ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2016 August 8; 8(22): 915-956

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

A peer-reviewed, online, open-access journal of hepatology

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World Journal of Hepatology is now indexed in PubMed, PubMed Central, and Scopus.

FLYLEAF **I-IV** Editorial Board

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

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

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World J Hepatol 2016 August 8; 8(22): 915-923
ISSN 1948-5182 (online)

REVIEW

Multimodal brain monitoring in fulminant hepatic failure

Fernando Mendes Paschoal Jr, Ricardo Carvalho Nogueira, Karla De Almeida Lins Ronconi, Marcelo de Lima Oliveira, Manoel Jacobsen Teixeira, Edson Bor-Seng-Shu

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Conflict-of-interest statement: The authors declare there is no conflict of interest regarding the publication of this paper.

Manuscript source: Unsolicited manuscript

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Received: February 20, 2016
Peer-review started: February 22, 2016
First decision: March 30, 2016
Revised: April 22, 2016
Accepted: June 14, 2016
Article in press: June 16, 2016
Published online: August 8, 2016

Abstract

Acute liver failure, also known as fulminant hepatic failure (FHF), embraces a spectrum of clinical entities characterized by acute liver injury, severe hepatocellular dysfunction, and hepatic encephalopathy. Cerebral edema and intracranial hypertension are common causes of mortality in patients with FHF. The management of patients who present acute liver failure starts with determining the cause and an initial evaluation of prognosis. Regardless of whether or not patients are listed for liver transplantation, they should still be monitored for recovery, death, or transplantation. In the past, neuromonitoring was restricted to serial clinical neurologic examination and, in some cases, intracranial pressure monitoring. Over the years, this monitoring has proven insufficient, as brain abnormalities were detected at late and irreversible stages. The need for real-time monitoring of brain functions to favor prompt treatment and avert irreversible brain injuries led to the concepts of multimodal monitoring and neurophysiological decision support. New monitoring techniques, such as brain tissue oxygen tension, continuous electroencephalogram, transcranial Doppler, and cerebral microdialysis, have been developed. These techniques enable early diagnosis of brain hemodynamic, electrical, and biochemical changes, allow brain anatomical and physiological monitoring-guided therapy, and have improved patient survival rates. The purpose of this review is to discuss the multimodality methods available for monitoring patients with FHF in the neurocritical care setting.

Key words: Fulminant hepatic failure; Cerebral edema; Multimodality methods; Intracranial hypertension; Liver transplantation

Core tip: Cerebral edema and intracranial hypertension are common causes of mortality in patients with fulminant hepatic failure (FHF). The management of

patients who present acute liver failure starts with determining the cause and an initial evaluation of prognosis. Regardless of whether or not patients are listed for liver transplantation, they should still be monitored for recovery, death, or transplantation. The purpose of this review is to discuss the multimodality methods available for monitoring patients with FHF in the neurocritical care setting.

Paschoal Jr FM, Nogueira RC, Ronconi KDAL, de Lima Oliveira M, Teixeira MJ, Bor-Seng-Shu E. Multimodal brain monitoring in fulminant hepatic failure. *World J Hepatol* 2016; 8(22): 915-923 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i22/915.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i22.915

INTRODUCTION

Fulminant hepatic failure (FHF) is a complex clinical condition that is only partially understood and remains a major clinical challenge[1,2]. Hepatic encephalopathy (HE) associated with intracranial hypertension is a severe neurologic complication and the leading cause of death among patients with FHF[3].

The management of patients who present acute liver failure starts with determining the cause and an initial evaluation of prognosis[3]. In the past, neuromonitoring was restricted to serial clinical neurologic examination and, in some cases, intracranial pressure (ICP) monitoring. Over the years, this monitoring has proven insufficient because brain abnormalities were detected at late and irreversible stages[4]. The need for real-time monitoring of brain functions to favor prompt treatment and avert irreversible brain injuries led to the concepts of multimodal monitoring and neurophysiological decision support. New monitoring techniques, such as brain tissue oxygen tension, continuous electroencephalogram (cEEG), transcranial Doppler (TCD), and cerebral microdialysis (MD), have been developed. These techniques enable early diagnosis of brain hemodynamic, electrical, and biochemical changes, allow brain anatomical and physiological monitoring-guided therapy, and have improved patient survival rates[4,5].

The purpose of this review is to discuss the multimodality methods available for the monitoring of patients in the neurocritical care setting.

MONITORING INTRACRANIAL PHYSIOLOGICAL VARIABLES

Invasive ICP monitoring

ICP monitoring is indicated for brain swelling due to FHF and involves the use of catheters, which can be implanted into epidural, subdural-subarachnoid, or intraventricular spaces through a burr hole. The latest catheters allow real-time and continuous ICP data acquisition. The objective of ICP monitoring is to maintain ICP below 20 mmHg and have adequate cerebral perfusion pressure (CPP) = arterial blood pressure (ABP) - ICP. The ideal management of CPP should take cerebral metabolic and hemodynamic data into account in order to avoid excessive cerebral hyperemia, as well as uncoupling of cerebral blood flow and metabolism[6,7]. Despite a lack of evidence that treatment of elevated ICP can improve survival rates of patients with FHF, it is generally accepted that Grade 3-4 HE patients, especially those awaiting liver transplantation, should undergo ICP monitoring[6,7]. ICP higher than 40 mmHg and prolonged low CPP < 50 mmHg are strongly associated with poor neurological recovery in FHF patients who are traditionally bad candidates for liver transplantation[8].

Continuous perioperative measurement of ICP has been associated with a FHF survival rate of 54%-74%. Invasive ICP monitoring is especially risky in FHF patients with coagulopathy, for whom the incidence of intracranial bleeding due to catheter placement ranges from 5% to 22%[9,10]. Recombinant factor Ⅶ (rFⅦa) can be an alternative method for preventing intracranial hemorrhage associated with ICP placement. Acidosis can lead to low effectiveness of rFⅦa, therefore requiring its correction before use[5,11].

Cerebral edema and intracranial hipertensina (IH) are complications in approximately 75% to 80% of patients with FHF and grade Ⅲ or Ⅳ encephalopathy, which remains a leading cause of death. The physiopathology of these two complications still remains poorly understood, but may be related to vasogenic edema, cytotoxic edema, or cerebral hyperemia[8,12]. Vasogenic edema is the consequence of a breakdown of the blood brain barrier, while cytotoxic edema is related to the glutamine osmotic effects in the astrocytes that results in cerebral edema. On the other hand, hyperemia can be caused by failure of the sodium-potassium adenosine triphosphatase pump[8] and/or the accumulation of certain substances such as cytokines, products of the necrotic liver, or glutamine, which lead to vasodilatation of the microcirculation. Brain edema and hyperemia can lead to IH, with decreased cerebral perfusion pressure, cerebral ischemia, and herniation[8,12].

NON-INVASIVE ICP MONITORING

Optic nerve ultrasound

The optic nerve has a sheath which is continuous with the dura mater of the brain. The subarachnoid space of the optic nerve sheath communicates with the brain and the subarachnoid space, meaning that optic nerve sheath diameter (ONSD) can be influenced by changes in the pressure of cerebrospinal fluid in the cranial cavity. ONSD has been increasingly used to monitor ICP in many different clinical settings, and is measured by an ultrasound probe placed on the eyes[13,14]. A linear correlation between ICP and ONSD measurements has been reported, and a significant reduction in ONSD occurs after draining the cerebrospinal fluid. The cut-off value of ONSD suggested to indicate ICP greater than 20 mmHg was 5.2 mm[15]. However, scant information

is available regarding the use of ONSD in patients undergoing liver transplantation. Kim *et al*[13] concluded that patients undergoing liver transplantation are susceptible to severe bleeding disorders and elevated ICP during the procedure, reporting two cases of patients who underwent liver transplantation at different stages. In one case with severe hepatic encephalopathy, ONSD was measured before transplantation, yielding a value of 6.4 mm. Meanwhile, measurements made in the other case after reperfusion of the graft yielded a value of 5.7 mm. These data demonstrate that measurement of ONSD is a useful method for evaluating patients with FHF undergoing liver transplantation.

Transcranial color-coded duplex ultrasonography

Midline shift (MLS) is a known prognostic factor for unfavorable outcome after the development of intracranial hemorrhage in patients with severe brain injury[16]. In clinical practice, the repetition of computed tomography is mostly used to monitor MLS. However, the examination leads to increased radiation exposure and requires the transport of critically-ill patients, which are associated with increased morbidity and mortality in these patients[17]. Transcranial color-coded duplex sonography (TCCD) represents a non-invasive bedside alternative to radiological methods. TCCD measurements are valid for the diagnosis and monitoring of various neurological diseases, including IH[18,19]. Furthermore, monitoring MLS *via* TCCD safely predicts early mortality and prognosis of conservative clinical treatment of hemispheric ischemic stroke[18]. Unlike ischemic stroke, intracranial hemorrhage MLS is caused by both the volume of hematoma and the formation of edema, which can make outcomes difficult to predict[20]. Patients with FHF who develop brain swelling and IH can benefit from this method, although it has not yet been described in the literature.

Brain computer tomography and magnetic resonance images

Brain images have traditionally been used to diagnose strokes, but are also useful in ruling out other causes of changes in mental status[21]. Furthermore, a non-contrast computer tomography (CT) scan of the brain can disclose brain swelling, compressed basal cisterns, hydrocephalus, mass effect, and midline shift, which can be indicative of increased ICP. However, the absence of these findings does not exclude the presence of brain swelling[22], which may be better visualized through magnetic resonance imaging (MRI) of the brain[21]. The imbalance in the homeostasis of cell volume consequent to elevation of cerebral ammonia concentration can be disclosed in MRI by the proton spectroscopy findings of decreased myo-inositol and choline signals[23]. Moreover, magnetization transfer ratio measurements of fast fluid-attenuated inversion recovery sequences and diffusion-weighted images can be used to detect abnormalities in white matter, thereby reflecting elevated ammonia concentrations in the central nervous system that facilitate the diagnosis of brain swelling in patients with FHF[23,24].

Cerebral blood flow monitoring

Cerebral blood flow (CBF) can generally be maintained in the presence of varying CPP. However, this relationship is not linear in severe brain injury due to impaired cerebral autoregulation[25,26]. In such cases, assessment based on CPP alone can be inaccurate, as measurements assume that cerebral vascular resistance remains constant, which is not the case in serious brain injuries[24]. Therefore, direct monitoring of CBF can help in the management of patients with severe brain injury.

The gold standard method for CBF study is the Kety-Schmidt technique. This technique assesses the area between the curves of arterial and venous washout of a freely diffusible indicator such as nitrous oxide and calculates global CBF from the absorption rate of the indicator in brain tissue[26,27]. Radioisotopes such as krypton-85 and xenon-133 can also be used for CBF study in combination with compact scintillation detectors and microprocessors, as well as the indocyanine green dye dilution technique, which involves non-invasive near-infrared spectroscopy (NIRS) and the thermodilution method[28,29]. The principle of spectroscopy is based on the application of light in the near-infrared wavelength to assess, quantitatively and qualitatively, the molecular components related to tissue oxygenation. Based on deoxyhemoglobin and oxyhemoglobin concentrations in the tissue, NIRS is a non-invasive method which allows for the gathering of information for calculating tissue oxygenation[30]. Other techniques that evaluate CBF include: CT with xenon, CT by single photon emission tomography (SPECT), positron emission oxygen-15 tomography (PET), perfusion CT, and perfusion imaging by MRI[31,32]. SPECT studies the spatial distribution of the radioactive isomer technetium-99 (Tc-99) and its local metabolism in the brain. Since these radionuclides are unusual in the human body, Tc-99 metabolism or its connection may not be identical to the native molecule, and therefore difficulties in the interpretation of results may occur[33]. SPECT provides only a relative measurement of radioactivity and allows for the comparison of physiological parameters such as blood flow in different areas of the brain[34,35].

Cerebrovascular resistance, according to Davies *et al*[10], tends to decrease during the course of FHF and can be influenced by the use of pharmacological agents (*i.e.*, sedatives and inotropes). Previous studies have shown increased blood flow in the basal ganglia of patients with minimal HE, which suggests an increased supply of ammonia to these areas, with resultant astrocyte dysfunction and cognitive impairment.

Nielsen *et al*[36] evaluated CBF of FHF patients *via* the NIRS method. This method detects changes in cerebral perfusion pressure and constitutes a non-invasive method that, in conjunction with transcranial Doppler, may detect brain hyperperfusion before the manifestation of increased intracranial pressure.

