ 47
Citruline	Cirrhotic	48 $\pm$ 7	44 $\pm$ 7	48 $\pm$ 7	58 $\pm$ 5	50 $\pm$ 7	59 $\pm$ 10
Valine	Cirrhotic	146 $\pm$ 23	160 $\pm$ 19	185 $\pm$ 19	209 $\pm$ 29 <sup>c</sup>	224 $\pm$ 36	243 $\pm$ 29
Cysteine	Cirrhotic	53 $\pm$ 3	55 $\pm$ 4	61 $\pm$ 7	60 $\pm$ 4	57 $\pm$ 3	63 $\pm$ 4 <sup>d</sup>
Methionine	Cirrhotic	34 $\pm$ 4	41 $\pm$ 4	45 $\pm$ 4	54 $\pm$ 6 <sup>f</sup>	58 $\pm$ 6	64 $\pm$ 8
Isoleucine	Cirrhotic	43 $\pm$ 2	57 $\pm$ 6	63 $\pm$ 6 <sup>d</sup>	86 $\pm$ 12	97 $\pm$ 16	107 $\pm$ 11
Leucine	Cirrhotic	73 $\pm$ 10	93 $\pm$ 12	107 $\pm$ 14	135 $\pm$ 25 <sup>c</sup>	149 $\pm$ 31	160 $\pm$ 20
Tyrosine	Cirrhotic	109 $\pm$ 13	115 $\pm$ 8	123 $\pm$ 11	139 $\pm$ 8 <sup>c</sup>	144 $\pm$ 8	159 $\pm$ 15
Phenylalanine	Cirrhotic	58 $\pm$ 7	66 $\pm$ 5	77 $\pm$ 10	84 $\pm$ 10 <sup>f</sup>	86 $\pm$ 9	98 $\pm$ 12
Ornithine	Cirrhotic	62 $\pm$ 7	69 $\pm$ 6	73 $\pm$ 7	81 $\pm$ 4 <sup>f</sup>	90 $\pm$ 8	94 $\pm$ 9
Lysine	Cirrhotic	148 $\pm$ 10	179 $\pm$ 22	211 $\pm$ 27 <sup>c</sup>	240 $\pm$ 32	248 $\pm$ 40	253 $\pm$ 32
Histidine	Cirrhotic	68 $\pm$ 8	80 $\pm$ 8	89 $\pm$ 7	90 $\pm$ 8	91 $\pm$ 10	93 $\pm$ 7
Arginine	Cirrhotic	91 $\pm$ 9	99 $\pm$ 10	109 $\pm$ 14	134 $\pm$ 11 <sup>c</sup>	139 $\pm$ 18	152 $\pm$ 19
BCAA/AAA	Cirrhotic	1.69 $\pm$ 0.3	1.74 $\pm$ 0.2	1.81 $\pm$ 0.2	1.93 $\pm$ 0.3	2.03 $\pm$ 0.3 <sup>c</sup>	2.08 $\pm$ 0.4 <sup>c</sup>

The results are expressed as means  $\pm$  SEM. Plasma amino acids are given in nmol/mL. First value significantly different from basal value: <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ , <sup>f</sup> $P < 0.02$ .



**Figure 1** Plasma alpha-amino nitrogen concentration in response to a 20 g protein meal. Cirrhotic patients ( $n = 6$ ), (closed circles); healthy subjects ( $n = 6$ ), (asterisk) (mean  $\pm$  SEM).

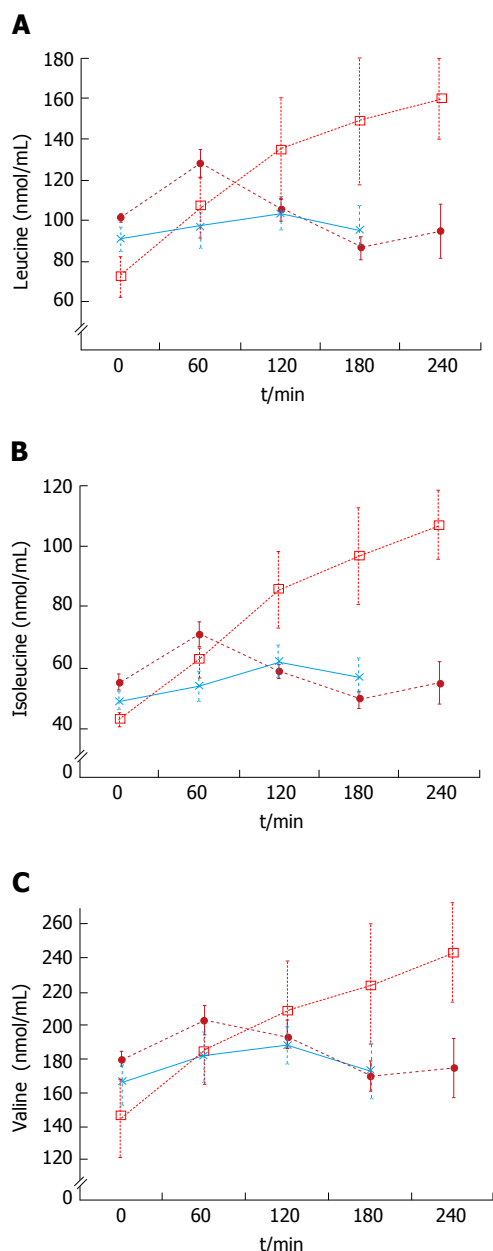
(type and dose of protein or amino acid formula) have varied widely, with very few controlled studies involving a “natural” meal<sup>[14,31,35,37]</sup>. This precludes the opportunity to make firm interpretations of the metabolic alterations in cirrhotic patients. In the present study we therefore investigated the plasma amino acid response to a *natural* meal administered to biopsy proven cirrhotic patients (Child-Pugh class A and C).

On the other hand ammonia is a toxic nitrogenous product of protein and amino acid metabolism<sup>[38]</sup> which under normal circumstances is mainly detoxified by the

liver. In patients with cirrhosis there is an increase in circulating ammonia caused by impaired hepatic detoxification and the presence (as in the decompensated cirrhotics group) of porto-systemic shunting<sup>[19,39]</sup>. Thus the rationale for a *protein tolerance test* is that if the patient develops HE after the test the risk of developing it after the shunt procedure is likely to be relatively high-information which helps surgeons decide which particular type of porto-systemic shunt or device to perform or use respectively.

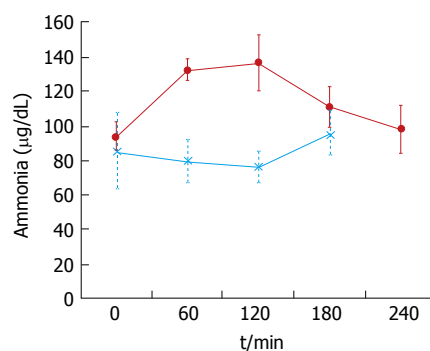
#### **Twenty grams protein natural meal**

After intake of a mixed meal, there were only small differences for most plasma amino acids between cirrhotic patients and controls (Table 3). Only isoleucine, tyrosine and particularly leucine showed modest, but significantly higher increases in cirrhotic patients after the meal (Table 3 and Figure 2). The mean AAA concentration was also higher, but not significantly so. The higher BCAA and AAA increases observed in cirrhotics may be explained by their peripheral insulin resistance<sup>[14]</sup> which results in reduced muscle uptake of BCAA and a decreased inhibition of muscle catabolism after food intake. In previous studies, patients at different stages of liver disease were given either a protein load (ranging from 27 to 48 g)<sup>[3,14-16,31]</sup> or BCAA-enriched formulae<sup>[3,9]</sup> and showed amino acid “intolerance” to that load of protein or



**Figure 2** Plasma leucine (A), isoleucine (B) and valine (C) concentrations in response to protein meals. Twenty grams protein meal, cirrhotics ( $n = 8$ ), (closed circles); 20 g protein meal, healthy subjects ( $n = 6$ ), (asterix) (mean  $\pm$  SEM); 1 g/kg body weight protein meal, cirrhotics ( $n = 6$ ), (open squares).

amino acids. The term “intolerance” here being based on a persistent increase of amino acids in plasma<sup>[40,41]</sup>. It is known, however, that patient selection and factors such as protein type and dosage influence the plasma amino acid response<sup>[35,42-44]</sup>. Additionally the description “protein intolerant” is better reserved for patients who develop HE during protein intake. The BCAA/AAA ratio, showed a slight but significant decrease 120 min after the meal (Table 3). This is in agreement with previous reports suggesting that this ratio may be useful for detecting differences in amino acid metabolism in different groups of cirrhotics<sup>[26,36]</sup>. The differences found in our study suggest subtle alterations in the metabolism of BCAA and AAA evident 2 h after a protein meal, although the meal



**Figure 3** Plasma ammonia concentrations in cirrhotic patients. Blood ammonia after a 20-g protein meal. Cirrhotic patients ( $n = 6$ ), (closed circles); healthy subjects ( $n = 6$ ), (asterix) (mean  $\pm$  SEM).