TCD is a non-invasive method that measures cerebral blood flow velocity (CBFV). Access of ultrasound waves to the intracranial environment is possible through the "ultrasonic windows", namely the temporal, orbital, suboccipital, and submandibular windows. Thus, placing one transducer against these ultrasonic windows allows the obtention of the spectra of blood flow velocity *vs* time for some cerebral arteries[37].

The previously mentioned arteries can be assessed every 1 mm to 2 mm along their lengths given the pulsed emission ultrasonic waves, which allow controlled modulation depth of the sampling area[38]. The examiner should acquire the most intense audible signal and best blood flow velocity spectra possible by adjusting the position and transducer angle so that the incidence angle between the emitted ultrasound beam and blood vessel is close to zero[39]; thus, more accurate measurements of blood flow velocity can be made.

TCD has proven a valuable method in studies of cerebral hemodynamics due to its high temporal resolution, non-invasiveness, portability, and ability to measure CBFV in real time. CBFV indirectly represents CBF if the cross-sectional area of the vessel is assumed to remain constant with fluctuations in arterial pressure. There is evidence that, despite variations in ABP, the caliber of the vessel does not change significantly[40,41], thereby validating the method for clinical use.

TCD can provide indirect information on CBF and ICP in patients with FHF[22]. Changes in the shape of the spectral diastolic wave can be an early sign of IH and impaired cerebral perfusion pressure. In addition, the final stages of IH can lead to large attenuation of diastolic blood flow velocity (BFV)[42].

ICP changes can influence cerebral blood circulation, which may be assessed with TCD. Currently, TCD publications are trying to predict ICP curves in a non-invasive manner. The pulsatility index (PI) is defined by the following formula: Systolic velocity - diastolic velocity/mean velocity, and is increased when cerebrovascular resistance is elevated. Increased ICP may lead to PI elevation, especially if there is an impairment of cerebral autoregulation. In this case, when diastolic blood pressure equals ICP, there is cessation of intracranial diastolic flow[43]; a further increase in ICP (oscillating flow) may appear during flow progress in the systole. During diastole, critically high ICP, CVR, and distended intracranial arteries eject the blood in a retrograde direction. When net forward flow is seriously reduced, severe ischemic brain damage or brain death may occur. In critical IH, the intracranial waveform degrades to become a small systolic spike and then disappears altogether[44]. The relationship between TCD-hemodynamic patterns and the different states of ICP reinforces the idea that TCD can be useful for determining the optimal range of arterial blood pressure for adequate cerebral blood flow dynamics in FHF patients[45,46].

Cerebral autoregulation (CA) is impaired in patients with FHF, and CBF has been described as correlating with ICP in FHF[23]. CA is characterized by CBF remaining relatively constant despite variations in CPP. This physiological response acts to protect the brain from the harmful effects (*i.e.*, ischemia or hyperemia) of large oscillations in perfusion pressure. Lassen *et al*[47] use the term "autoregulation" to explain the relatively constant values of blood flow encountered during hypotension induction. However, autoregulation has been confused with other dynamic adjustment processes. Strictly speaking, autoregulation refers only to the brain's vascular response to changes in CPP, and is sometimes referred to specifically as pressure autoregulation. Brain vessels also dilate or contract as a physiological response to cellular metabolic activity, but should not strictly be called autoregulation. The influence of neuronal metabolism on CBF should be referred to as metabolic regulation of the flow-metabolism coupling[47,48].

The methods used to estimate changes in cerebral perfusion are TCD ultrasound and clearance of xenon-133, while CT demonstrates stable CBF. Other techniques reflect tissue perfusion and estimate changes in CBF such as jugular arteriovenous difference in oxygen ($AVDO_2$), electromagnetic flow meters, near-infrared spectroscopy, laser Doppler flowmetry, and venous occlusion plethysmography[49].

With changes in technology, particularly the advent of TCD and high temporal resolution examination, it has become possible to calculate an index for static CA[50], which relates cerebrovascular resistance to blood pressure, according to the following formula[51]: ΔCVR%/ΔCPP% (CVR - cerebrovascular resistance); where it is assumed that CPP = ABP - ICP, with the value of ICP being negligible and thus ABP replacing CPP[50].

However, the nature of the estimates, the need for invasive procedures to change ABP, the inherent risk of exposing the patient to exhaustion of self-regulatory reserves, and the emergence of new dynamic CA study methods has reduced the use of the static method for evaluating CA in clinical studies[51,52].

Abdo *et al*[46] evaluated BFV by TCD in five patients with FHF and compared the results against a control group who had associated critical neurological conditions without FHF. Despite the limitations of the study, the authors concluded that patients with FHF may have a dominant pattern of brain hypoperfusion, with an average velocity below normal values and an increased pulsatility index, possibly due to an increase in ICP. The authors suggested that proper measurement by this method improves brain perfusion and prevents hypoxia in these patients. Another study that used TCD demonstrated that CA of CBF was re-established after the onset of HE improvement in patients with FHF[53].

NEUROPHYSIOLOGICAL MONITORING

Electroencephalogram

Electroencephalogram (EEG) is a non-invasive method which analyzes spontaneous brain electrical activity and is performed by placing electrodes on the scalp with the aid of a conductive paste which, besides affixing the

electrodes, allows for the proper acquisition of the signals that constitute the brain's electrical activity[54]. Initially, a spontaneous recording of brain electrical activity is made while the patient is awake and conscious. If possible, this activity is also recorded during drowsiness and sleep. Recording during these different states increases the sensitivity of the method in detecting various defects, including patients with severe brain pathologies[21,54].

Continuous video EEG (cEEG) provides long-term monitoring of brain electrical activity in critically-ill patients with altered mental status and in those at risk of secondary ischemia following acute brain injury. The main indications of cEEG are the detection of non-convulsive seizures or status epilepticus in order to investigate causes of impairment of consciousness, and to determine the prognosis of brain injury. EEG changes in hepatic encephalopathy may range from low alpha-rhythm frequency (8 Hz) mixed with bilateral theta activity, which can later develop into theta-delta with deceleration throughout both hemispheres, with or without three-phase curves. With increasing stupor, sleep activity disintegrates. In severe coma, arrhythmic delta activity diminishes, both in frequency and amplitude, and progresses to electrocerebral silence[54].

The presence of subclinical seizure is often poorly recognized in patients with grade Ⅲ and Ⅳ HE, emphasizing the importance of EEG monitoring in these patients. Cerebral ischemia has often been known to precede the onset of seizures in patients with FHF[54]. Seizures are susceptible to cerebral hypoxia and contribute to the development and perpetuation of brain swelling. During FHF, the increase in extracellular brain glutamate concentrations predisposes patients to epileptic activity[21]. Although no definitive recommendations can be made at the time of writing, the morbidity of untreated subclinical crisis should be considered concomitant with the prudent administration of anti-epileptic drugs until additional studies are established.

Bispectral index

The bispectral index (BIS) is a neurophysiological monitoring system that continuously analyzes electroencephalograms to determine the level of consciousness of patients undergoing general anesthesia. The notion of "anesthetic depth" is usually associated with training experiences or memories during surgery, in which anesthesia does not prevent consciousness or even waking-up during general anesthesia. Although EEG is the gold standard used to determine electrical activity in comatose patients, standard EEG monitoring may not be feasible for all patients who require intensive care during pretransplant[55,56].

Studies show that monitoring by BIS, which was developed in order to assist with the clinical evaluation of the degree of hypnosis with anesthesia, is useful for monitoring cases of FHF[55-57]. The BIS monitor uses the EEG signal derived from electrodes placed on the forehead that provide continuous monitoring. While monitoring for BIS has been developed to assess the level of awareness during anesthesia, this method may also be useful to assess the degree of recovery of consciousness alongside improved liver function after liver transplantation. Hwang *et al*[9] showed that the BIS may be useful for evaluating state of consciousness during the peritransplant and intensive care periods for FHF patients who develop HE.

BRAIN OXYGENATION MONITORING

Brain oxygenation monitoring after brain injury can lead to the detection or prevention of secondary ischemic episodes. The four methods used to measure cerebral oxygenation are: Jugular bulb oximetry, measurement of direct tissue oxygen tension, NIRS, and PET oxygen-15[32].

Jugular bulb oximetry

Catheterization of the jugular bulb and obtention of venous blood samples allow for an estimate of blood flow and cerebral metabolism. Monitoring blood oxygen saturation in veins that drain the brain provides an estimate of overall metabolic demand compared to oxygenation deprivation[32]. The parameter can be used as a measure of jugular venous oxygen content, as well as arteriovenous oxygen difference[57].

Monitoring the oxygen saturation of the jugular vein provides an estimate of overall metabolic demand compared to oxygenation. The parameter used can be jugular venous oxygen content or arteriovenous oxygen difference ($AVDO_2$ = $CMRO_2$/CBF; $CMRO_2$ = cerebral metabolic rate of oxygen consumption). The extent of arteriovenous oxygen difference indicates the amount of oxygen extracted by the brain. Under normal conditions, this value is a 2.8 μmol/mL (range 2.2-3.3 μmol/mL) or 6.3% volume (volume varies from 5%-7.5% oxygen) change in $CMRO_2$ or cerebral blood flow extraction[24]. A reduction in cerebral blood flow, without changes in the energetic demands, increases oxygen extraction in the cerebral tissue. Thus, the jugular vein oxygen decreases and the difference between arterial and jugular venous oxygen increases. On the other hand, a disproportionate increase in cerebral blood flow or a decrease in energy consumption decreases $AVDO_2$[57]. The limitation of the method is the non-detection of oxygen consumption changes in small brain regions[58].

Brain tissue oxygen

Quantitation of tissue oxygen pressure ($PtiO_2$) in the brain reflects the partial pressure of oxygen at the end of the capillary circuit. In ischemic situations, a fall in $PtiO_2$ is accompanied by a decrease in pH (lactic acidosis) and an increase in tissue carbon dioxide pressure, with a lack of metabolic exchange between cells and the capillary circuit. Low values indicate $PtiO_2$ tissue hypoxia and help guide therapy[59]. The patient should exhibit adequate hemoglobin content, balanced hemoglobin affinity for oxygen, and appropriate systemic arterial oxygen

content. Commonly-used sensors determine mean tissue oxygen pressure in an area of 17 mm^3. The catheter is introduced into the cerebral white matter to a depth of 25 mm below the dura mater. The cathode comprises a gold and silver anode immersed in an electrolyte solution[58,59]. The oxygen molecules diffuse into the catheter, producing a reversible reaction at the cathode in which oxygen combines with water and forms ions (OH^-). These reactions generate an electric current detected by a voltmeter, with the electrical signal subsequently digitized and transformed into a numeric value on the monitor display panel. Positioning the catheter in a circulatory border territory between the anterior and middle cerebral arteries allows for the early detection of changes in this area, which is more sensitive to flow variations[59].

Based on previous studies, the cutoff point value for cerebral ischemia monitoring with $PtiO_2$ appears to lie within the 8 to 25 mmHg range. $PtiO_2$ monitoring can provide real-time information on the regulation of brain blood flow and has been shown to have a clear impact on the management of patients with severe brain injuries, such as traumatic brain injury and hemispheric infarcts[60]. Patients with FHF who develop brain swelling and IH can benefit from this method.

Near infrared spectroscopy

As described above, this is a non-invasive technique for measuring regional cerebral oxygen saturation, as well as analyzing the difference in oxygenated hemoglobin and deoxygenated absorption spectra[61].

Studies in patients with FHF demonstrate that the monitoring of brain oxygenation provides valuable data for the clinical management of this population[62]. Oxygen and cerebral glucose consumption have been observed before signs of brain swelling, suggesting that cerebral oxygen metabolism is intact at this stage[62]. In another study, $CMRO_2$ was found to be decreased in all patients with FHF[61]. There was also evidence of cerebral ischemia, as indicated by increased $AVDO_2$. In the study, it was concluded that hyperemia alone was not related to the outcome, despite having occurred more frequently during elevated ICP. All patients with malignant intracranial hypertension previously had hyperemia[62,63]. Nielsen *et al*[36] reported that both pressure and arterial oxygen saturation were maintained during infusion with norepinephrine. Additionally, hemoglobin concentration in blood flow was not compromised. Cerebral arterial oxygenation is capable of detecting brain perfusion changes during norepinephrine infusion in patients with acute liver failure. This suggests that NIRS can be valuable in monitoring critical changes in the cerebral oxygenation and blood volume of these patients.

METABOLIC MONITORING

Brain metabolism can be evaluated by PET and MR spectroscopy, jugular oxygen saturation, monitoring of CBF, and MD. PET scans provide an estimate of the topographic view of glucose metabolism, while MRI spectroscopy qualitatively shows the lactate content of a particular brain structure[58].

MD techniques provide information on tissue metabolism, including the availability of substrates such as glucose and the production of local metabolites. This technique is based on the exchange of solutes through a semipermeable membrane that simulates the operation of a capillary and has the basic objective of monitoring the tissue availability of the different metabolites released by cells[64].

The tip of the catheter contains a semipermeable membrane that separates a solution of known composition from the extracellular fluid space. MD fluid is then analyzed to quantify metabolites. This technique allows for the study of the release of excitatory neurotransmitters such as glutamate and aspartate, as well as other neuromodulators, thereby indirectly analyzing the ischemic excitotoxicity phenomenon. It also allows for the analysis of the concentration of tissue degradation products such as glycerol. The catheter's semipermeable membrane used to study the cited substances only allows for the passage of ions of molecules with a molecular weight of less than 20000 daltons[64,65].

Glucose is most frequently determined as the cellular energy substrate. In conditions where there is a decrease in both cerebral tissue glucose and $PtiO_2$, a reduction of capillary blood flow may be inferred[63,64]. Lactate studies can indicate the intensity of anaerobic metabolism, while glycerol studies can evaluate tissue damage since glycerol is one of the structural components in the tissue lipid layer of cell membranes[66]. Glutamate is an important excitatory neurotransmitter in the mammalian nervous system, with aspartate following in importance. These amino acids are released in the synaptic cleft after neuronal depolarization. This depolarization can be associated with tissue ischemia in states of massive release[67]. In situations of excitotoxicity, massive release of glutamate into the synaptic cleft can be seen. Thus, large inputs of calcium into the cell are observed; as a consequence, there is production of oxygen free radicals in cell membranes and the release of more fatty acids and glycerol[66]. It is recommended that the MD catheter be placed in so-called "penumbra" areas adjacent to focal lesions in order to allow monitoring of potentially recoverable brain regions[68,69]. MD is currently considered one of the most important *in vitro* sampling methods in physiology and pharmacology. Applied in neurointensive care, it is the only tool that allows continuous measurement of chemicals in the brain extracellular space and elucidation of non-ischemic forms of cerebral hypoxia[67].

The tissue volume evaluated by the MD catheter is a cylinder equivalent to the length of the dialysis membrane (10 mm) with a diameter of a few millimeters (0.6 mm). MD pumps perfuse the catheter with an artificial cerebrospinal fluid, which equilibrates with the interstice around the catheter. Balance occurs by diffusion through the dialysis membrane. Using a dialysis membrane with a 10 mm 0.3 perfusion flow L/min, the

concentration of dialyzed glucose, lactate, pyruvate, and glutamate is approximately 70% of the concentration of interstitial fluid. Samples are continuously collected and analyzed at the bedside every hour, or as needed, with the results being analyzed on trend curves[70]. When monitoring biochemical markers it is established that: Lactate/pyruvate ratio is the best marker of cerebral cortex state and early biomarkers in secondary ischemic injury glycerol and glutamate are additional markers of tissue hypoxia[70].

Brain swelling predominantly involving glial cells is often reported as a serious complication of FHF. The swelling of astrocytes may result in elevated ICP and cerebral herniation syndrome in patients with FHF[70]. Tofteng *et al*[71] found brain chemical changes in the MD of a young man with severe acute liver failure and brain swelling in the liver transplant, and found that both extracellular glutamate and glycerol levels were elevated before liver transplant, and tending to decrease after grafting. These results indicate changes in glutamate neurotransmission, arachidonic acid metabolism, lactate, and flow through the blood-brain barrier in patients with FHF.

In another study, Tofteng *et al*[72] investigated whether an increased concentration of glutamate and brain extracellular lactate preceded episodes of elevated ICP in patients with FHF (7 women and 3 men; age range 20-55 years) by inserting MD and ICP catheters into the brain. A total of 352 MD samples were collected for a median of 3 d, allowing for the analysis of approximately 1760 dialyzed samples at the bedside. It has been shown that patients with FHF feature elevated concentrations of extracellular glutamate and cerebral lactate. However, high levels of glutamate are not correlated with increased intracranial pressure, while high levels of lactate precede episodes of elevated ICP. Hyperglycolysis to lactate accumulation is involved in brain microvascular vasodilation and ICP increase in patients with FHF. Therefore, it can be concluded that brain MD at the bedside can be a valuable tool for monitoring these patients.

CONCLUSION

Patients with FHF are usually submitted for brain monitoring after undergoing liver transplantation or when they have a neurological decline. Brain monitoring in this critical phase is essential for maintaining hemodynamic, metabolic, and electrical parameters at acceptable levels. There are a myriad of methods for real time measuring of the aforementioned parameters, with each method having a particular contribution in the detection of "a brain at risk". The key point for proper patient management in order to prevent neurological complications is to combine the different methods in a multimodal approach.

The multimodal technique of extended neuromonitoring offers an advanced option for further development and investigations in animal models of FHF. Furthermore, identification of patients at risk for neurologic complications before and after liver transplant may allow for prompt neuroprotective interventions, including the optimal control of blood pressure.

REFERENCES

1 **Kjaergard LL**, Liu J, Als-Nielsen B, Gluud C. Artificial and bioartificial support systems for acute and acute-on-chronic liver failure: a systematic review. *JAMA* 2003; **289**: 217-222 [PMID: 12517233 DOI: 10.1001/jama.289.2.217]

2 **O'Grady J**. Modern management of acute liver failure. *Clin Liver Dis* 2007; **11**: 291-303 [PMID: 17606208 DOI: 10.1016/j.cld.2007.04.011]

3 **Larsen FS**. Cerebral circulation in liver failure: Ohm's law in force. *Semin Liver Dis* 1996; **16**: 281-292 [PMID: 8989814 DOI: 10.1055/s-2007-1007241]

4 **Vespa PM**, Nenov V, Nuwer MR. Continuous EEG monitoring in the intensive care unit: early findings and clinical efficacy. *J Clin Neurophysiol* 1999; **16**: 1-13 [PMID: 10082088 DOI: 10.1097/00004691-199901000-00001]

5 **Shami VM**, Caldwell SH, Hespenheide EE, Arseneau KO, Bickston SJ, Macik BG. Recombinant activated factor VII for coagulopathy in fulminant hepatic failure compared with conventional therapy. *Liver Transpl* 2003; **9**: 138-143 [PMID: 12548507 DOI: 10.1053/jlts.2003.50017]

6 **Hanid MA**, Davies M, Mellon PJ, Silk DB, Strunin L, McCabe JJ, Williams R. Clinical monitoring of intracranial pressure in fulminant hepatic failure. *Gut* 1980; **21**: 866-869 [PMID: 6777264 DOI: 10.1136/gut.21.10.866]

7 **Frühauf NR**, Radunz S, Grabellus F, Laube T, Uerschels AK, Kaiser GM. Neuromonitoring in a porcine model of acute hepatic failure. *Lab Anim* 2011; **45**: 174-178 [PMID: 21508115 DOI: 10.1258/la.2011.010083]

8 **Donovan JP**, Shaw BW, Langnas AN, Sorrell MF. Brain water and acute liver failure: the emerging role of intracranial pressure monitoring. *Hepatology* 1992; **16**: 267-268 [PMID: 1618475 DOI: 10.1002/hep.1840160138]

9 **Hwang S**, Lee SG, Park JI, Song GW, Ryu JH, Jung DH, Hwang GS, Jeong SM, Song JG, Hong SK, Lim YS, Kim KM. Continuous peritransplant assessment of consciousness using bispectral index monitoring for patients with fulminant hepatic failure undergoing urgent liver transplantation. *Clin Transplant* 2010; **24**: 91-97 [PMID: 19925461 DOI: 10.1111/j.1399-0012.2009.01148.x]

10 **Davies MH**, Mutimer D, Lowes J, Elias E, Neuberger J. Recovery despite impaired cerebral perfusion in fulminant hepatic failure. *Lancet* 1994; **343**: 1329-1330 [PMID: 7910328 DOI: 10.1016/S0140-6736(94)92471-6]

11 **Darlington DN**, Kheirabadi BS, Scherer MR, Martini WZ, Dubick MA. Acidosis and correction of acidosis does not affect rFVIIa function in swine. *Int J Burns Trauma* 2012; **2**: 145-157 [PMID: 23272296]

12 **Bingaman WE**, Frank JI. Malignant cerebral edema and intracranial hypertension. *Neurol Clin* 1995; **13**: 479-509 [PMID: 7476816]

13 **Kim YK**, Seo H, Yu J, Hwang GS. Noninvasive estimation of raised intracranial pressure using ocular ultrasonography in liver transplant recipients with acute liver failure -A report of two cases-. *Korean J Anesthesiol* 2013; **64**: 451-455 [PMID: 23741570 DOI: 10.4097/kjae.2013.64.5.451]

14 **Soldatos T**, Chatzimichail K, Papathanasiou M, Gouliamos A. Optic nerve sonography: a new window for the non-invasive evaluation of intracranial pressure in brain injury. *Emerg Med J* 2009; **26**: 630-634 [PMID: 19700575 DOI: 10.1136/emj.2008.058453]

15 **Moretti R**, Pizzi B, Cassini F, Vivaldi N. Reliability of optic nerve ultrasound for the evaluation of patients with spontaneous intracranial hemorrhage. *Neurocrit Care* 2009; **11**: 406-410 [PMID: 19636971 DOI: 10.1007/s12028-009-9250-8]

16 **Hallevy C**, Ifergane G, Kordysh E, Herishanu Y. Spontaneous supratentorial intracerebral hemorrhage. Criteria for short-term functional outcome prediction. *J Neurol* 2002; **249**: 1704-1709