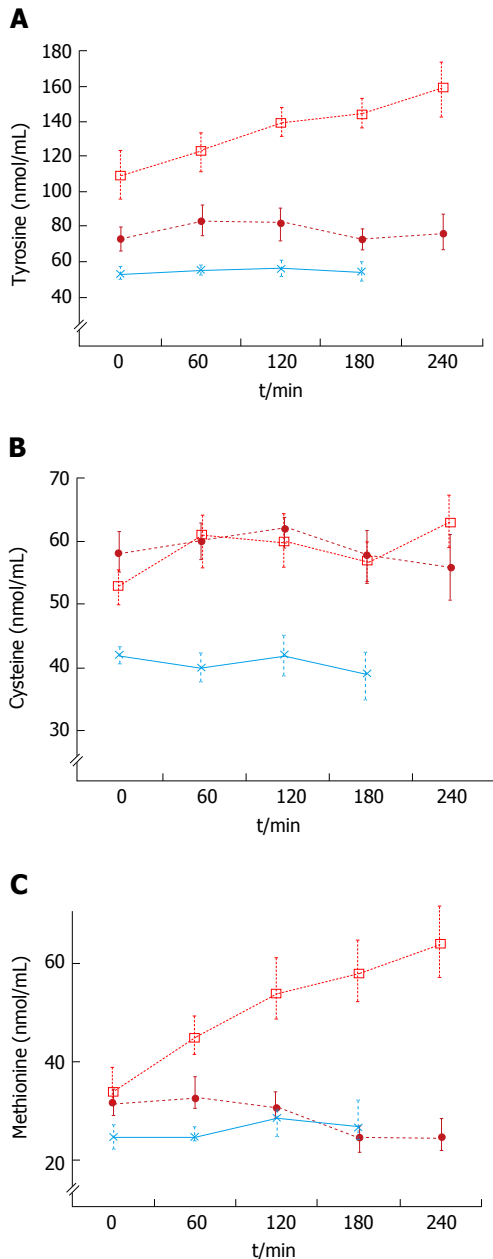
seemed otherwise well tolerated.

In the stable cirrhotic patients we observed a significant increase in the venous plasma ammonia concentration 60 min after food intake (Figure 3) although this protein meal had little effect on alpha amino nitrogen levels (Figure 1). This may be explained by the considerably larger amino nitrogen pool (13.8 mmol N) compared with that of ammonia (0.16 mmol N)<sup>[45]</sup> which might be more sensitive to cyclic changes in absorptive periods and by protein breakdown in the small intestine<sup>[46]</sup>. Additionally, a healthy liver has a huge capacity for increasing urea synthesis after protein ingestion, when ammonia is released from the gut into the portal blood. In patients with cirrhosis, liver ammonia clearance is diminished by the decreased functional liver mass, portosystemic shunting and loss of normal perisinusoidal glutamine synthetase activity<sup>[47-49]</sup>. Nevertheless, the increases in ammonia were modest and most importantly, we did not observe any overt (clinically detectable) HE. The NCT was carried out to test the patients for covert HE which is not clinically detectable. No patients had covert HE after ingestion of the meal. These results support more the role of those factors affecting the clearance of blood ammonia rather than the effect of diet in the development of HE<sup>[39]</sup>.

In this study, the plasma amino acid response to a 20 g natural protein meal was almost the same in cirrhotic patients and controls and we suggest that cirrhotic patients, with a reasonably good liver function have a good tolerance to a natural protein meal. This concurs with current guidelines for protein intake in patients with liver disease<sup>[20-22]</sup>.

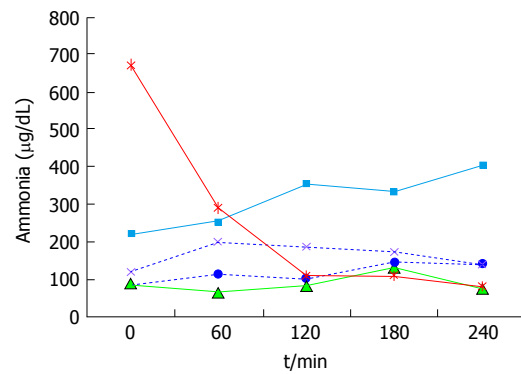
### High protein meal to decompensated patients with cirrhosis

The baseline results showed that the BCAA/AAA ratio was lower in decompensated cirrhotics than in patients with stable cirrhosis and healthy subjects (Table 4). This characteristic pattern of plasma amino acids has previously been described by us and others<sup>[14,26,29,31,34]</sup>. In this group administration of a high (1 g/kg body weight) protein meal led to significant increases in most plasma amino acid levels (Figures 2 and 4, Table 4).



**Figure 4** Plasma tyrosine (A), cysteine (B) and methionine (C) concentrations in response to protein meals. Twenty grams protein meal, cirrhotics ( $n = 8$ ), (closed circles); 20 g protein meal, healthy subjects ( $n = 6$ ), (asterisk) (mean  $\pm$  SEM); 1 g/kg body weight protein meal, cirrhotics ( $n = 6$ ), (open squares).

These results agree in general with those reported by Marchesini *et al.*<sup>[14]</sup> and by Schulte-Frohlinde *et al.*<sup>[31]</sup>. The fact that we found slightly lower increases of leucine, methionine, valine, arginine and glycine in our study might be explained by the type of meal administered (balanced and protein of mixed origin vs meat only in other reports)<sup>[31,35,43]</sup>, and by differences in the degree of liver disease in the study populations<sup>[14-16]</sup>. It is established that a balanced diet increases protein tolerance<sup>[43]</sup>. Apart from the expected increases in valine and methionine levels, our results showed that tyrosine, leucine, isoleucine, phenylalanine, arginine and glycine were also regularly increased after the meal (Figures 2 and 4, Table



**Figure 5** Plasma ammonia concentrations in cirrhotic patients. Individual results after a 1g/kg per body weight protein meal.

4). We also observed a significant increase in the BCAA/AAA ratio which remained elevated up to three hours. The increment in the BCAA/AAA ratio may have resulted from extreme elevations of the BCAAs included in this ratio, the more advanced degree of the disease and/or a paradoxical tendency to normalization of the BCAA/AAA ratio seen after a high protein dose. This should be further investigated.

Current nutrition guidelines recommend high protein diets (1.2-1.5 g/kg body weight/day) for liver cirrhosis<sup>[20-22]</sup> but this is mainly based on applied therapeutic interventions rather than on tolerance or challenge tests<sup>[20-22,40]</sup>. In this study we present experimental evidence supporting those recommendations.

In contrast to the patients with stable cirrhosis, no specific pattern in plasma ammonia concentration was observed in the high protein group (Figure 5), although the concentration in some of the patients reached higher levels than those seen after a standard (20 g) protein meal. The variability of the response in this group suggests an abnormal ammonia metabolism which would be in accordance with the Child-Pugh's grade of liver insufficiency (*i.e.*, C) and the presence of portal hypertension.

No patients experienced overt HE in spite of the amino acid elevations; only one of the six decompensated cirrhotic patients showed mild electroencephalographic changes compatible with covert HE. Previously, protein loads were thought to be a common precipitating factor for HE<sup>[23,27]</sup>. However, protein restriction worsens the nutritional status of cirrhotic patients<sup>[10,50]</sup> and a report by Córdoba *et al.*<sup>[49]</sup> showed that diets with a normal-high protein content (1.2 g/kg per day) are metabolically more adequate than low-protein diets and can be administered safely to cirrhotic patients with episodic HE. Restriction of dietary protein did not have any beneficial effect<sup>[49]</sup>.

**Limitations:** We studied a small group of patients with decompensated cirrhosis. As they were following a protocol in preparation for a porto-caval shunt operation a protein tolerance test was done in order to predict the likelihood of the development of HE after the procedure. Those patients represented the high protein meal group

in this study. As that was not part of the protocol a control group for this part was not included, although we recognize that it would have given us more complete information and provided a better comparison group than just the standard protein group.

In conclusion, after a natural meal containing 20 g of protein, the overall plasma amino acid response in patients with cirrhosis was similar to that of healthy subjects. Plasma ammonia levels increased slightly but, importantly, no evidence of either covert or overt HE was observed. Patients with decompensated cirrhosis showed higher post-prandial concentrations of amino acids in response to a high protein meal. However, we did not observe any overt HE, hence the obvious benefits of a high protein regime should be considered in these patients<sup>[30,50,51]</sup>. In this patient group we therefore recommend following the current nutritional guidelines: protein intake of 1.2-1.5 g/kg body weight distributed daily in frequent small meals. If patients develop HE on a high-protein diet, consider supplementation with BCAA<sup>[12,13,20]</sup>.

## ACKNOWLEDGMENTS

We thank Ms. Angela Madden for her technical support in the preparation and analysis of the diets. Dr. Campollo O was a fellow of the Programa Universitario de Investigación en Salud (PUIS), UNAM and was supported by a scholarship from DGAPA, Universidad Nacional Autónoma de México.

## COMMENTS

### Background

The plasma amino acid increase after ingestion of amino acids or protein tends to be associated with an increase in plasma ammonia, which in turn has been implicated in the development of hepatic encephalopathy. There has been long standing discussion over the adequate amount of protein to be administered to patients with liver cirrhosis in spite of generally accepted nutritional guidelines for these patients. Despite current nutrition guidelines recommend high protein diets, recommendations have not been completely adopted in some places where protein restriction is still considered as a general rule and proper dietary management is not readily followed.