[PMID: 12529793 DOI: 10.1007/s00415-002-0911-1]
17 **Voigt LP**, Pastores SM, Raoof ND, Thaler HT, Halpern NA. Review of a large clinical series: intrahospital transport of critically ill patients: outcomes, timing, and patterns. *J Intensive Care Med* 2009; **24**: 108-115 [PMID: 19188270 DOI: 10.1177/0885066608329946]
18 **Schlachetzki F**, Herzberg M, Hölscher T, Ertl M, Zimmermann M, Ittner KP, Pels H, Bogdahn U, Boy S. Transcranial ultrasound from diagnosis to early stroke treatment: part 2: prehospital neurosonography in patients with acute stroke: the Regensburg stroke mobile project. *Cerebrovasc Dis* 2012; **33**: 262-271 [PMID: 22261817 DOI: 10.1159/000334667]
19 **Pérez ES**, Delgado-Mederos R, Rubiera M, Delgado P, Ribó M, Maisterra O, Ortega G, Alvarez-Sabin J, Molina CA. Transcranial duplex sonography for monitoring hyperacute intracerebral hemorrhage. *Stroke* 2009; **40**: 987-990 [PMID: 19164795 DOI: 10.1016/s1073-5437(09)79362-3]
20 **Gerriets T**, Stolz E, König S, Babacan S, Fiss I, Jauss M, Kaps M. Sonographic monitoring of midline shift in space-occupying stroke: an early outcome predictor. *Stroke* 2001; **32**: 442-447 [PMID: 11157180 DOI: 10.1161/01.str.32.2.442]
21 **Prakash R**, Mullen KD. Mechanisms, diagnosis and management of hepatic encephalopathy. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 515-525 [PMID: 20703237 DOI: 10.1038/nrgastro.2010.116]
22 **Mohsenin V**. Assessment and management of cerebral edema and intracranial hypertension in acute liver failure. *J Crit Care* 2013; **28**: 783-791 [PMID: 23683564 DOI: 10.1016/j.jcrc.2013.04.002]
23 **Rovira A**, Alonso J, Córdoba J. MR imaging findings in hepatic encephalopathy. *AJNR Am J Neuroradiol* 2008; **29**: 1612-1621 [PMID: 18583413 DOI: 10.1007/978-1-61779-836-8_10]
24 **Robertson CS**, Contant CF, Gokaslan ZL, Narayan RK, Grossman RG. Cerebral blood flow, arteriovenous oxygen difference, and outcome in head injured patients. *J Neurol Neurosurg Psychiatry* 1992; **55**: 594-603 [PMID: 1640238 DOI: 10.1136/jnnp.55.7.594]
25 **Panerai RB**. The critical closing pressure of the cerebral circulation. *Med Eng Phys* 2003; **25**: 621-632 [PMID: 12900178 DOI: 10.1016/s1350-4533(03)00027-4]
26 **KETY SS**, SCHMIDT CF. Measurement of cerebral blood flow and cerebral oxygen consumption in man. *Fed Proc* 1946; **5**: 264 [PMID: 21064908 DOI: 10.1007/978-3-7091-9101-9_2]
27 **Cook DJ**, Anderson RE, Michenfelder JD, Oliver WC, Orszulak TA, Daly RC, Bryce RD. Cerebral blood flow during cardiac operations: comparison of Kety-Schmidt and xenon-133 clearance methods. *Ann Thorac Surg* 1995; **59**: 614-620 [PMID: 7887699 DOI: 10.1016/0003-4975(94)00956-2]
28 **Obrist WD**, Thompson HK, Wang HS, Wilkinson WE. Regional cerebral blood flow estimated by 133-xenon inhalation. *Stroke* 1975; **6**: 245-256 [PMID: 1154462 DOI: 10.1016/0304-3959(85)90215-5]
29 **Keller E**, Nadler A, Alkadhi H, Kollias SS, Yonekawa Y, Niederer P. Noninvasive measurement of regional cerebral blood flow and regional cerebral blood volume by near-infrared spectroscopy and indocyanine green dye dilution. *Neuroimage* 2003; **20**: 828-839 [PMID: 14568455 DOI: 10.1016/S1053-8119(03)00315-X]
30 **Mélot C**, Berré J, Moraine JJ, Kahn RJ. Estimation of cerebral blood flow at bedside by continuous jugular thermodilution. *J Cereb Blood Flow Metab* 1996; **16**: 1263-1270 [PMID: 8898700 DOI: 10.1097/00004647-199611000-00022]
31 **Yonas H**, Jungreis C. Xenon CT cerebral blood flow: past, present, and future. *AJNR Am J Neuroradiol* 1995; **16**: 219-220 [PMID: 7900599 DOI: 10.5005/jp/books/11824_25]
32 **Latchaw RE**. Cerebral perfusion imaging in acute stroke. *J Vasc Interv Radiol* 2004; **15**: S29-S46 [PMID: 15101514 DOI: 10.1097/01.RVI.0000112976.88422.86]
33 **Lammertsma AA**. PET/SPECT: functional imaging beyond flow. *Vision Res* 2001; **41**: 1277-1281 [PMID: 11322972 DOI: 10.1016/s0042-6989(00)00262-5]
34 **O'Carroll RE**, Hayes PC, Ebmeier KP, Dougall N, Murray C, Best JJ, Bouchier IA, Goodwin GM. Regional cerebral blood flow and cognitive function in patients with chronic liver disease. *Lancet* 1991; **337**: 1250-1253 [PMID: 1674063 DOI: 10.1016/0140-6736(91)92920-w]
35 **Catafau AM**, Kulisevsky J, Bernà L, Pujol J, Martin JC, Otermin P, Balanzó J, Carrió I. Relationship between cerebral perfusion in frontal-limbic-basal ganglia circuits and neuropsychologic impairment in patients with subclinical hepatic encephalopathy. *J Nucl Med* 2000; **41**: 405-410 [PMID: 10716310 DOI: 10.1016/s0002-9270(01)04132-6]
36 **Nielsen HB**, Tofteng F, Wang LP, Larsen FS. Cerebral oxygenation determined by near-infrared spectrophotometry in patients with fulminant hepatic failure. *J Hepatol* 2003; **38**: 188-192 [PMID: 12547407 DOI: 10.1016/s0168-8278(02)00377-x]
37 **Ringelstein E**. A practical guide to transcranial Doppler sonography, 1989
38 **McCartney JT**, Gomez CR. Handbook of transcranial Doppler. New York: Springer-Verlag, 1997
39 **Torbey MT**, Hauser TK, Bhardwaj A, Williams MA, Ulatowski JA, Mirski MA, Razumovsky AY. Effect of age on cerebral blood flow velocity and incidence of vasospasm after aneurysmal subarachnoid hemorrhage. *Stroke* 2001; **32**: 2005-2011 [PMID: 11546889 DOI: 10.1161/hs0901.094622]
40 **Newell DW**, Aaslid R, Lam A, Mayberg TS, Winn HR. Comparison of flow and velocity during dynamic autoregulation testing in humans. *Stroke* 1994; **25**: 793-797 [PMID: 7909175 DOI: 10.1161/01.str.25.4.793]
41 **Serrador JM**, Picot PA, Rutt BK, Shoemaker JK, Bondar RL. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 2000; **31**: 1672-1678 [PMID: 10884472 DOI: 10.1161/01.str.31.7.1672]
42 **Kudo M**. Cerebral vascular resistance in hepatic insufficiency. *J Gastroenterol Hepatol* 2001; **16**: 845-847 [PMID: 11555094 DOI: 10.1046/j.1440-1746.2001.02552.x]
43 **Kawakami M**, Koda M, Murawaki Y. Cerebral pulsatility index by transcranial Doppler sonography predicts the prognosis of patients with fulminant hepatic failure. *Clin Imaging* 2010; **34**: 327-331 [PMID: 20813293 DOI: 10.1016/j.clinimag.2009.09.006]
44 **Paschoal FM**, Bor-Seng-Shu E, Teixeira MJ. Transcranial Doppler ultrasonography with jugular vein compression can detect impairment of intracranial compliance. *Clin Neurol Neurosurg* 2013; **115**: 1196-1198 [PMID: 23128012 DOI: 10.1016/j.clineuro.2012.09.028]
45 **Bor-Seng-Shu E**, Teixeira MJ, Hirsch R, Andrade AF, Marino R Jr. Transcranial Doppler sonography in two patients who underwent decompressive craniectomy for traumatic brain swelling: report of two cases. *Arq Neuropsiquiatr* 2004; **62**: 715-721 [PMID: 15334237 DOI: 10.1590/S0004-282X2004000400028]
46 **Abdo A**, López O, Fernández A, Santos J, Castillo J, Castellanos R, González L, Gómez F, Limonta D. Transcranial Doppler sonography in fulminant hepatic failure. *Transplant Proc* 2003; **35**: 1859-1860 [PMID: 12962825 DOI: 10.1016/s0041-1345(03)00592-x]
47 **Lassen NA**. Cerebral blood flow and oxygen consumption in man. *Physiol Rev* 1959; **39**: 183-238 [PMID: 13645234]
48 **MacKenzie ET**, Strandgaard S, Graham DI, Jones JV, Harper AM, Farrar JK. Effects of acutely induced hypertension in cats on pial arteriolar caliber, local cerebral blood flow, and the blood-brain barrier. *Circ Res* 1976; **39**: 33-41 [PMID: 1277403 DOI: 10.1161/01.res.39.1.33]
49 **Rangel-Castilla L**, Gasco J, Nauta HJ, Okonkwo DO, Robertson CS. Cerebral pressure autoregulation in traumatic brain injury. *Neurosurg Focus* 2008; **25**: E7 [PMID: 18828705 DOI: 10.3171/foc.2008.25.10.e7]
50 **Panerai RB**. Cerebral autoregulation: from models to clinical applications. *Cardiovasc Eng* 2008; **8**: 42-59 [PMID: 18041584 DOI: 10.1007/s10558-007-9044-6]
51 **Tiecks FP**, Lam AM, Aaslid R, Newell DW. Comparison of static and dynamic cerebral autoregulation measurements. *Stroke* 1995; **26**: 1014-1019 [PMID: 7762016 DOI: 10.1161/01.str.26.6.1014]
52 **Aaslid R**. Cerebral autoregulation and vasomotor reactivity. *Front Neurol Neurosci* 2006; **21**: 216-228 [PMID: 17290140 DOI: 10.1159/000092434]

53 **Strauss G**, Hansen BA, Kirkegaard P, Rasmussen A, Hjortrup A, Larsen FS. Liver function, cerebral blood flow autoregulation, and hepatic encephalopathy in fulminant hepatic failure. *Hepatology* 1997; **25**: 837-839 [PMID: 9096585 DOI: 10.1002/hep.510250409]

54 **Ellis AJ**, Wendon JA, Williams R. Subclinical seizure activity and prophylactic phenytoin infusion in acute liver failure: a controlled clinical trial. *Hepatology* 2000; **32**: 536-541 [PMID: 10960446 DOI: 10.1053/jhep.2000.9775]

55 **Vivien B**, Paqueron X, Le Cosquer P, Langeron O, Coriat P, Riou B. Detection of brain death onset using the bispectral index in severely comatose patients. *Intensive Care Med* 2002; **28**: 419-425 [PMID: 11967595 DOI: 10.1007/s00134-002-1219-4]

56 **Dahaba AA**, Worm HC, Zhu SM, Bao FP, Salah A, Zakaria S, Bornemann H, Stadlbauer V, Rehak PH, Metzler H, Stauber RE. Sensitivity and specificity of bispectral index for classification of overt hepatic encephalopathy: a multicentre, observer blinded, validation study. *Gut* 2008; **57**: 77-83 [PMID: 17698861 DOI: 10.1136/gut.2007.129130]

57 **Lewis SB**, Myburgh JA, Reilly PL. Detection of cerebral venous desaturation by continuous jugular bulb oximetry following acute neurotrauma. *Anaesth Intensive Care* 1995; **23**: 307-314 [PMID: 7573917]

58 **Feldman Z**, Robertson CS. Monitoring of cerebral hemodynamics with jugular bulb catheters. *Crit Care Clin* 1997; **13**: 51-77 [PMID: 9012576 DOI: 10.1016/s0749-0704(05)70296-7]

59 **Sarrafzadeh AS**, Kiening KL, Unterberg AW. Neuromonitoring: brain oxygenation and microdialysis. *Curr Neurol Neurosci Rep* 2003; **3**: 517-523 [PMID: 14565908 DOI: 10.1007/s11910-003-0057-2]

60 **Tolias CM**, Reinert M, Seiler R, Gilman C, Scharf A, Bullock MR. Normobaric hyperoxia--induced improvement in cerebral metabolism and reduction in intracranial pressure in patients with severe head injury: a prospective historical cohort-matched study. *J Neurosurg* 2004; **101**: 435-444 [PMID: 15352601 DOI: 10.3171/jns.2004.101.3.0435]

61 **Jöbsis FF**. Non-invasive, infra-red monitoring of cerebral O2 sufficiency, blood volume, HbO2-Hb shifts and blood flow. *Acta Neurol Scand Suppl* 1977; **64**: 452-453 [PMID: 268870]

62 **Strauss GI**, Møller K, Larsen FS, Kondrup J, Knudsen GM. Cerebral glucose and oxygen metabolism in patients with fulminant hepatic failure. *Liver Transpl* 2003; **9**: 1244-1252 [PMID: 14625823 DOI: 10.1016/j.lts.2003.09.020]

63 **Aggarwal S**, Obrist W, Yonas H, Kramer D, Kang Y, Scott V, Planinsic R. Cerebral hemodynamic and metabolic profiles in fulminant hepatic failure: relationship to outcome. *Liver Transpl* 2005; **11**: 1353-1360 [PMID: 16237715 DOI: 10.1002/lt.20479]

64 **Johnston AJ**, Gupta AK. Advanced monitoring in the neurology intensive care unit: microdialysis. *Curr Opin Crit Care* 2002; **8**: 121-127 [PMID: 12386512 DOI: 10.1097/00075198-200204000-00006]

65 **Ungerstedt U**, Rostami E. Microdialysis in neurointensive care. *Curr Pharm Des* 2004; **10**: 2145-2152 [PMID: 15281890 DOI: 10.2174/1381612043384105]

66 **Rosenbloom AJ**, Sipe DM, Weedn VW. Microdialysis of proteins: performance of the CMA/20 probe. *J Neurosci Methods* 2005; **148**: 147-153 [PMID: 16043227 DOI: 10.1016/j.jneumeth.2005.04.018]

67 **Bor-Seng-Shu E**, Figueiredo EG, Fonoff ET, Fujimoto Y, Panerai RB, Teixeira MJ. Decompressive craniectomy and head injury: brain morphometry, ICP, cerebral hemodynamics, cerebral microvascular reactivity, and neurochemistry. *Neurosurg Rev* 2013; **36**: 361-370 [PMID: 23385739 DOI: 10.1007/s10143-013-0453-2]

68 **de Lima Oliveira M**, Kairalla AC, Fonoff ET, Martinez RC, Teixeira MJ, Bor-Seng-Shu E. Cerebral microdialysis in traumatic brain injury and subarachnoid hemorrhage: state of the art. *Neurocrit Care* 2014; **21**: 152-162 [PMID: 24072457 DOI: 10.1007/s12028-013-9884-4]

69 **de Lima Oliveira M**, Paiva W, Teixeira MJ, Bor-Seng-Shu E. Brain metabolic crisis in traumatic brain injury: what does it mean? *J Neurotrauma* 2014; **31**: 1750-1751 [PMID: 24915159 DOI: 10.1089/neu.2014.3386]

70 **Bellander BM**, Cantais E, Enblad P, Hutchinson P, Nordström CH, Robertson C, Sahuquillo J, Smith M, Stocchetti N, Ungerstedt U, Unterberg A, Olsen NV. Consensus meeting on microdialysis in neurointensive care. *Intensive Care Med* 2004; **30**: 2166-2169 [PMID: 15549254 DOI: 10.1007/s00134-004-2461-8]

71 **Tofteng F**, Jorgensen L, Hansen BA, Ott P, Kondrup J, Larsen FS. Cerebral microdialysis in patients with fulminant hepatic failure. *Hepatology* 2002; **36**: 1333-1340 [PMID: 12447856 DOI: 10.1002/hep.1840360607]

72 **Tofteng F**, Larsen FS. Monitoring extracellular concentrations of lactate, glutamate, and glycerol by in vivo microdialysis in the brain during liver transplantation in acute liver failure. *Liver Transpl* 2002; **8**: 302-305 [PMID: 11910577 DOI: 10.1053/jlts.2002.32283]

P- Reviewer: Hashimoto N **S- Editor**: Gong ZM
L- Editor: Rutherford A **E- Editor**: Li D

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DOI: 10.4254/wjh.v8.i22.924

World J Hepatol 2016 August 8; 8(22): 924-932
ISSN 1948-5182 (online)

MINIREVIEWS

Cholesterol metabolism in cholestatic liver disease and liver transplantation: From molecular mechanisms to clinical implications

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Author contributions: Nemes K wrote the first draft; Åberg F made critical revisions and constructed the figures; Gylling H and Isoniemi H further revised the manuscript; all authors have approved the final version of the manuscript and its submission.

Conflict-of-interest statement: The authors declare no conflict of interests for this article.

Manuscript source: Invited manuscript

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Received: March 31, 2016
Peer-review started: April 6, 2016
First decision: May 17, 2016
Revised: June 7, 2016
Accepted: July 11, 2016
Article in press: July 13, 2016

Published online: August 8, 2016

Abstract

The aim of this review is to enlighten the critical roles that the liver plays in cholesterol metabolism. Liver transplantation can serve as gene therapy or a source of gene transmission in certain conditions that affect cholesterol metabolism, such as low-density-lipoprotein (LDL) receptor gene mutations that are associated with familial hypercholesterolemia. On the other hand, cholestatic liver disease often alters cholesterol metabolism. Cholestasis can lead to formation of lipoprotein X (Lp-X), which is frequently mistaken for LDL on routine clinical tests. In contrast to LDL, Lp-X is non-atherogenic, and failure to differentiate between the two can interfere with cardiovascular risk assessment, potentially leading to prescription of futile lipid-lowering therapy. Statins do not effectively lower Lp-X levels, and cholestasis may lead to accumulation of toxic levels of statins. Moreover, severe cholestasis results in poor micellar formation, which reduces cholesterol absorption, potentially impairing the cholesterol-lowering effect of ezetimibe. Apolipoprotein B-100 measurement can help distinguish between atherogenic and non-atherogenic hypercholesterolemia. Furthermore, routine serum cholesterol measurements alone cannot reflect cholesterol absorption and synthesis. Measurements of serum non-cholesterol sterol biomarkers - such as cholesterol precursor sterols, plant sterols, and cholestanol - may help with the comprehensive assessment of cholesterol metabolism. An adequate cholesterol supply is essential for liver-regenerative capacity. Low preoperative and perioperative serum cholesterol levels seem to predict mortality in liver cirrhosis and after liver transplantation. Thus, accurate lipid profile evaluation is highly important in liver disease and after liver transplantation.

Key words: Cholesterol metabolism; Cholestasis; Liver transplantation; Non-cholesterol sterols; Cholestanol; Donor; Low density lipoprotein receptor mutation; Apolipoprotein B-100; Lipoprotein-X

Core tip: The liver plays key roles in cholesterol metabolism. Cholestatic liver disease leads to alterations of cholesterol metabolism: Cholesterol homeostasis is disturbed and cholesterol synthesis and especially cholesterol absorption are reduced, and lipoprotein X may develop. The latter can interfere with cardiovascular risk assessment. Apolipoprotein B-100 measurement may be useful in such cases. Cholesterol metabolism in cholestasis could be better described using cholesterol precursor sterols, diet-derived plant sterols, and cholestanol (the liver-synthesized derivate of cholesterol). Accurate lipid profile evaluation is particularly important after liver transplantation, when both atherogenic and non-atherogenic hypercholesterolemia may co-exist.

Nemes K, Åberg F, Gylling H, Isoniemi H. Cholesterol metabolism in cholestatic liver disease and liver transplantation: From molecular mechanisms to clinical implications. *World J Hepatol* 2016; 8(22): 924-932 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i22/924.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i22.924

INTRODUCTION

Abundant in the bloodstream and in cell membranes, cholesterol is a critical component of vertebrate cell-membrane structure and function, allowing cells to maintain the permeability and fluidity that is fundamental for all animal life[1,2]. Cholesterol biosynthesis defects, such as Smith-Lemli-Opitz syndrome and lathosterolosis, reveal cholesterol's importance in normal embryonic development. Lathosterolosis is a defect of postsqualene cholesterol biosynthesis that results in deficient transformation of lathosterol into 7-dehydrocholesterol by sterol-C5-desaturase/dehydrogenase. This disorder is characterized by high serum levels of the cholesterol precursor lathosterol, and low cholesterol levels in cells, plasma, and tissues-which causes multiple congenital anomalies, including microcephaly and progressive cholestasis leading to liver failure[3].

The tightly inter-regulated whole-body cholesterol homeostasis includes the following main components: Intestinal cholesterol absorption, hepatic *de-novo* cholesterol synthesis, and cholesterol excretion from the body. Recent advances in the field have further clarified the mechanisms of intestinal transporters and regulatory pathways[4-11]. The brain is home to about 23% of total body cholesterol, which is mainly synthesized *in situ* following blood-brain barrier establishment since dietary cholesterol does not cross this boundary[3]. In contrast to other species, humans exhibit a high cholesterol synthesis rate in the brain only after birth[3].

Under normal circumstances, the liver is the primary site of cholesterol biosynthesis and storage[12]. The liver is also the principal site of cholesterol excretion, converting cholesterol to bile acids and removing free cholesterol as neutral sterols *via* biliary excretion[4,5,13,14]. Since the liver plays a central role in cholesterol metabolism, liver disease can impact cholesterol metabolism, depending on the type of liver injury (parenchymal, cholestatic, or mixed)[15]. In one case of lathosterolosis, liver transplantation (LT) removed the liver disease, reversing the cholesterol metabolism defect and somewhat improving the postnatal neurological symptoms[3]. Conversely, various changes in cholesterol metabolism can be indicators of hepatic and biliary dysfunction.

In the present review, we aimed to summarize current concepts regarding the regulation of cholesterol metabolism in health and in cholestatic liver disease. We discuss difficulties in assessing cholesterol metabolism, and summarize the cholesterol metabolism disturbances seen in cholestatic liver disease and before and after LT. Cholesterol metabolism in the setting of non-alcoholic steatohepatitis was recently reviewed[16], and is not discussed here.

OVERVIEW OF CHOLESTEROL METABOLISM

The following are the main components involved in liver-related cholesterol metabolism and the control of plasma cholesterol levels: (1) intestinal absorption of dietary and biliary cholesterol; (2) bile acid synthesis; (3) endogenous cholesterol synthesis; (4) biliary excretion of cholesterol; (5) low-density lipoprotein (LDL) receptor activity; (6) very-low-density lipoprotein (VLDL) particle synthesis and transport into circulation; and (7) reverse cholesterol transport from peripheral tissues for biliary or non-biliary excretion [trans-intestinal cholesterol efflux (TICE)], the latter of which has been demonstrated only in animal models[4-11,13,17-19].

ABSORPTION OF DIETARY AND BILIARY CHOLESTEROL IN THE SMALL INTESTINE

Intestine-driven pathways are an important component of cholesterol homeostasis, through which cholesterol is both taken up from and pumped back to the intestinal lumen. Intestinal cholesterol absorption is a selective multistep process that is regulated by multiple sterol-transporter genes at the enterocyte level[17,18]. Uptake of free cholesterol from mixed micelles in the intestinal lumen to enterocytes occurs *via* the specific transporter protein Niemann-Pick C1 Like 1 (NPC1L1), which is highly expressed in the brush-border membrane of small-intestinal enterocytes[10,11,18,19] (Figure 1). These enterocytes then selectively efflux about half of the free cholesterol and about 90% of plant sterols back to the

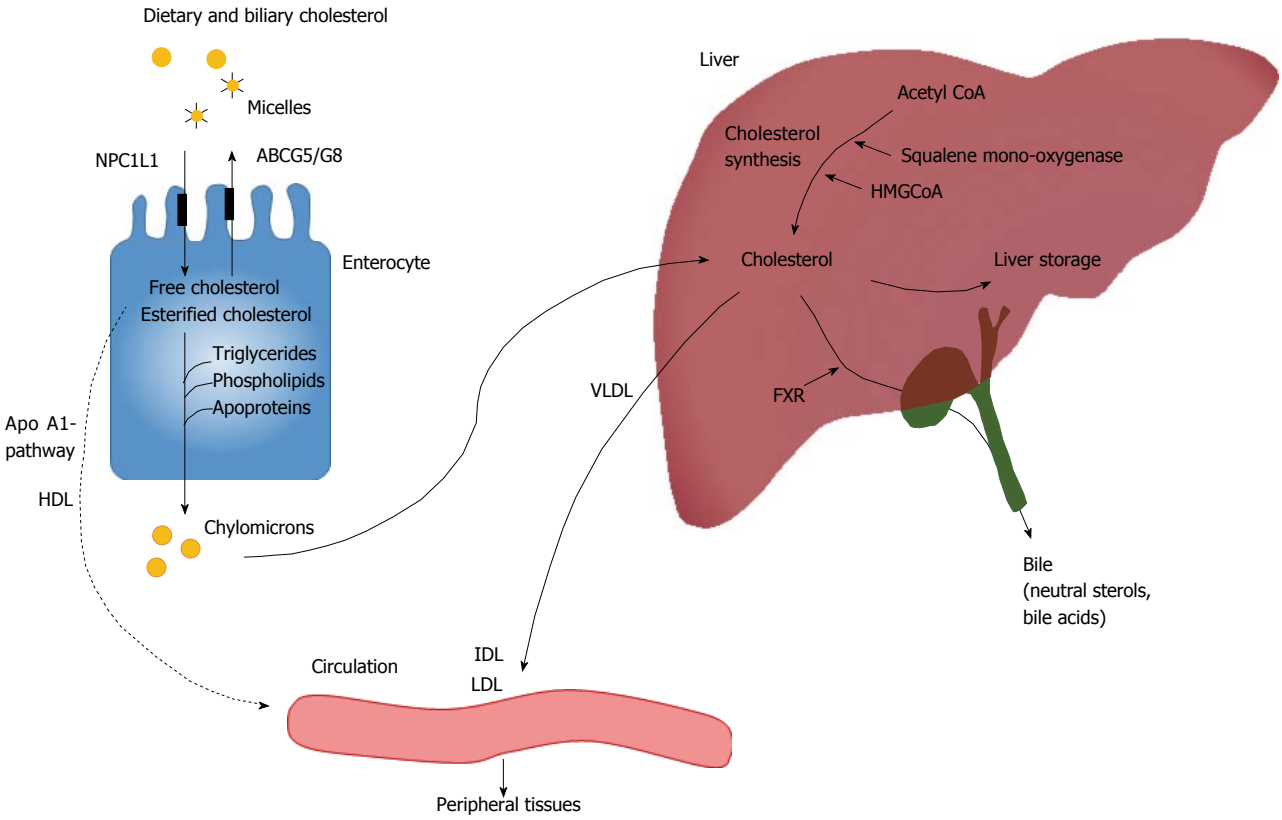

Figure 1 From the gut to the circulation: An overview of the pathways involved in cholesterol absorption and synthesis. IDL: Intermediate density lipoprotein; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; NPC1L1: Niemann-Pick C1 like 1; ABCG5: Adenosine triphosphate-binding cassette transporter G5 heterodimer; HMGCoA: 3-hydroxy-3-methyl-glutaryl CoA; FXR: Farnesoid X receptor.

intestinal lumen *via* the adenosine triphosphate (ATP)-binding cassette (ABC) G5/G8 transporters[20].

Lipoproteins synthesized in the liver and intestine play central roles in mediating the cholesterol transport to and from tissues through the bloodstream. Within enterocytes, free cholesterol is esterified and assembled - together with triglycerides, phospholipids, and apolipoproteins - to form chylomicrons (lipoproteins). Chylomicrons next enter the lymphatic system and the blood circulation at the thoracic duct, such that chylomicron remnants can be transported to the liver[21] (Figure 1). Some of the cholesterol in enterocytes is generated by endogenous synthesis[4,22]. Cholesterol is also reportedly secreted *via* an apolipoprotein A1-dependent pathway to form high-density lipoproteins (HDL) in the extracellular milieu, which then enter circulation[21].