### Research frontiers

While current nutrition guidelines are mainly based on applied therapeutic interventions there have been few reports investigating the tolerance to dietary protein nor they have studied protein tolerance or challenge tests. In this study the authors investigated the plasma amino acid response to standard and high protein natural meals in patients with liver cirrhosis and looked for evidence of protein intolerance by testing for the presence of either covert or overt hepatic encephalopathy.

### Innovations and breakthroughs

Several studies have investigated the effect of protein ingestion on circulating amino acid levels in patients with liver cirrhosis. However, both the type and dosage of protein feed or formula and/or the routes of administration have been varied and the selection of patients has been heterogeneous. The authors aimed to improve on previous methodology by selecting a more homogeneous group of patients with biopsy proven cirrhosis, and by using natural mixed protein meals at two protein levels: A standard (20 g) meal and a high (1 g/kg per body weight) protein meal. In this study they provide experimental evidence to support current nutritional guidelines.

## Applications

Current nutritional guidelines recommend normal to high protein diets (1.2-1.5 g/kg body weight/d) which the authors experimented in this study with good results. They did not observe any overt hepatic encephalopathy hence the obvious benefits of a high protein regime. If patients develop hepatic encephalopathy on a high-protein diet, temporary reduction of protein intake and supplementation with branched chain amino acids should be considered.

## Terminology

Protein tolerance test: A high protein meal (load) has been used to predict the likelihood of hepatic encephalopathy developing following a porto-caval shunt procedure for the treatment of portal hypertension. Therefore patients are studied before and after ingestion of a high protein meal (1 g protein/kg body weight) and clinical and psychological (*i.e.*, "number connection test") evaluations are performed to study overt and covert hepatic encephalopathy.

## Peer-review

The manuscript is well-structured, the rationale behind the study is clear, and the results are relevant for the field of nutrition in liver disease.

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**P- Reviewer:** Alonso-Martinez JL, Ruiz-Margain A, Tarantino G

**S- Editor:** Gong ZM **L- Editor:** A **E- Editor:** Li D



## Inducible protein-10 as a predictive marker of antiviral hepatitis C treatment: A systematic review

Bastian Neesgaard, Morten Ruhwald, Nina Weis

Bastian Neesgaard, Nina Weis, Department of Infectious Diseases, Copenhagen University Hospital Hvidovre, 2650 Hvidovre, Denmark

Morten Ruhwald, Department of Infectious Disease Immunology, Section of Human Immunology, Statens Serum Institute, 2300 Copenhagen S, Denmark

Nina Weis, Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, 2100 Copenhagen Ø, Denmark

**Author contributions:** Neesgaard B and Weis N contributed equally to this work; Neesgaard B and Weis N designed the research; Neesgaard B and Weis N performed the research; Neesgaard B, Ruhwald M and Weis N analyzed the data; Neesgaard B and Weis N wrote the paper.

**Supported by** Amagar and Hvidovre Hospital Research Foundation of 45000 Dkr. (to Bastian Neesgaard); and The Family Hede Nielsen Foundation of 10000 Dkr. (to Bastian Neesgaard).

**Conflict-of-interest statement:** Ruhwald M is registered as inventor on a patent application disclosing IP-10 based liver fibrosis monitoring, using DBS, which could be viewed as a conflict-of-interest. Otherwise all the authors declare that they have no competing interests.

**Data sharing statement:** The technical appendix, and dataset are available from the corresponding author at [nina.weis@regionh.dk](mailto:nina.weis@regionh.dk).

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**Manuscript source:** Unsolicited manuscript

**Correspondence to:** Nina Weis, MD, PhD, Associate Professor,

Department of Infectious Diseases, Copenhagen University Hospital Hvidovre, Kettegårds allé 30, 2650 Hvidovre, Denmark. [nina.weis@regionh.dk](mailto:nina.weis@regionh.dk)  
Telephone: +45-38-623514  
Fax: +45-38-623504

Received: October 22, 2016

Peer-review started: October 28, 2016

First decision: December 1, 2016

Revised: December 30, 2016

Accepted: January 16, 2017

Article in press: January 18, 2017

Published online: May 18, 2017

### Abstract

#### AIM

To investigate interferon- $\gamma$ -inducible protein-10's (IP-10) potential to anticipate rapid (RVR)- and sustained virological responses (SVR) to chronic hepatitis C (CHC) treatment.

#### METHODS

We included case series examining RVR or SVR in relation to 24 or 48 wk treatment for CHC, in patients treatment free for at least six months, with genotype 1 or 4, and in relation to 24 wk treatment for genotype 2 and 3, with pegylated interferon in combination with ribavirin. Patients had to have both a baseline IP-10 level as well as a hepatitis C virus (HCV)-RNA determination 4 wk after treatment initiation or 24 wk after end of treatment. Studies including patients with liver diseases other than CHC, human immunodeficiency virus-infection, treatment with immunosuppressants or cytostatics, alcohol dependency or active intravenous drug-use were excluded. We found 81 articles by searching the MEDLINE and EMBASE databases. Eight studies were eligible for inclusion. Their quality were assessed using an 18 point checklist for case series, developed using a modified Delphi technique. Information was extracted from the articles, and no raw data was requisitioned. The review protocol was

registered at the International Prospective Register of Systematic Reviews (reg. number: CRD42014008736).

## RESULTS

Three studies reported on baseline IP-10 level in association with RVR. A significant association was found for HCV genotype 1 infection by two studies. Only two studies reported on HCV genotype 4 infected and genotype 2 and 3 infected patients, respectively. A trend was seen for an association between RVR and baseline IP-10 for genotype 4, while no association was found for genotype 2 and 3. Seven studies provided information regarding baseline IP-10 and SVR. Following the pattern regarding rapid virological response all five studies examining SVR in relation to baseline IP-10 levels for HCV, genotype 1 infected patients showed a significant association. Likewise a significant association was seen for HCV, genotype 4 infected, while no association was found for HCV, genotype 2 and 3 infected. Though only two studies examined the association for HCV genotype 4 infected and HCV genotype 2 and 3 infected respectively.

## CONCLUSION

We found indications of a possible association between baseline IP-10 level and virological responses in patients with CHC genotype 1 and 4.

**Key words:** Chronic hepatitis C; Inducible protein-10's; Sustained virological response; Interferon- $\gamma$ -inducible protein-10; CXCL-10; Chemokine; Genotype; Pegylated interferon; Ribavirin; Rapid virological response

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**Core tip:** This is the first systematic review examining the association between baseline levels of interferon- $\gamma$ -inducible protein-10 (IP-10) and virological response to treatment with pegylated interferon and ribavirin among patients chronically infected with hepatitis C virus, genotype 1-4. We found a possible correlation for genotype 1 and 4 infected patients, indicating that baseline IP-10 levels could predict which patients, infected with genotype 1 or 4, would have the highest likelihood of benefitting from antiviral treatment with pegylated interferon and ribavirin. These findings can be especially relevant in countries, where treatments with direct acting antivirals are not readily applicable.

Neesgaard B, Ruhwald M, Weis N. Inducible protein-10 as a predictive marker of antiviral hepatitis C treatment: A systematic review. *World J Hepatol* 2017; 9(14): 677-688 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v9/i14/677.htm> DOI: <http://dx.doi.org/10.4254/wjh.v9.i14.677>

## INTRODUCTION

Every year 3-4 million individuals are infected with hepatitis C virus (HCV) of whom only 20%-35% clear the

infection, meaning that 2.4-3.2 million individuals remain chronically infected, defined as detectable HCV-RNA in two consecutive measurements  $\geq$  six months apart. Globally, the prevalence of chronic hepatitis C (CHC) is estimated to 150 million people, with CHC being the leading cause of chronic liver disease<sup>[1]</sup>. CHC can lead to formation of connective tissue (fibrosis) in the liver. However, the rate and severity of the inflammation and fibrosis vary<sup>[2,3]</sup>. Though only 5%-20% of HCV infected patients develop cirrhosis, these patients have an increased risk of developing hepatocellular carcinoma, a condition responsible for more than 300000 deaths annually<sup>[1]</sup>.

Until recently, the standard of care for CHC was lengthy dual therapy with pegylated interferon plus ribavirin (peg-IFN/RBV), either as 180  $\mu$ g peg-IFN- $\alpha$ -2a weekly or peg-IFN- $\alpha$ -2b 1.5  $\mu$ g/kg per week in combination with ribavirin 15 mg/kg per day (minimum 1000 mg daily and maximum 1400 mg daily), fixed doses of 1000 mg for patients < 75 kg and 1200 mg for patients > 75 kg with genotype 1 or 4 or flat dosing of 800 mg daily for genotypes 2 and 3 - a treatment with modest success rates, severe adverse events and variation in treatment response between genotypes<sup>[4]</sup>. Therefore, a great effort has been put into identifying biomarkers to predict rapid virological response (RVR), defined as undetectable serum HCV-RNA at week four of antiviral treatment, and sustained virological response (SVR), defined as the undetectable HCV-RNA 24 wk after discontinuing antiviral treatment. One of the most promising chemokine biomarker candidate is interferon- $\gamma$  inducible protein-10 (IP-10). Both intrahepatic IP-10 mRNA and plasma levels of IP-10 are elevated in individuals with CHC<sup>[5,6]</sup>, strongly indicating that intrahepatic IP-10 is the source of plasma IP-10. Several studies have suggested that pretreatment levels of IP-10 have the capability to predict RVR and SVR<sup>[6-9]</sup>. In addition, hepatic inflammation and fibrosis have been shown to correlate with IP-10 levels<sup>[10-12]</sup>, and it has been proposed, that plasma levels of IP-10 can predict the risk of fibrosis progression<sup>[13]</sup>. Later years have seen the forthcoming of the new direct acting antivirals (DAA), and all current treatment recommendations for CHC patients from the European Association for the Study of the Liver contain at least one DAA<sup>[14]</sup>. The current DAAs are the NS5B polymerase inhibitor, sofosbuvir, the NS3/4A protease inhibitor simeprevir and the NS5A-replication-inhibitors daclatasvir and ledispavir or the so-called 3D regimen containing the dual NS3/4A protease inhibitors Paritaprevir/Ritonavir, the NS5A inhibitor Ombitasvir and the NS5B palm polymerase inhibitor Dasabuvir. This has yielded the possibility for treating CHC patients with interferon free, all-oral regimens, with high SVR-rates and fewer adverse events<sup>[14-18]</sup>. Despite of these great advantages, the cost of DAAs will without doubt substantially delay their introduction as standard treatment in low and middle-income countries by years to come. Moreover, even in high income countries, treatment with DAA therapy is reserved for patients with advanced liver disease, despite the fact that a majority of patients are expected to benefit from the treatment. Therefore,

peg-IFN/RBV treatment still has a role to play in treatment of patients with CHC, and the need for markers that can predict successful treatment outcomes to peg-INF- $\alpha$ /RBV are still needed.

Several studies have independently shown an association between virological response and baseline IP-10 concentrations for CHC patients infected with genotype 1 and 4<sup>[19-21]</sup>. However, the association seems to be lacking for CHC patients, infected with HCV genotype 2 and 3<sup>[21,22]</sup>. Despite this being the case, a systematic review to address and clarify the differences in IP-10 properties, in relation to the different HCV genotypes, is missing. The aim of this systematic review was therefore to examine IP-10's ability to predict RVR and SVR in patients with CHC genotypes 1-4 treated with peg-IFN/RBV. We succeeded in doing so, with data presented in the following.

## MATERIALS AND METHODS

On initiation of this review a protocol was made and registered at the International Prospective Register of Systematic Reviews (PROSPERO) - registration number: CRD42014008736. Protocol can be found at <https://www.crd.york.ac.uk/PROSPERO/>.

### Literature search

Using the search profiles listed in the Appendix I in the supporting information, suitable literature was identified in MEDLINE and EMBASE. The first article sorting was performed by rating the article headlines, while the second sorting was performed on abstract level. Papers passing both sorting rounds were considered for the review, and thoroughly scrutinized based on pre-defined inclusion and exclusion criteria as listed below. The initial search provided 81 articles; 34 in MEDLINE and 47 in EMBASE. After the first- and second-sorting, 14 articles remained from MEDLINE and 14 articles from EMBASE of which 10 were duplicates. One article was found by manual searching the references, bringing the total number of articles after the third sorting to 19. During the third sorting, 11 articles were excluded<sup>[6,8,22-30]</sup>. This left 8 studies for inclusion<sup>[7,9,19,21,31-34]</sup>. Overview of the entire sorting process is shown in Figure 1.

### Inclusion criteria

Case series examining RVR or SVR in relation to 24 or 48 wk treatment with either 180  $\mu$ g Peg-IFN- $\alpha$ -2a weekly or peg-INF- $\alpha$ -2b 1.5  $\mu$ g/kg per week in combination with ribavirin 15 mg/kg per day (minimum 1000 mg daily and maximum 1400 mg daily) or fixed doses of 1000 mg for patients < 75 kg and 1200 mg for patients > 75 kg or flat dosing of 800 mg daily, in CHC patients infected with HCV, genotypes 1 or 4, treatment free for at least six months prior to inclusion, with both a baseline IP-10 level- and HCV-RNA determination, as well as a HCV-RNA determination four weeks after treatment initiation to assess RVR and/or 24 wk after end of treatment to assess SVR.

Case series studies examining RVR or SVR, in rela-

tion to 24 wk treatment with either 180  $\mu$ g Peg-IFN- $\alpha$ -2a per week or peg-INF- $\alpha$ -2b 1.5  $\mu$ g/kg per week in combination with ribavirin 800 mg daily or fixed doses of 1000 mg for patients < 75 kg and 1200 mg for patients > 75 kg, in CHC patients infected with HCV, genotypes 2 or 3, treatment free for at least six months prior to inclusion, with both a baseline IP-10 level- and HCV-RNA determination 4 wk after treatment initiation to assess RVR and/or 24 wk after end of treatment to assess SVR.

### Exclusion criteria

Liver diseases other than CHC, Co-infection with human immunodeficiency virus (HIV), co-infection with hepatitis B virus (HBV), alcohol dependency (regular intake of  $\geq$  75 g/d), active intravenous drug-use, treatment with immunosuppressants or cytostatica and prior treatment for CHC within the last 6 mo.

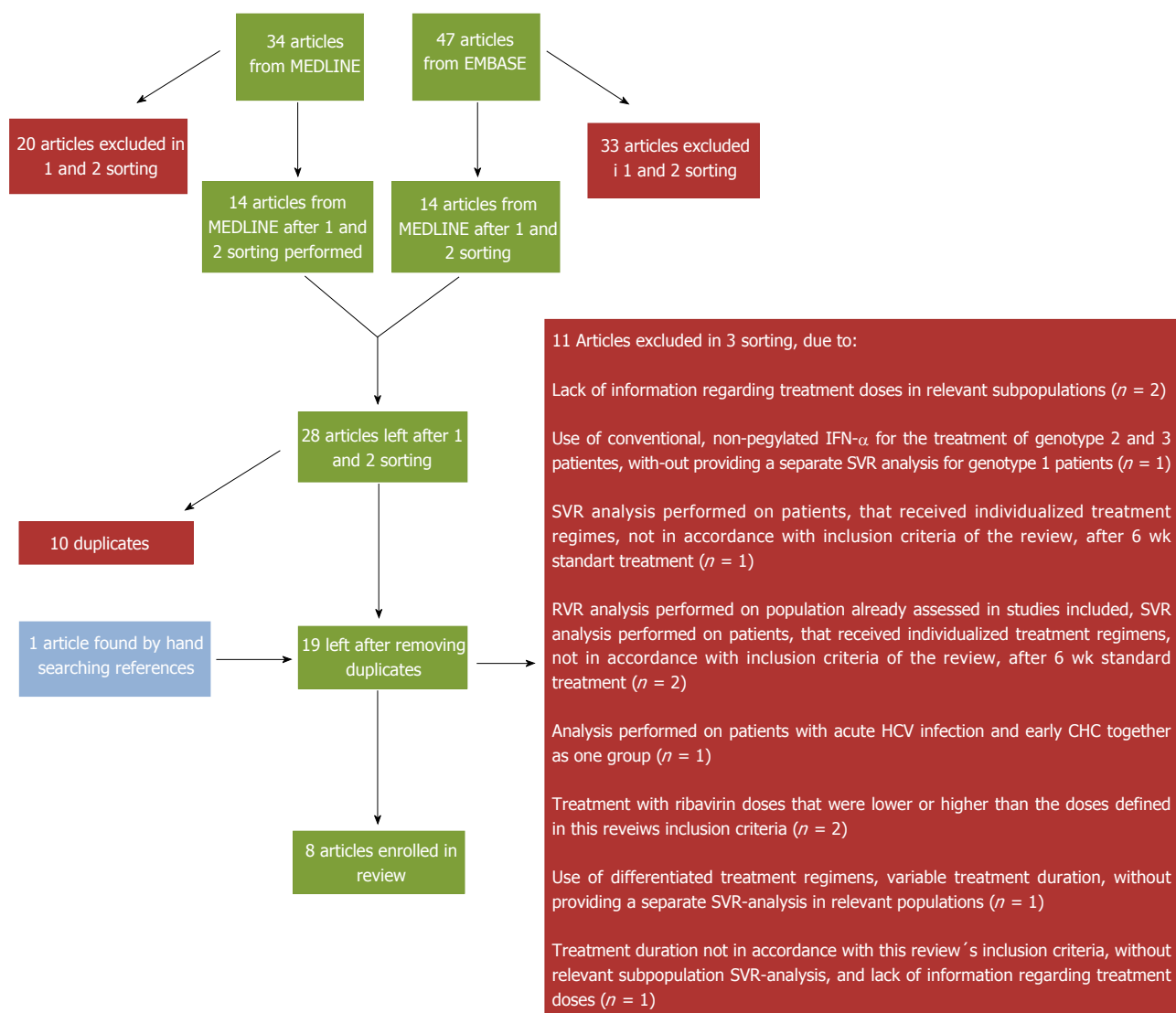
### Quality assessment

The quality of the 8 included articles were appraised using an 18 point checklist for case series, developed using a modified Delphi technique<sup>[35]</sup>. Each criterion can be answered with "yes", "no" or "partially reported/unclear", with the 18 criteria being weighted equally. In line with a pilot study conducted testing the assessment tool, we choose to rate studies with 14 or more "yes responses" as "high-quality studies", and studies with 13 or less "yes responses" as "low-quality studies". No studies were excluded on the basis of the criteria scores. The full checklist can be found in the Appendix II in the supporting information. Table 1 shows the sum score of the checklist. The baseline demographics regarded as important for the appraisal of the studies were: Number of patients included, patient ethnicity, patient age, male/female ratio, HCV RNA, liver enzyme level [alanine transaminase (ALT) or aspartate transaminase (AST)], body mass index (BMI), genotype, liver fibrosis stage and distribution on interleukin 28B (IL28B) single nucleotide polymorphism (SNPs).