CHOLESTEROL SYNTHESIS IN THE LIVER AND PERIPHERAL TISSUE

Cholesterol primarily enters blood circulation from two sources: From intestinal cholesterol absorption, and from the *de novo* cholesterol synthesis that is ubiquitous in all nucleated cells[1]. The majority of the body's endogenous cholesterol is produced by the liver[4]. Through a complex 37-step process, cholesterol is synthesized from simpler precursor molecules, starting with acetyl CoA[12]. The rate-limiting factors in the cholesterol synthesis pathway include two enzymes: The target of statins 3-hydroxy-3-methyl-glutaryl CoA reductase and squalene mono-oxygenase, which oxidizes the precursor squalene to lanosterol[1,23] (Figure 1).

The membrane of the endoplasmic reticulum (ER) contains an intracellular feedback system-a tightly controlled protein network that modulates the transcription of genes that mediate cholesterol synthesis and uptake (Figure 2). Sterol regulatory element-binding protein isoform 2 (SREBP-2) is an ER membrane-bound transcription factor that activates genes encoding the enzymes required for cholesterol synthesis[1,6-8,24]. A key event in cholesterol synthesis is the gated movement of SREBP-2 from the ER to the Golgi complex. A crucial ER membrane component, the cleavage-activating protein (SCAP), acts as both an escort for SREBP-2 and a sterol sensor. Immediately after SREBP-2 synthesis in the ER, its COOH-terminal regulatory domain binds to the COOH-terminal domain of SCAP. When cells become cholesterol depleted, SCAP escorts SREBP-2 from the ER to the Golgi apparatus, where SREBP-2 is cleaved by two proteases and then trans-located to the nucleus, where it activates transcription of multiple target genes for cholesterol synthesis. Upon accumulation of excess cellular cholesterol, the SCAP-SREBP complex binds to the resident ER protein INSIG-2, remaining in the ER in a sterol-regulated manner and thereby blocking cholesterol synthesis[25] (Figure 2). Interactions between cholesterol, SCAP, and the SCAP-binding protein INSIG-2

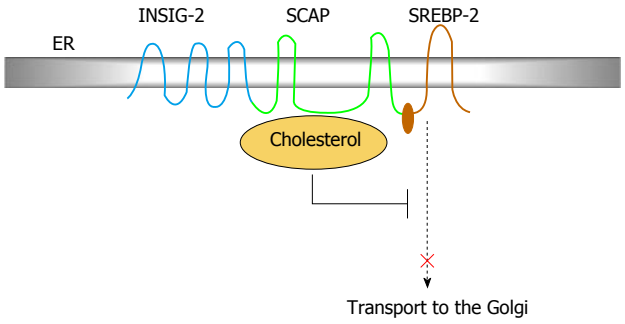

Figure 2 One key event in cholesterol homeostasis is the gated movement of sterol regulatory element-binding protein isoform 2 from the endoplasmic reticulum to the Golgi complex, involving the cleavage-activating protein, and INSIG-2. SREBP-2: Sterol regulatory element-binding protein isoform 2; ER: Endoplasmic reticulum; SCAP: Cleavage-activating protein.

create a sensitive switch that can respond to minor alterations of intracellular cholesterol levels, thus exerting precise control over the cholesterol composition of cell membranes.

In liver cells, free cholesterol can be excreted as neutral sterols into bile or transformed into bile acids, or it can be esterified and either stored in the liver as cholesterol esters or assembled into VLDL and secreted into circulation. The microsomal transfer protein assembles VLDL from cholesterol esters, triglycerides, phospholipids, free cholesterol, and apolipoprotein B-100 (apo B-100) as its structural protein. Triglycerides in VLDL are subsequently broken down by the enzymes lipoprotein lipase and hepatic lipase, producing intermediate-density lipoproteins (IDLs), followed by LDLs that transport cholesterol to peripheral tissues[21].

CHOLESTEROL ELIMINATION

Free cholesterol is toxic and mammalian somatic cells cannot catabolize it; thus, the removal of excess intracellular cholesterol by a distinct regulatory system is crucial (Figure 3). Liver X receptors (LXRs) act as whole-body cholesterol sensors. Under physiological conditions, cholesterol pool expansion and high intracellular cholesterol levels raise the intracellular concentration of oxygenated cholesterol metabolites termed oxysterols, which are important intermediate or end products in cholesterol excretion pathways. Oxysterols trigger liver-specific LXR activation, generating a transcriptional response that results in net elimination of cholesterol from the body *via* mobilization of cholesterol from peripheral tissues and promotion of hepatic excretion[4]. Quantitatively, the most important oxygenation reactions are those involved in the early steps of converting cholesterol into bile acids, a metabolically strictly controlled process. Cholesterol 7α-hydroxylase (CYP7A1) is the initial and rate-limiting enzyme of bile-acid synthesis[7,26]. *CYP7A1* gene transcription is inhibited by the farnesoid X nuclear receptor, thereby producing negative feedback that reduces the bile acid synthesis from cholesterol. The farnesoid X receptor-agonist obeticholic acid is currently under investigation for possible use in therapy for primary biliary cholangitis (PBC)[27].

The most important target genes of LXRs include ABCG5/G8, NPC1L1, acetyl-CoA cholesterol acyltransferase 2 (ACAT-2), and ATP-binding cassette transporter sub-family member A 1 (ABCA1; also known as the cholesterol efflux regulatory protein)[4]. Activation of these genes can increase intestinal and hepatic cholesterol excretion (ABCG5/G8), reduce cholesterol absorption (NPC1L1), and reduce cholesterol storage (ACAT-2). ABCA1 is involved in reverse cholesterol transport, in which surplus free cholesterol from peripheral tissues is eliminated from the body *via* biliary excretion or through the non-biliary TICE pathway[4,5] (Figure 3).

HDL mediates the transfer of cholesterol from peripheral tissues to the liver. Nascent cholesterol-poor pre-β HDL particles take up free cholesterol from peripheral tissues *via* ABCA1, after which this free cholesterol is esterified by lecithin-cholesterol acyltransferase. The esterified cholesterol is moved to the HDL particle's hydrophobic core, and progressive lipidation of the HDL particle causes it to mature, enlarge, and become more spherical. The cholesterol esters in mature HDL particles can be removed from circulation by hepatic scavenger receptor B1, or *via* transfer to apo B-100-containing lipoproteins (VLDL, IDL, and LDL) in a manner mediated by the cholesterol-ester transfer protein. By means of the LDL receptor and the LDL receptor-related protein, the liver can take up the apo B-100-containing lipoprotein particles from circulation[21].

DIFFICULTIES OF ASSESSING CHOLESTEROL METABOLISM IN CHOLESTASIS

Changes in cholesterol metabolism are not mirrored by routine serum cholesterol and lipoprotein measurements[28]. Moreover, the direct methods available to evaluate cholesterol metabolism are complex and laborious, and require labeling techniques, feces collection, and dietary recalls over several days.

In clinical research under steady state conditions, several non-cholesterol sterols that are measurable in serum can serve as valid biomarkers of cholesterol metabolism, especially when expressed as ratios to cholesterol[13,29-31]. Cholesterol precursor sterols, such as desmosterol and lathosterol, are markers of cholesterol synthesis[29]. On the other hand, diet-derived plant sterols (*e.g.*, campesterol and sitosterol) and the liver-synthesized cholesterol metabolite cholestanol are markers of cholesterol absorption efficiency[30]. These markers have been investigated in PBC before and after LT[15,28,32-34]. Compared to that in healthy controls, intestinal cholesterol absorption is reportedly reduced by 2/3 in cases of prolonged severe intrahepatic cholestasis leading to cirrhosis and end-stage liver failure, as seen in PBC[35]. Cholestasis impairs the intestinal absorption of all types of sterols due to poor micellar formation secondary

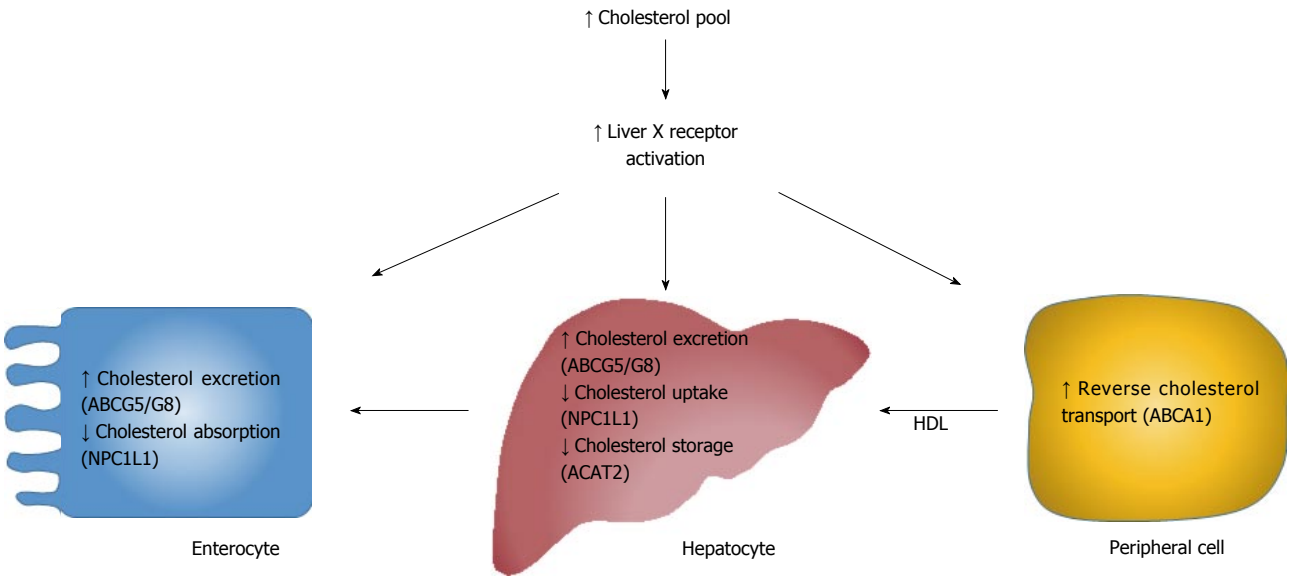

Figure 3 Cholesterol elimination- mechanisms and key transporters. Expansion of the cholesterol pool activates liver X receptor, thereby generating transcriptional responses resulting in cholesterol excretion and catabolism. NPC1L1: Niemann-Pick C1 like 1; ABCA1: ATP-binding cassette transporter sub-family member A 1, also known as the cholesterol efflux regulatory protein; ACAT2: Acetyl-CoA acyltransferase 2; ABCG5: Adenosine triphosphate-binding cassette transporter G5 heterodimer; HDL: High density lipoprotein.

to reduced bile formation and excretion. However, striking increases of serum and hepatic plant sterol and cholestanol levels are also observed, indicating that the serum levels of plant sterols and cholestanol do not correctly mirror cholesterol absorption in cholestasis[15,28]. These changes can be used as biomarkers of the degree of cholestasis, with serum cholestanol/cholesterol being an even more sensitive marker of cholestasis among early-stage PBC patients than serum bilirubin[33]. Moreover, in end-stage cholestasis, serum cholestanol levels increase to levels that are otherwise only seen in the rare genetic disorder cerebrotendinous xanthomatosis[36]. This genetic disorder manifests with extremely high cholesterol deposits in tissues, including nerve tissues, resulting in severe neurologic symptoms[36].

The liver is almost solely responsible for the secretion of sterols (*e.g.*, cholesterol, plant sterols, and cholestanol) from the human body *via* bile, which is regulated by hepatic proteins, including ABCG5/G8, NPC1L1, and LXRs[4-11]. ABCG5/G8 is active in cholesterol and sterol excretion across the canalicular membrane into bile. To our knowledge, no human studies have been performed to clarify how these sterol transporters function on the biliary canalicular level in intrahepatic cholestasis. Mutations in the genes of these transporters cause phytosterolemia, characterized by increased intestinal absorption and reduced biliary secretion of plant sterols, cholesterol, and cholestanol. Interestingly, Miettinen *et al*[37] reported the case of a patient with phytosterolemia who presented with cholestatic liver disease necessitating LT. Following LT, the grossly elevated pre-transplant serum levels of plant sterols decreased to values only slightly above normal. This case highlights that the liver apparently plays a predominant role in maintaining sterol balance, since the intestinal ABCG5/G8 defect was not altered by LT[37].