## RESULTS

### Patient baseline demographic

All information was extracted from the articles, no raw data was requisitioned. Overall, presentation of baseline demographic data was missing in one study. Instead, this study provided baseline demographics in the following subpopulations: IL28 *rs12979860* (CC, CT, TT), *rs12980275* (AA, AG, GG), *rs8099917* (TT, TG, GG)<sup>[34]</sup>. Only baseline characteristics for *rs12979860* are reported in the review, as these were representative for the study population. All studies provided baseline information on total number of patients included, gender and age. Four studies failed to provide BMI<sup>[7,9,19,31]</sup>, and four studies did not supply exact information regarding patient ethnicity<sup>[7,9,19,32]</sup>. Information regarding number of patients included, ethnicity, age, male/female ratio, and BMI is reported in Table 2. ALT or AST values were not reported by two studies<sup>[33,34]</sup>, one of these however stated that all patients included had two



**Figure 1 Flow chart depicting the sorting of articles.** The chart depicts the number of articles found by searching the MEDLINE and EMBASE databases 04.15.2014, the number of articles excluded during the first and second sorting, the number of duplicates, the number of articles found by manual searching references, and the number of articles excluded in the third sorting, with indication of the reason for exclusion. Articles progressing down the chart from the original search to final inclusion are marked with green boxes, articles found by manual search are marked with blue boxes, and articles excluded are marked with red boxes. SVR: Sustained virological responses; RVR: Rapid virological responses; CHC: Chronic hepatitis C; HCV: Hepatitis C virus.

serum ALT values above the upper limit of normal within 6 mo of treatment initiation<sup>[34]</sup>. Effect on liver parenchyma and HCV-RNA load are shown in Table 3. Regarding fibrosis stage, four studies used the Ishak score<sup>[7,31,33,34]</sup>, two studies used the Scheuer score<sup>[9,32]</sup>, and two studies used the Metavir staging system<sup>[19,21]</sup>. Overview of genotype and fibrosis stage is presented in Table 4. Information regarding treatment regimens can be found in Table 5. Four studies provided information on IL28B SNP distribution. An overview of SNPs can be seen in Table 6.

### Rapid virological response

An overview is presented in Table 7. Lagging *et al.*<sup>[34]</sup> (2011) examined IP-10's ability to predict virological response and treatment outcome in 170 patients with genotype 1, from the DITTO-HCV study group. After six weeks, patients were randomized to individualized treatment,

or continued on the standard combination therapy as no sub analysis on SVR for patient receiving therapy, corresponding with the review's inclusion criteria for the course of 24-48 wk, was provided. Only results regarding RVR are featured in the review. The study found that patients obtaining RVR had significantly lower median baseline IP-10 levels than patients without a RVR. These findings were similar to results reported by Fattovich *et al.*<sup>[21]</sup> that patients infected with HCV, genotype 1, who achieved RVR, had a significant lower mean baseline IP-10, than those who did not. However, this association was not seen for patients infected with HCV genotype 2 or 3. The study also enrolled genotype 4 infected patients, but due to insufficient numbers ( $n = 15$ ), these were excluded. Al-Ashgar *et al.*<sup>[19]</sup>, 2013 studied the relationship between IP-10 and virological response in patients infected with genotype 4, and showed a trend

**Table 1 Overview of the modified Delphi, 18-point quality assessment checklist for studies included in the review**

Ref.	Yes response	No response	Partiel reported/unclear	Assessment
Fattovich <i>et al</i> <sup>[21]</sup>	16	2	0	High-quality
Diago <i>et al</i> <sup>[7]</sup>	15	2	1	High-quality
Apolinario <i>et al</i> <sup>[9]</sup>	14	2	2	High-quality
Darling <i>et al</i> <sup>[31]</sup>	14	1	3	High-quality
Lagging <i>et al</i> <sup>[34]</sup>	14	3	1	High-quality
Al-Ashgar <sup>[19]</sup>	13	3	2	Low-quality
Derbala <i>et al</i> <sup>[32]</sup>	13	2	3	Low-quality
Kurelac <i>et al</i> <sup>[33]</sup>	12	3	3	Low-quality

Studies was ranked as high quality if they provided  $\geq 14$  "yes" answers, or low quality if they provided  $\leq 13$  "yes" answers.

towards lower mean baseline IP-10 in patients with RVR, than in those without, though the association was not significant.

### SVR

An overview is presented in Table 8. Following the pattern regarding RVR, all five studies examining SVR in relation to baseline IP-10 levels for HCV genotype 1 infected patients, showed a significant association.

Apolinario *et al*<sup>[9]</sup> enrolled 63 Spanish patients from clinical trials and out patient clinics. Forty-three patients had genotype 1, while 20 had a non-1 genotype. Among the 43 HCV genotype 1 infected patients, mean baseline IP-10 levels were significantly lower in patients who reached a SVR compared to those who did not. Because some of the genotype non-1 infected patients received 48 wk of therapy, the results for these are not provided in Table 7. Diago *et al*<sup>[7]</sup> also found a significant association between mean baseline IP-10 and SVR for their overall population of Spanish patients. An association that remained significant, when the analysis was restricted to HCV genotype 1 infected patients. The same significant association between lower baseline IP-10 and SVR for HCV genotype 1 infected, were reported for Italian, Croatian and American patients<sup>[21,31,33]</sup>. An interesting aspect of the study by Kurelac *et al*<sup>[33]</sup>, 2012 was, that the greatest difference in IP-10, between patients with a SVR vs non-SVR, was seen at treatment week 4, where median IP-10 levels were 185 pg/mL (63-518) and 424 pg/mL (90-815) ( $P < 0.0001$ ), respectively. Darling *et al*<sup>[31]</sup> noted, that the significant association between baseline IP-10 levels and SVR remained when patients were grouped as Caucasian Americans (CA) or African Americans (AA) ( $447 \pm 44$  pg/mL vs  $677 \pm 69$  pg/mL,  $P < 0.001$  and  $418$  pg/mL  $\pm 35$  vs  $716$  pg/mL  $\pm 55$ ,  $P < 0.001$ , respectively). Fattovich *et al*<sup>[21]</sup> were the only ones that reported on HCV genotype 2 and 3 patients. As with the results regarding RVR, no association was found between SVR and baseline IP-10. Derbala *et al*<sup>[32]</sup> and Al-Ashgar *et al*<sup>[19]</sup> studied HCV genotype 4 infected Egyptian and Saudi patients, respectively, and showed significantly higher values of baseline IP-10 in non-SVRs

than in SVRs. Interestingly, a sub analysis, performed by Al-Ashgar *et al*<sup>[19]</sup> on genotype 4a and 4d, showed that this correlation was present for genotype 4d ( $465.9$  pg/mL  $\pm 349.1$  vs  $904.9$  pg/mL  $\pm 532.1$ ,  $P < 0.001$ ), but not for genotype 4a ( $564.7$  pg/mL  $\pm 288.9$  vs  $568$  pg/mL  $\pm 384.9$ ,  $P = 0.300$ ). Derbala *et al*<sup>[32]</sup> failed to provide information on the exact levels of IP-10, and instead provided a graphic depiction, which could not be interpreted to adequate results.

### DISCUSSION

Several studies have independently shown levels of IP-10 to be associated with both RVR and SVR to peg-IFN/RBV treatment for CHC patients infected with HCV, genotype 1 and 4, but not for genotype 2 and 3. We conducted this systematic review to assess variation in IP-10's predictive ability for RVR and SVR to peg-INF/RBV treatment in patients chronically infected with HCV genotypes 1-4.

Our main findings indicate that a correlation exist between baseline IP-10 and SVR- and in part for RVR - for genotype 1 and possibly for genotype 4, however not for genotype 2 or 3.

Three studies provided information on baseline IP-10 in relation to RVR<sup>[19,21,34]</sup>. Studies reporting on HCV, genotype 1 infected patients, found significant lower baseline IP-10 values in patients achieving RVR compared to those who did not<sup>[21,34]</sup>. Only a trend, failing to reach significance, was described between baseline IP-10 and RVR in genotype 4 infected patients<sup>[19]</sup> and no significant relation was found in relation to genotype 2 or -3<sup>[21]</sup>. Seven studies provided information on baseline IP-10 in relation to SVR<sup>[7,9,19,21,31-33]</sup>. All five studies reporting on HCV genotype 1 infected patients<sup>[7,9,21,31,33]</sup> found significantly lower IP-10 levels of SVR than non-SVR. Diago *et al*<sup>[7]</sup> did not provide separate results for the group of genotype non-1 patients included, which was surprising, as three quarters of the patients were infected with HCV, genotype 1, and could very well be the reason for finding a significant association in the overall population, when all genotypes were analyzed together. In line with this, Apolinario *et al*<sup>[9]</sup> stated that no associations was found for their genotype non-1 group. However lacking differentiation into sub genotypes, compromise the value of information, especially as no association were found for HCV, genotype 2 or 3 infected<sup>[21]</sup>, and both studies reporting on genotype 4 infected patients<sup>[19,32]</sup> found significant lower baseline IP-10 level in their populations, when comparing patients achieving SVR vs non-SVR. It should be noted, that while Fattovich *et al*<sup>[21]</sup> considered two-sided  $P$ -values  $< 0.05$  as statistical significant, only results of statistical tests with a  $P$ -value  $< 0.01$  were considered of interest, because of the multiple comparisons between subjects with and without SVR. Therefore IP-10 was not considered to be associated with SVR, for HCV genotype 3 infected individuals, even though the  $p$ -value was found to be 0.02.

One study observed that the greatest difference in IP-10 levels was found at week 4. Patients, who at this

**Table 2** Baseline total patient number, number of male patients, mean age, body mass index and ethnicity for the 8 included studies

Ref.	Patients		Mean age (yr)	BMI (kg/m <sup>2</sup> )	Ethnicity		
	Males	Total (n)			Caucasian	African American	Asian
Apolinario <i>et al</i> <sup>[9]</sup>	40	63	41 (± 9.3) <sup>5</sup>	Information not provided	Information not provided <sup>6</sup>		
<sup>1</sup> Lagging <i>et al</i> <sup>[34]</sup>	169	252 <sup>2</sup>			252 <sup>9</sup>		
IL28B rs12979860 CC	64	93	41.6 (± 10.1) <sup>5</sup>	25.1 (± 3.6) <sup>5</sup>			
IL28B rs12979860 CT	77	123	41.9 (± 9.5) <sup>5</sup>	25.0 (± 3.5) <sup>5</sup>			
IL28B rs12979860 TT	28	36	41.9 (± 11.4) <sup>5</sup>	25.0 (± 3.5) <sup>5</sup>			
Diago <i>et al</i> <sup>[7]</sup>	77	137	42 (± 9.7) <sup>5</sup>	Information not provided	Information not provided		
Fattovich <i>et al</i> <sup>[21]</sup>	133	226 <sup>3</sup>	46 (± 11) <sup>5</sup>	24.7 (± 3.8) <sup>5</sup>	226		
Kurelac <i>et al</i> <sup>[33]</sup>	17	46	41.5 (± 12.4) <sup>5</sup>	23.7 (21.9-25) <sup>4</sup>	46		
Darling <i>et al</i> <sup>[31]</sup>	176	272	48.4 (± 7.4) <sup>5</sup>	Information not provided	138	134	
Darbala <i>et al</i> <sup>[32]</sup>	144	159	46.47 (± 8.83) <sup>5</sup>	30.18 (± 5.05) <sup>5</sup>	Information not provided <sup>7</sup>		
Al-Ashgar <i>et al</i> <sup>[19]</sup>	41	64	38.7 (± 11.5) <sup>5</sup>	Information not provided	Information not provided <sup>8</sup>		

<sup>1</sup>Study did not provide baseline characteristics for their entire population, but instead provided baseline demographics in accordance with IL-28 genotype (only baseline characteristics for rs12979860 are reported in the review, as these were representative for the study population); <sup>2</sup>253 was reported to be enrolled, however when adding the males and female patients, it sums to 252; <sup>3</sup>Only 226 out of 280 patients had serum available for iP-10 testing; <sup>4</sup>Median (25-75 percentiles); <sup>5</sup>Mean (SD); <sup>6</sup>51 patients from clinical trials had Spanish nationality; <sup>7</sup>Egyptian nationality; <sup>8</sup>Saudi nationality; <sup>9</sup>95% of the original DITTO patient population were Caucasian. BMI: Body mass index.

**Table 3** Hepatitis C virus-RNA and patient liver enzyme status for the 8 included studies

Ref.	HCV-RNA				All patients	Liver enzyme level	
	High viral load		Low viral load			AST	ALT
	<i>n</i>	Limit	<i>n</i>	Limit			
Apolinario <i>et al</i> <sup>[9]</sup>	28	≥ 6.3 log IU/mL <sup>5</sup>	35	< 6.3 log IU/mL <sup>5</sup>			118 IU/L (± 64) \$
<sup>1</sup> Lagging <i>et al</i> <sup>[34]</sup>							
IL28B <i>rs12979860 CC</i>					6.3 log IU/mL (± 0.8) <sup>3</sup>	Information not provided	
IL28B <i>rs12979860 CT</i>					6.1 log IU/mL (± 0.7) <sup>3</sup>		
IL28B <i>rs12979860 TT</i>					5.9 log IU/mL (± 0.8) <sup>3</sup>		
Diago <i>et al</i> <sup>[7]</sup>	85	≥ 5.7 log IU/mL <sup>5</sup>	52	< 5.7 log IU/mL <sup>5</sup>			117.2 IU/L (± 81.6) <sup>3</sup>
Fattovich <i>et al</i> <sup>[21]</sup>	147	≥ 5.6 log IU/mL <sup>5</sup>			5.74 log IU/mL (± 0.9) <sup>3,5</sup>		92 IU/L (± 78) <sup>3</sup>
Kurelac <i>et al</i> <sup>[33]</sup>					5.55 log IU/mL (5.52-6.1) <sup>2,5</sup>	Information not provided	
Darling <i>et al</i> <sup>[31]</sup>					6.66 log IU/mL (± 6.76) <sup>3,5</sup>		
Darbala <i>et al</i> <sup>[32]</sup>					4.95 log IU/mL (3.6-5.63) <sup>4,5</sup>		
Al-Ashgar <i>et al</i> <sup>[19]</sup>	45	≥ 5.78 log IU/mL	19	< 5.78 log IU/mL <sup>5</sup>		67.5 IU/L (43.5-106.8) <sup>2</sup>	56.0 IU/L (32.0-86.0) <sup>2</sup>

<sup>1</sup>Lagging *et al* provided baseline demographics in accordance with IL-28 genotype (only baseline characteristics for rs12979860 are reported in the review, as these were representative for the study population); <sup>2</sup>Median (25-75 percentiles); <sup>3</sup>Mean (SD); <sup>4</sup>Median (IQR); <sup>5</sup>Recalculated into log IU/mL. HCV-RNA is shown as number of patients with high or low viral load or as the mean or median for the entire population. Depending of the presentation in the original article, levels of ALT, AST or both are shown. HCV: Hepatitis C virus; ALT: Alanine transaminase; AST: Aspartate transaminase.

point had IP-10 levels higher than 250 pg/mL, had a 40-fold risk of not reaching SVR compared to patients with IP-10 levels lower than 250 pg/mL<sup>[33]</sup>. This might indicate, that IP-10 levels at treatment week 4, could be used to assess if peg-INF/RBV treatment should be discontinued or not in genotype 1 patients - and perhaps could also be used to evaluate the need for adjacent DAA treatment (*i.e.*, using a 4 wk lead in phase with peg-INF/RBV treatment before apprising the need for DAAs). However, the small number of patients participating calls for caution when interpreting these results, and further studies of IP-10 levels at treatment week 4 should be encouraged.

One study<sup>[31]</sup> showed that the correlation between baseline IP-10 and SVR remained significant even when the population was grouped according to ethnicity ( $P < 0.001$ ). The latter is interesting as AA ethnicity is otherwise considered an unfavorable prognostic factor for obtaining SVR<sup>[36-38]</sup>, and might imply that IP-10 could help aid the

decision as to whom would have the greatest potential benefit from peg-INF/RBV treatment regardless of ethnicity. In this context it is interesting that it has previously been shown that HCV infected AA had higher IP-10 levels than corresponding CA patients, while uninfected AA had IP-10 levels similar to uninfected CA<sup>[28]</sup>. The effect of race on Interferon Stimulated Genes, once at the stage of CHC, should therefore be examined further.

Findings, regarding SVR for HCV genotype 2 and 3, followed the same pattern as the results for RVR with no association between baseline IP-10 and SVR present for genotype 2 or 3<sup>[21]</sup>. Supporting our findings, this lacking correlation in patients with HCV genotype 2 and 3, has also been shown when treating patients with standard and low (90 µg once weekly) peg-INF/RBV regimens<sup>[22]</sup>.

As mentioned, a significant correlation between IP-10 and SVR was reported by both studies, including HCV genotype 4 infected patients<sup>[19,32]</sup>. One of these<sup>[19]</sup> also performed differentiated analyses on HCV genotype 4

**Table 4 Genotype and liver fibrosis stage for the 8 included studies**

Ref.	Genotype (n)				Method	Liver fibrosis stage						
	1	2	3	4		0	1	2	3	4	5	6
Apolinario <i>et al</i> <sup>[9]</sup>	43	20			Scheuer score	28		35				
Lagging <i>et al</i> <sup>[34]</sup>	170 <sup>1</sup>	23 <sup>1</sup>	49 <sup>1</sup>	11 <sup>1</sup>	Ishak score	11	61	65	30	15	20	14
IL28B <i>rs12979860</i> CC	44	13	33	3		3	18	27	11	5	12	5
IL28B <i>rs12979860</i> CT	96	7	15	5		7	35	27	17	7	6	6
IL28B <i>rs12979860</i> TT	30	3	1	3		1	8	11	2	3	2	3
Diago <i>et al</i> <sup>[7]</sup>	103	9	25		Ishak score	106				31		
Fattovich <i>et al</i> <sup>[21]</sup>	92	87	47		Metavir <sup>2</sup>	121			21			
Kurelac <i>et al</i> <sup>[33]</sup>	46				Ishak		34			12		
Darling <i>et al</i> <sup>[31]</sup>	272				Ishak	220				52		
Darbala <i>et al</i> <sup>[32]</sup>				159	Scheuer score		109		50			
Ashgar <i>et al</i> <sup>[19]</sup>				64	Metavir	34 <sup>3</sup>			10 <sup>3</sup>			

<sup>1</sup>Baseline information for the sub analysis of IL28 12979860. Two hundred and fifty-two patients are reported to be enrolled, however adding the genotypes yields 253 patients. Likewise biopsies from 228 patients are described, however when adding the Ishak scores only yields 216 patients; <sup>2</sup>Biopsies only available for 142 patients; <sup>3</sup>Histology available for 44 patients; <sup>4</sup>Lagging *et al* provided baseline demographics in accordance with IL-28 genotype (only baseline characteristics for rs12979860 are reported in the review, as these were representative for the study population). A box stretching over two - or more genotypes or fibrosis stage, indicates that the number refers to the combined group.

**Table 5 Overview of the treatment regimens for pegylated interferon in combination with ribavirin, for the 8 studies included, in relation to dose and duration**

Ref.	Genotype		Duration	Interferon treatment	Ribavirin treatment
Apolinario <i>et al</i> <sup>[9]</sup>	Multi-centerpatients	1	48 wk	180 µg peg-INF-α-2a once weekly	800 mg per day or 1000 mg < 75 kg, 1200 mg > 75 kg
		Non-1	24-48 wk		
	Out patiens	1	48 wk	peg-INF-α2b 1.5 µg/kg per week	1000-1200 mg per day
		Non-1	24 wk		
Lagging <i>et al</i> <sup>[34]</sup>	1		6 wk <sup>1</sup>	180 µg peg-INF-α-2a once weekly	1000 mg < 75 kg, 1200 mg > 75 kg per day
Diago <i>et al</i> <sup>[7]</sup>	1		48 wk	peg-INF-α-2b 1.5 µg/kg per week or 180 µg peg-INF-α-2a/week	1000 mg < 75 kg, 1200 mg > 75 kg per day
	Non-1		24 wk		
Fattovich <i>et al</i> <sup>[21]</sup>	1 and 4		48 wk	peg-INF-α-2b 1.5 µg/kg per week or 180 µg peg-INF-α-2a/week	800-1200 mg per day
	2 and 3		24 wk		
Kurelac <i>et al</i> <sup>[33]</sup>	1		48 wk	peg-INF-α-2b 1.5 µg/kg per week	Weight based ribavirin treatment <sup>2</sup>
Darling <i>et al</i> <sup>[31]</sup>	1		48 wk	180 µg peg-INF-α-2a once weekly	1000-1200 mg per day
Derbala <i>et al</i> <sup>[32]</sup>	4		48 wk	Peg-IFN once weekly <sup>3</sup>	1000 mg < 75 kg, 1200 mg > 75 kg per day
Al-Ashgar <sup>[19]</sup>	4		48 wk	180 µg peg-INF-α-2a once weekly	1000 mg < 75 kg, 1200 mg > 75 kg

<sup>1</sup>After 6 wk, patients were randomized to differentiated treatment regimes; <sup>2</sup>No further information on ribavirin treatment was provided; <sup>3</sup>No further information on the subtype of peg-IFN was provided. Apolinario *et al*<sup>[9]</sup> feature patients from both an outpatient clinic as well as patients from two multicenter trials receiving different treatment regimens, illustrated by the segregation in the genotype column. peg-IFN: Pegylated interferon.

subtypes, 4a and 4d, showing a significant association only for the latter ( $P = 0.330$  and  $P < 0.001$ , respectively). It would have been interesting to examine if this was also the case for RVR, as it could be speculated that the association between baseline IP-10 and RVR in HCV genotype 4 infected patients failed to show significance, because both subtype 4a and 4d were analyzed as a whole. Therefore, subsequent studies making RVR and SVR assessments should be encouraged to perform differential analysis on individual viral subtypes, in order to uncover more specific associations. The setup for this study, did not allow us to investigate, what specific mechanisms account for the differences in correlation between baseline IP-10 and HCV genotype 1 and 4 compared with HCV genotype 2 and 3. However it is of great interest that these differences occur, and should be investigated further. Inversely patients infected with HCV genotype 2 or 3 generally has a more favorable response to treatment with PEG-IFN and RBV. Therefore, in a clinical setting the underlying mechanism

might not be relevant, as genotype 2 and 3 patients would readily be treated, whereas clinicians might be more reluctant to initiate peg-INF treatment to genotype 1 and 4 - infected individuals and here IP-10 levels might help to show which patients should undergo treatment.

This review focused on the association between pre-treatment IP-10 levels and virological responses. However, IL28B SNPs should be addressed when considering IP-10, as they are strongly linked with treatment response to Peg-INF/ RBV<sup>[39-45]</sup>. Especially are homozygote genotypes at markers *rs8099917* (TT), *rs12979860* (CC) and *rs12980275* (AA) associated with a favorable outcome to treatment. While IL28B polymorphisms were not found to be predictive for treatment response in HCV genotype 2 and 3 infected individuals by Fattovich *et al*<sup>[21]</sup>, pretreatment IL28B polymorphisms, HCV-RNA- and IP-10 levels independently predict RVR in HCV genotype 1 infected individuals, with RVR in turn being the strongest predictor of SVR. Combining the IL28B polymorphisms and HCV-

**Table 6** Overview of the marker distribution, in the four studied that supplied information on interleukin 28B single nucleotide polymorphism

Ref.	Genotype (n)	rs12979860			rs12980275			rs8099917			rs11881222		
		CC	CT	TT	AA	AG	GG	TT	TG	GG	AA	AG	GG
Lagging <i>et al</i> <sup>[30]</sup>	1 (253)	93	123	37	101	115	37	153	90	10			
Fattovich <i>et al</i> <sup>[21]</sup>	1 (92)	33	44	15	33	45	14	49	38	5			
	2 (87)	34	43	10	34	42	11	47	34	6			
	3 (47)	25	21	1	25	20	2	34	13	0			
Darling <i>et al</i> <sup>[31]</sup>	1 (201)	63	103	44									
Derbala <i>et al</i> <sup>[32]</sup>	4 (159)	57	77	25				96	55	8	64	75	20

Genotype column indicates specific genotype, and total number of patients with the specific genotype. Each marker column is divided into allelic distribution for the IL28B SNP genotype. SNP: Single nucleotide polymorphism.

**Table 7** Overview of rapid virological response in the 3 studies providing information on baseline inducible protein-10's, and hepatitis C virus RNA levels at week 4

Ref.	Patients (n)	IP-10 measurement method	Genotype (n)	Baseline IP-10 concentration, grouped by rapid virological response (pg/mL)			Overall RVR (n)	
				RVR	Non-RVR	P-value	RVR	Non-RVR
Lagging <i>et al</i> <sup>[34]</sup>	170	ELISA (Quantikine, R and D systems, Minneapolis, MN, United States)	1 (170)	222	401	$P < 0.01$ (median)	33	137
<sup>1</sup> Fattovich <i>et al</i> <sup>[21]</sup>	226	ELISA (Quantikine, R and D systems, Minneapolis, MN, United States)	1 (92)	2.4 ( $\pm 0.28$ )	2.6 ( $\pm 0.25$ )	$P < 0.01$ (log mean $\pm$ SD)	172	108
			2 (87)	2.38 ( $\pm 0.31$ )	2.3 ( $\pm 0.30$ )	$P > 0.05$ (log mean $\pm$ SD)		
			3 (47)	2.45 ( $\pm 0.23$ )	2.48 ( $\pm 0.39$ )	$P > 0.05$ (log mean $\pm$ SD)		
Al-Ashgar <i>et al</i> <sup>[19]</sup>	64	ELISA (Quantikine, R and D systems, Minneapolis, MN, United States)	4 (64)	483.9 ( $\pm 261.6$ )	609.9 ( $\pm 424.3$ )	$P > 0.05$ (mean $\pm$ SD)	12	52

<sup>1</sup>Entire patient population was 280, genotype 4 infected were removed from the analyses, and IP-10 results was available for 226 patients. IP-10: Inducible protein-10; RVR: Rapid virological response.

RNA yielded a specificity of 98% but a low sensitivity of 39%. By including IP-10 values in the equation, the sensitivity and the negative predictive value was raised from 81% to 94%, however lowering the positive predictive value from 87% to 76%. This is consistent with other findings in HCV genotype 1 infected, homozygous carriers of the favorable IL28B SNPs, with low IP-10 level, which also significantly predicted a first phase decline of HCV RNA, which translated into increased rates of RVR and SVR<sup>[30]</sup>. While the two latter studies was carried out solely on Caucasian patients infected with HCV genotype 1, the additive predictive effect has also been shown for both HCV genotype 1 infected AA and CA patients<sup>[31]</sup>, and HCV genotype 4 infected patients<sup>[32]</sup>, respectively. Although low in numbers, these results could indicate, that both variables should be considered in a clinical context, before initiating treatment with Peg-INF/RBV in patients infected with HCV genotype 1 or 4. Further studies examining the association in HCV genotype 2 and 3 infected patients should be encouraged.

Conducting a systematic review with clear and stringent in- and exclusions criteria, is an obvious strength of this study, ensuring homogeneity between the studies included, hereby allowing an unbiased assessment of the current evidence. Another strength of our study was that we assessed the quality of the studies included, and provided a detailed declaration of the studies aim,

method - including treatment regimens and duration, as well as baseline patient demographics for the individual studies - supplying a solid ground for interpreting the results put forth. Although some authors recommend the use of quality assessments, other consider them misleading<sup>[46]</sup>, and there remains uncertainties about the relationship between methodology, validity and the use of sum scores to judge the quality of studies<sup>[47]</sup>. Therefore we chose not to exclude any articles based on their quality score (e.g., high quality or low quality), but instead presented the ratings of the studies in the review to serve as an objective guide to interpret the review's results, rather than a tool for selecting studies for the review. As seen by the exclusion criteria, we wished to eliminate the possible uncertainties that could arise by including studies treating HIV/HCV - or HBV/HCV co-infected patients. Therefore, it should be mentioned that even though there was no indication towards inclusion of co-infected patients, three of the included studies, based in the United States, Croatia and Egypt, contained no clear exclusion criteria for HIV- or HBV- infection<sup>[31-33]</sup>.

Only a limited number of articles fulfilled the in- and exclusion criteria to be assessed in this review. Hence, more work is needed to establish a sufficient ground for final conclusions to be made. Further, there was an overweight of studies that addressed the association between SVR and baseline IP-10 in CHC patients infected

**Table 8 Overview of sustained viral response in the 8 studies providing information on baseline inducible protein-10's, and hepatitis C virus-RNA levels 24 wk after end-of- treatment**

Ref.	Patients (n)	IP-10 measurement method	Genotype (n)	Baseline IP-10 concentration, grouped by sustained virological response (pg/mL)			Overall SVR	
				SVR	Non-SVR	P-value	SVR	Non-SVR
Apolinario <i>et al</i> <sup>[9]</sup>	63	ELISA (OptEIA, Pharmingen, San Diego, CA, United States)	1 (43)	245 (± 154)	381 (± 138)	$P < 0.05$ (mean ± SD)	36	27
Diago <i>et al</i> <sup>[7]</sup>	137	ELISA (Human immunoassay kit; BioSource Europe SA, Nivelles, Belgium)	1 (103)	347 (± 197.4)	500.6 (± 311.2)	$P < 0.01$ (mean ± SD)	79 <sup>2</sup>	58 <sup>2</sup>
			1 (103)	332.4 (± 222.1)	476.8 (± 305.3)	$P < 0.01$ (mean ± SD)		
			2 (9)					
<sup>1</sup> Fattovich <i>et al</i> <sup>[21]</sup>	226	ELISA (Quantikine, R and D systems, Minneapolis, MN, United States )	1 (92)	2.47 ± 0.23	2.65 ± 0.28	$P < 0.001$ (log mean ± SD)	209 <sup>2</sup>	71 <sup>2</sup>
			2 (87)	2.37 ± 0.31	2.33 ± 0.35	$P > 0.05$ (log mean ± SD)		
			3 (47)	2.42 ± 0.21	2.67 ± 0.46	$P < 0.05^a$ (log mean ± SD)		
Kurelac <i>et al</i> <sup>[33]</sup>	46	ELISA (Quantikine, R and D systems, Minneapolis, MN, United States )	1 (46)	185 (63-518)	395.5 (111-926)	$P < 0.0001$ (median, range)	26	20
Darling <i>et al</i> <sup>[31]</sup>	272	ELISA (Quantikine, R and D systems, Minneapolis, MN, United States )	1 (272)	437 (± 31)	704 (± 44)	$P < 0.001$ (mean ± SD)	157	115
Derbala <i>et al</i> <sup>[32]</sup>	159	Luminex, Cytokine multiplex immunoassay kit (Merck Millipore, Billerica, MA, United States)	4 (159)	Exact data not provided, only graphic presentation		$P < 0.001$ (median, IQR)	98	61
Al-Ashgar <i>et al</i> <sup>[19]</sup>	64	ELISA (Quantikine, R and D systems, Minneapolis, MN, United States )	4 (64)	462 (± 282.6)	840.4 (± 490.6)	$P < 0.01$ (mean ± SD)	41	23

<sup>1</sup>Entire patina population was 280, genotype 4 removed from the analyses, and IP-10 results was available for 226 patients Note that M. Derbala *et al* did not provide written specification on IP-10 levels for SVR compared to non-SVR, and only supplied a graphic depiction, which could not be interpreted to adequate results; <sup>2</sup>SVR for the entire population. <sup>a</sup> $P = 0.02$ . Only the results of statistical tests with a  $P$  value  $< 0.01$  were considered of interest, because of the multiple comparisons between subjects with and without SVR. SVR: Sustained viral response; IP-10: Inducible protein-10.

with HCV genotype 1, whereas there was only a small fraction addressing the association between SVR and baseline IP-10 for genotype 2, 3 and 4, as well as studies examining the relationship between RVR and baseline IP-10 constituting an insufficient base for assessing baseline IP-10's predictive ability in these regards.

In this systematic review, we found correlations between baseline IP-10 levels and SVR in patients chronically infected with HCV genotype 1 and 4, while no such association was found for patients infected with HCV genotype 2 or 3. Likewise, we found indications of a possible correlation between baseline IP-10 and RVR for HCV genotype 1 infected patients, while no such association were found for HCV genotype 2 or 3 patients, and only a trend was found for HCV genotype 4 infected patients. However, the amount of information regarding baseline RVR for genotypes 1-4, and SVR's relation with baseline IP-10 for genotypes 2, 3 and 4 were insufficient for final conclusions.

## COMMENTS

### Background

Until recently, the standard of care for chronic hepatitis C (CHC) patients was lengthy dual therapy with pegylated interferon plus ribavirin (peg-IFN/RBV), a treatment with modest success rates, severe adverse events and variation in treatment response between hepatitis C virus (HCV) genotypes. Therefore, efforts to identifying biomarkers that can predict virological responses to treatment have been made. Interferon- $\gamma$  inducible protein-10 (IP-10) is one such promising

marker, with several studies independently showing an association between virological response and baseline IP-10 concentrations for CHC patients infected with HCV genotype 1 and 4. However, the association seems to be lacking for CHC patients, infected with HCV genotype 2 and 3.

### Research frontiers

IP-10 has been shown to be expressed at higher levels in HCV genotype 1 infected CHC patients with moderate to severe fibrosis compared to patients with mild or non fibrosis. Therefore, studies are being made to examine if this correlation is also found in HCV genotype 2 and 3 infected CHC patients. In addition to this, examinations of baseline IP-10 ability to predict fibrosis progression in CHC patients are pending. IP-10 research in relation to CHC is therefore expanding from the possible correlation between virological response to treatment with peg-IFN/RBV at baseline, to also include fibrosis score at baseline and fibrosis progress over time.

### Innovations and breakthroughs

Despite the work done so far to correlate IP-10 levels to treatment response, this is to our knowledge, the first systematic review to address and clarify the differences in IP-10 properties, in relation to the different HCV genotypes and virological response. The authors found indications of correlations between baseline IP-10 levels and SVR in CHC patients infected with HCV genotype 1 and 4, but not in patients infected with HCV genotype 2 or 3. Likewise, the authors found indications of a possible correlation between baseline IP-10 and RVR for HCV genotype 1 infected patients, while no such association were found for HCV genotype 2 or 3 patients, and only a trend was found for HCV genotype 4 infected patients.

### Applications

Despite of the great advantages with the new treatment options with direct acting antivirals (DAA), the cost of DAAs will without doubt substantially delay their introduction as standard treatment in low and middle-income countries by

years to come. In addition, DAA in high-income countries is still reserved for patients with advanced liver disease. Therefore, peg-IFN/RBV treatment still has a role to play in treatment of patients with CHC. Their findings of a possible correlation between baseline IP-10 levels and SVR in CHC patients infected with HCV genotype 1 and 4 but not for genotypes 2 and 3 could be beneficial in a clinical setting. Genotype 2 and 3 patients would readily be treated, as these patients generally have a favorable outcome to peg-IFN/RBV compared to genotype 1 and 4 infected individuals. In such patients, IP-10 levels might help to show which patients would have the best prognosis for a positive outcome to treatment.

### Terminology

Interferon- $\gamma$  inducible protein-10, more commonly denoted IP-10 or CXCL10, is a non-ELR-CXC chemokine, binding to the CXC-receptor-3. It functions as a chemotactic, attracting T lymphocytes and NK cells to the site of inflammation. Within the liver, IP-10 mRNA is produced by hepatocytes in inflammatory areas, and both intrahepatic IP-10 mRNA - and plasma levels of IP-10 are elevated in individuals with CHC, indicating that intrahepatic IP-10 is the source of plasma IP-10. The hypothesis therefore is that IP-10 can function as proxy for the level of liver inflammation, which in turns lead to fibrosis formation.

### Peer-review

This is a well written and comprehensive systemic review to explore the association between baseline levels of interferon- $\gamma$ -inducible protein-10 and virological response to treatment with pegylated interferon and ribavirin among patients chronically infected with hepatitis C virus, genotype 1-4.

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