LIPOPROTEIN X

Despite reduced cholesterol synthesis, high serum total and LDL cholesterol concentrations and even xanthomata are common features in PBC and other forms of cholestatic liver disease[35,38-47]. In PBC, serum total cholesterol varies widely, ranging from 2.9 to 46.1 mmol/L (112-1779 mg/dL), and even up to 83 mmol/L (3204 mg/dL)[43].

High serum LDL cholesterol concentration is associated with atherosclerosis. Apo B-100 is present in all liver-derived atherogenic lipoproteins-including VLDL, IDL, LDL, and lipoprotein (a). However, in chronic cholestasis, LDL cholesterol measured using standard hospital laboratory methods is frequently elevated due to abnormal lipoprotein X (Lp-X), which is distinct from apo B-100-containing lipoproteins. Lp-X is characterized by a vesicular structure comprising a 30- to 70-nm lipid bilayer enclosing an aqueous compartment. Lp-X possesses strikingly high contents of unesterified cholesterol and phospholipids; low contents of cholesterol esters and triglycerides; small amounts of albumin and apolipoproteins C, E, and A-1; and no or a low concentration of apo B-100. Lp-X and LDL have the same density and are thus indistinguishable by standard lipoprotein ultracentrifugation. On the other hand, the physical size of Lp-X is in the range of VLDL or larger. Routine clinical laboratory methods currently used to measure LDL cholesterol are markedly affected by the presence of Lp-X, leading to false interpretations of elevated LDL cholesterol levels. Nuclear magnetic resonance spectroscopy measurements of lipoproteins reveal that Lp-X exist in PBC patients more commonly than currently recognized[48]. This phenomenon explains

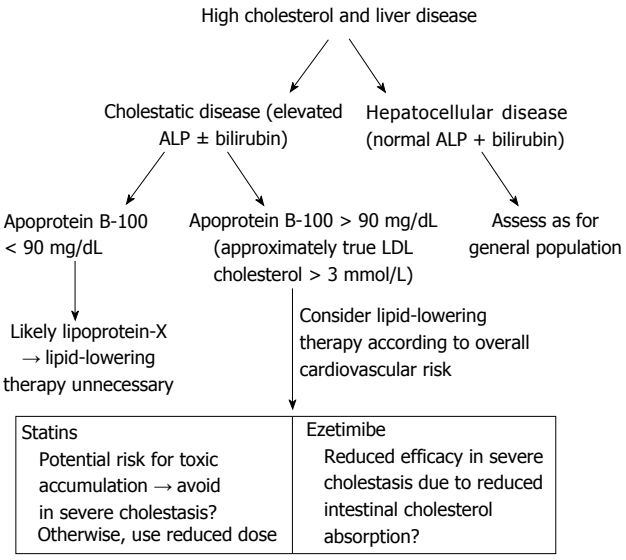

Figure 4 Algorithm for assessing hyperlipidemia in the setting of chronic liver disease. LDL: Low density lipoprotein; ALP: Alkaline phosphatase.

why high LDL cholesterol concentrations within the context of PBC, when actually caused by Lp-X, show no association with atherosclerotic events[38-41].

Lp-X formation is typically associated with a low apo B-100 concentration together with a high total cholesterol concentration[44]. The usual target level of apo B-100 is below 90 mg/dL, corresponding to a true LDL cholesterol concentration of below 3.0 mmol/L (116 mg/dL)[49-51]. The ratio of apo B-100 to total cholesterol is normally around 1:2, but may be 1:10 in cases of severe Lp-X formation[45]. Since an elevated apo B-100 concentration is a risk factor for atherosclerosis[49-51], apo B-100 concentrations should be measured when considering lipid-lowering treatment in PBC and other cholestatic conditions (Figure 4). Even when LDL cholesterol levels are high, cholesterol-lowering medication is unnecessary in cases where apo B-100 is below 90 mg/dL, since this suggests prevalence of non-atherogenic Lp-X. Lp-X resolves after successful cholestasis treatment[52].

Importantly, most statins are excreted into bile and, thus, cholestatic liver disease may lead to toxic levels of drug accumulation[41]. Furthermore, in Lp-X-related hypercholesterolemia, statin therapy does not effectively lower cholesterol levels because Lp-X does not undergo LDL receptor-mediated hepatic clearance[43,48]. Therefore, statins must be used cautiously in cholestatic conditions. Moreover, cholesterol absorption is low in severe cholestasis due to poor micellar formation, potentially diminishing the effect of ezetimibe, which lowers cholesterol levels by decreasing intestinal cholesterol absorption. In severe cholestasis, a lipid phenotype suggesting high cardiovascular risk necessitates accurate evaluation with consultation of a lipidologist. An additional caveat is that elevated Lp-X may affect various laboratory tests - for instance, potentially leading to pseudohyponatremia[45]. Although hypercholesterolemia is well-acknowledged in PBC, Lp-X formation is often neglected[53].

CHOLESTEROL AND LIVER REGENERATION

An ample cholesterol supply is critical for liver regeneration and for hepatocyte, stellate cell, and Kupffer cell function[54]. The importance of a circulating cholesterol supply for liver regeneration is exemplified following liver resection, where declining serum cholesterol coincides with intrahepatic cholesterol accumulation. In parallel, a serum total cholesterol concentration of below 2.8 mmol/L (108 mg/dL) in decompensated liver cirrhosis is associated with reduced transplant-free survival[55]. Additionally, among patients with non-cholestatic cirrhosis who underwent LT, a recipient serum total cholesterol level of below 1.8 mmol/L (69 mg/dL) at LT was associated with reduced post-LT graft outcome, independent of relevant donor, graft, and pre-operative recipient variables[56]. Both recipient cholesterol levels and the expressions of cholesterol metabolism genes in the liver graft could conceivably influence liver graft cholesterol availability and graft regeneration[56].

DONOR-DERIVED HYPERCHOLESTEROLEMIA

The LDL receptor is critical in mediating the catabolism of cholesterol-enriched particles and is abundant in the liver, with hepatocytes expressing up to 70%-80% of all LDL receptors in humans[57]. Pathogenic mutations in the LDL receptor gene cause familial hypercholesterolemia (FH) characterized by markedly elevated serum total and LDL cholesterol levels, tendon xanthomas, and early atherosclerosis. LT presents an effective therapy for homozygous FH.

On the other hand, we recently reported a case in which an LDL receptor mutation was unintentionally transmitted from a donor to an LT recipient, causing severe hypercholesterolemia in the recipient[58]. Prior to LT, the patient had hepatic epithelioid hemangioendothelioma without cirrhosis or cholestasis and exhibited no dyslipidemia. Following LT, the recipient's lipid levels were similar to those observed in FH, but her genomic DNA was normal in this regard. DNA was extracted from biopsy specimens of the liver allograft, and subjected to sequencing of the LDL receptor coding region, revealing a heterozygous splicing mutation in intron 9 that was previously reported as an FH-associated pathogenic mutation[58]. This finding essentially represents a transgenic model, consistent with previous evidence suggesting that most LDL cholesterol uptake in the body occurs in the liver and is mediated by LDL receptors. Since heterozygous FH is not extremely rare (prevalence 1/200 to 1/500[59]), our report raises concern of LT recipients acquiring unidentified FH from LT donors, especially

since FH manifestations are extrahepatic and thus easily overseen during donor evaluation[60].

POST-TRANSPLANT FOLLOW-UP

Hyperlipidemia reportedly occurs in 40%-66% of patients following LT[61]. Many mechanisms contribute to post-LT hypercholesterolemia and hypertriglyceridemia, including genetic susceptibility, diet, obesity, metabolic syndrome, diabetes, cholestatic problems, and immunosuppressive medication. The immunosuppressive drug cyclosporine induces hypercholesterolemia by inhibiting sterol 27-hydroxylase, a key enzyme in the bile synthesis pathway. Corticosteroids are usually tapered in the early post-LT period, and thus have minimal long-term influence on serum lipids[61]. Post-transplant cholestasis is also relatively common, and often secondary to anastomotic or non-anastomotic biliary stricturing[62]. Prolonged cholestasis may lead to Lp-X formation, but very few post-LT cases are reported[52,63,64].

Compared to the general population, LT recipients more commonly experience cardiovascular events, especially LT recipients with metabolic syndrome and/or diabetes[65]. The overall lipoprotein profile in LT recipients is generally proatherogenic, but variation exists[66], warranting an individualized detailed assessment of cardiovascular risk. Hepatic steatosis is considered a manifestation of metabolic syndrome and/or diabetes and is associated with a proatherogenic profile. Importantly, liver graft steatosis is increasingly detected. Thus, lipid profile assessment should include apo B-100 quantification in addition to the routine measurements of total, LDL, and HDL cholesterol, and total triglycerides. It is assumed that reducing intrahepatic lipids reduces the risks of hepatic and cardiovascular complications. Recent data suggest that treatment with a combination of dietary intervention, weight loss, and ezetimibe (which is well tolerated and can be combined with a statin) can reduce LDL cholesterol and apo B-100 concentrations in these patients[22,67-70].

CONCLUSION

Various liver disorders, particularly cholestasis, affect cholesterol metabolism and can cause variable hypercholesterolemia, including Lp-X appearance. Mistaking Lp-X for LDL cholesterol may interfere with cardiovascular risk assessment, leading to the prescription of futile lipid-lowering therapy. Lipid panel assessment should be regularly performed in all LT recipients, and at LT evaluation. Apo B-100 measurement can help in distinguishing between atherogenic and non-atherogenic hypercholesterolemia. Therefore, the measurement of apo B-100 can help in evaluating overall cardiovascular risk, as well as the effects of therapy during follow-up. This is particularly important after LT, when cholestasis and Lp-X may coexist with true atherogenic hypercholesterolemia and increased cardiovascular risk.

ACKNOWLEDGMENTS

I recently changed my name from Katriina Nikkilä to Katriina Nemes. My previous publication history has been released by using the name Katriina Nikkilä (*e.g.*, Nikkilä K) partly shown also in the actual references.

REFERENCES

1 **Ikonen E**. Cellular cholesterol trafficking and compartmentalization. *Nat Rev Mol Cell Biol* 2008; **9**: 125-138 [PMID: 18216769 DOI: 10.1038/nrm2336]
2 **Maxfield FR**, van Meer G. Cholesterol, the central lipid of mammalian cells. *Curr Opin Cell Biol* 2010; **22**: 422-429 [PMID: 20627678 DOI: 10.1016/j.ceb.2010.05.004]
3 **Calvo PL**, Brunati A, Spada M, Romagnoli R, Corso G, Parenti G, Rossi M, Baldi M, Carbonaro G, David E, Pucci A, Amoroso A, Salizzoni M. Liver transplantation in defects of cholesterol biosynthesis: the case of lathosterolosis. *Am J Transplant* 2014: **14**; 960-965 [PMID: 24621408 DOI: 10.1111/ajt.12645]
4 **Bonamassa B**, Moschetta A. Arteriosclerosis: lessons from LXR and the intestine. *Trends Endocrinol Metab* 2013; **24**: 120-128 [PMID: 23158108 DOI: 10.1016/j.tem.2012.10.004]
5 **Degirolamo C**, Sabbà C, Moschetta A. Intestinal nuclear receptors in HDL cholesterol metabolism. J *Lipid Res* 2015; **56**: 1262-1270 [PMID: 25070952 DOI: 10.1194/jlr.R052704]
6 **Horton JD**, Goldstein JL, Brown MS. SREBs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; **109**: 1125-1131 [PMID: 11994399 DOI: 10.1172/JCI15593]
7 **Weber LW**, Boll M, Stampfl A. Maintaining cholesterol homeostasis: Sterol regulatory element-binding proteins. *World J Gastroenterol* 2004; **10**: 3081-3087 [PMID: 15457548 DOI: 10.3748/wjg.v10.i21.3081]
8 **Eberlé D**, Hegarty B, Bossard P, Ferré P, Foufelle F. SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie* 2004; **86**: 839-848 [PMID: 15589694 DOI: 10.1016/j.biochi.2004.09.018]
9 **Moschetta A**. Nuclear receptors and cholesterol metabolism in the intestine. *Atheroscler Suppl* 2015; **17**: 9-11 [PMID: 25659870 DOI: 10.1016/S1567-5688(15)50003-2]
10 **Davis HR Jr**, Altmann SW. Niemann-Pick C1 Like 1 (NPC1L1) an intestinal sterol transporter. *Biochim Biophys Acta* 2009; **1791**: 679-683 [PMID: 19272334 DOI: 10.1016/j.bbalip.2009.01.002]
11 **Betters JL**, Yu L. NPC1L1 and cholesterol transport. *FEBS Lett* 2010; **584**: 2740-2747 [PIMD: 20307540 DOI: 10.1016/j.febslet.2010.03.030]
12 **Goldstein JL**, Brown MS. Regulation of the mevalonate pathway. *Nature* 1990; **343**: 425-430 [PMID: 1967820 DOI: 10.1038/343425a0]
13 **Gylling H**. Clinical utility of serum markers of cholesterol absorption and synthesis. *Curr Opin Lipidol* 2014; **25**: 207-212 [PMID: 24811297 DOI: 10.1097/MOL.0000000000000069]
14 **Turner S**, Voogt J, Davidson M, Glass A, Killion S, Decaris J, Mohammed H, Minehira K, Boban D, Murphy E, Luchoomun J, Awada M, Neese R, Hellerstein M. Measurement of reverse cholesterol transport pathways in humans: in vivo rates of free cholesterol efflux, esterification, and excretion. *J Am Heart Assoc* 2012; **1**: e001826 [PMID: 23130164 DOI: 10.1161/JAHA.112.001826]
15 **Nikkilä K**, Höckerstedt K, Miettinen TA. High cholestanol and low campesterol-to-sitosterol ratio in serum of patients with primary biliary cirrhosis before liver transplantation. *Hepatology* 1991; **13**: 663-669 [PMID: 2010161 DOI: 10.1002/hep.1840130409]
16 **Arguello G**, Balboa E, Arrese M, Zanlungo S. Recent insights on the role of cholesterol in non-alcoholic fatty liver disease. *Biochim Biophys Acta* 2015; **1852**: 1765-1778 [PMID: 26027904 DOI: 10.1016/j.bbadis.2015.05.015]
17 **Sudhop T**, Lütjohann D, von Bergmann K. Sterol transporters:

target of natural sterols and new lipid lowering drugs. *Pharmacol Ther* 2005; **105**: 333-341 [PMID: 15737409 DOI: 10.1016/j.pharmthera.2004.10.011]
18 **Hui DY**, Labonté ED, Howles PN. Development and physiological regulation of intestinal lipid absorption. III. Intestinal transporters and cholesterol absorption. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G839-G843 [PMID: 18276831 DOI: 10.1152/ajpgi.00061.2008]
19 **Phan BA**, Dayspring TD, Toth PP. Ezetimibe therapy: mechanism of action and clinical update. *Vasc Health Risk Manag* 2012; **8**: 415-427 [PMID: 22910633 DOI: 10.2147/VHRM.S33664]
20 **Ostlund RE Jr**, McGill JB, Zeng CM, Covey DF, Stearns J, Stenson WF, Spilburg CA. Gastrointestinal absorption and plasma kinetics of soy Delta(5)-phytostanols in humans. *Am J Physiol Endocrinol Metabol* 2002; **282**: E911-E 916 [PMID: 11882512 DOI: 10.1152/ajpendo.00328.2001]
21 **Hussain MM**. Intestinal lipid absorption and lipoprotein formation. *Curr Opin Lipidol* 2014; **25**: 200-206 [PMID: 24751933 DOI: 10.1097/MOL.0000000000000084]
22 **Arca M**. Alterations of intestinal lipoprotein metabolism in diabetes mellitus and metabolic syndrome. *Atheroscler Supp* 2015; **17**: 12-16 [PMID: 25659871 DOI: 10.1016/S1567-5688(15)50004-4]
23 **Buhaescu I**, Izzedine H. Mevalonate pathway: a review of clinical and therapeutical implications. *Clin Biochem* 2007; **40**: 575-584 [PIMD: 17467679 DOI: 10.1016/j.clinbiochem.2007.03.016]
24 **Radhakrishnan A**, Goldstein JL, McDonald JG, Brown MS. Switch-like control of SREBP-2 transport triggered by small changes in ER cholesterol: a delicate balance. *Cell Metab* 2008; **8**: 512-521 [PMID: 19041766 DOI: 10.1016/j.cmet.2008.10.008]
25 **Yabe D**, Brown MS, Goldstein JL. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. *Proc Natl Acad Sci USA* 2002; **99**: 12753-12758 [PMID: 12242332 DOI: 10.1073/pnas.162488899]
26 **Repa JJ**, Berge KE, Pomajzl C, Richardson JA, Hobbs H, Mangelsdorf DJ. Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by liver X receptors alfa and beeta. J *Biol Chem* 2002; **277**: 18793-18800 [PMID: 11901146 DOI: 10.1074/jbc.M109927200]
27 **Corpechot C**. Primary biliary cirrhosis beyond ursodeoxycholic acid**.** *Semin Liver Dis* 2016; **36**: 15-26 [PMID: 26870929 DOI: 10.1055/s-0035-1571273]
28 **Nikkilä K**, Höckerstedt K, Miettinen TA. Serum and hepatic cholestanol, squalene and noncholesterol sterols in man: a study on liver transplantation. *Hepatology* 1992; **15**: 863-870 [PMID: 1568728 DOI: 10.1002/hep.1840150519]
29 **Miettinen TA**, Tilvis RS, Kesäniemi YA. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am J Epidemiol* 1990; **131**: 20-31 [PMID: 2293749]
30 **Miettinen TA**, Tilvis RS, Kesäniemi YA. Serum cholestanol and plant sterol levels in relation to cholesterol metabolism in middle-aged men. *Metabolism* 1989; **38**: 136-140 [PMID: 2913464 DOI: 10.1016/0026-0495(89)90252-7]
31 **Miettinen TA**, Gylling H, Nissinen MJ. The role of serum non-cholesterol sterols as surrogate markers of absolute cholesterol synthesis and absorption. *Nutr Metab Cardiovasc Dis* 2011; **21**: 765-769 [PMID: 21899991 DOI: 10.1016/j.numecd. 2011.05.005]
32 **Nikkilä K**, Miettinen TA, Höckerstedt KV, Isoniemi H. Sterol parameters as markers of liver function in primary biliary cirrhosis before and after liver transplantation. *Transpl Int* 2005; **18**: 221-225 [PMID: 15691276 DOI: 10.1111/j.1432-2277.2004.00002.x]
33 **Nikkilä K**, Nissinen MJ, Gylling H, Isoniemi H, Miettinen TA. Serum sterols in patients with primary biliary cirrhosis and acute liver failure before and after liver transplantation. *J Hepatol* 2008; **49**: 936-945 [PMID: 18926587 DOI: 10.1016/j.jhep.2008.07.026]
34 **Nikkilä K**, Höckerstedt K, Miettinen TA. Liver transplantation modifies serum cholestanol, cholesterol precursor and plant sterol levels. *Clin Chim Acta* 1992; **208**: 205-218 [PMID: 1499139]
35 **Gylling H**, Färkkilä M, Vuoristo M, Miettinen TA. Metabolism of cholesterol and low- and high-density lipoproteins in primary biliary cirrhosis: cholesterol absorption and synthesis related to lipoproteins levels and their kinetics. *Hepatology* 1995; **21**: 89-95 [PMID: 7806174]
36 **Bhattacharyya AK**, Lin DS, Connor WE. Cholestanol metabolism in patients with cerebrotendinous xanthomatosis: absorption, turnover, and tissue deposition. *J Lipid Res* 2007; **48**: 185-192 [PMID: 17012751 DOI: 10.1194/jlr.M600113-JLR200]
37 **Miettinen TA**, Klett EL, Gylling H, Isoniemi H, Patel SB. Liver transplantation in a patient with sitosterolemia and cirrhosis. *Gastroenterology* 2006; **130**: 542-547 [PMID: 16472606 DOI: 10.1053/j.gastro.2005.10.022]
38 **Crippin JS**, Lindor KD, Jorgensen R, Kottke BA, Harrison JM, Murtaugh PA, Dickson ER. Hypercholesterolemia and atherosclerosis in primary biliary cirrhosis: what is the risk? *Hepatology* 1992; **15**: 858-862 [PMID: 1568727 DOI: 10.1002/hep.1840150518]
39 **Longo M**, Crosignani A, Battezzati PM, Squarcia Giussani C, Invernizzi P, Zuin M, Podda M. Hyperlipidaemic state and cardiovascular risk in primary biliary cirrhosis. *Gut* 2002; **51**: 265-269 [PMID: 12117892 DOI: 10.1136/gut.51.2.265]
40 **Chang PY**, Lu SC, Su TC, Chou SF, Huang WH, Morrisett JD, Chen CH, Liau CS, Lee YT. Lipoprotein-X reduces LDL atherogenicity in primary biliary cirrhosis by preventing LDL oxidation. *J Lipid Res* 2004; **45**: 2116-2122 [PMID: 15314101 DOI: 10.1194/jlr.M400229-JLR200]
41 **Sorokin A**, Brown JL, Thompson PD. Primary biliary cirrhosis, hyperlipidemia, and atherosclerotic risk: a systematic review. *Atherosclerosis* 2007; **194**: 293-299 [PMID: 17240380 DOI: 10.1016/j.atherosclerosis.2006.11.036]
42 **Citkowitz E**. Was the low-density lipoprotein cholesterol level really elevated? *Liver Transpl* 2011; **17**: 1234, author reply 1235 [PMID: 21506250 DOI: 10.1002/lt.22317]
43 **Wong ML**, Raghavan RP, Hedger NA, Ellis RD, Meeking DR, Albon L. The use of plasmapheresis in managing primary biliary cirrhosis presenting with profound hypercholesterolaemia. *The British Journal of Diabetes and Vascular Diseases* 2012; **12**: 156-158 [DOI: 10.1177/1474651412442410]
44 **Ooi YK**, Mietus-Snyder M, Torres C, Mohan P, Harahsheh A. Lipoprotein-X-accumulation: a mimic of familial hypercholesterolemia. *Consult Pediatricians* 2013; **12**: 63-65
45 **Hussain I**, Ahmad Z, Garg A. Extreme hypercholesterolemia presenting with pseudohyponatremia - a case report and review of the literature. *J Clin Lipidol* 2015; **9**: 260-264 [PMID: 25911084 DOI: 10.1016/j.jacl.2014.11.007]
46 **Joukhadar R**, Chiu K. Severe hypercholesterolemia in patients with graft-vs-host disease affecting the liver after stem cell transplantation. *Endocr Pract* 2012; **18**: 90-97 [PMID: 21940276 DOI: 10.4158/Ep11212.RA]
47 **Chow A**, Rifici VA, Schneider SH. Lipoprotein-X in a patient with lymphoplasmacytic sclerosing cholangitis: an unusual cause of secundary hypercholesterolemia. *AACE Clinical Case Reports* 2016; **2**: e20- e 24 [DOI: 10.4158/EP14249.CR]
48 **Foley KF**, Silveira MG, Hornseth JM, Lindor KD, McConnell JP. A patient with primary biliary cirrhosis and elevated LDL cholesterol. *Clin Chem* 2009; **55**: 187-191, discussion 191-192 [PMID: 19106186 DOI: 10.1373/clinchem.2008.108720]
49 Assessment by the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices. Cole TG, Contois JH, Csako G, McConnell JP, Remaley AT, Devaraj S, Hoefner DM, Mallory T, Sethi AA, Warnick GR. Association of apolipoprotein B and nuclear magnetic resonance spectroscopy-derived LDL particle number with outcomes in 25 clinical studies. *Clin Chem* 2013; **59**: 752-770 [PMID: 23386699 DOI: 10.1373/clinchem.2012.196733]
50 **Sniderman AD**, Williams K, Contois JH, Monroe HM, McQueen MJ, de Graaf J, Furberg CD. A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. *Circ Cardiovasc Qual Outcomes* 2011; **4**: 337-345 [MPID: 21487090 DOI: 10.1161/CIRCOUTCOMES.110.959247]

51 **Varvel SA**, Dayspring TD, Edmons Y, Thiselton DL, Ghaedi L, Voros S, McConnel JP, Sasinowski M, Dall T, Warnick GR. Discordance between apolipoprotein B and low-density lipoprotein particle number is associated with insulin resistance in clinical practice. *J Clin Lipidol* 2015; **9**: 247-255 [PMID: 25911082 DOI: 10.1016/j.jacl.2014.11.005]

52 **Jankowski K**, Wyzgal A, Wierzbicka A, Tronina O, Durlik M, Pruszczyk P. Rapid normalization of severe hypercholesterolemia mediated by lipoprotein X after liver transplantation in a patient with cholestasis - a case report. *Acta Biochim Pol* 2015; **62**: 621-623 [PMID: 26317127 DOI: 10.18388/abp.2015_971]

53 **Purohit T**, Cappell MS. Primary biliary cirrhosis: Pathophysiology, clinical presentation and therapy. *World J Hepatol* 2015; **7**: 926-941 [PMID: 25954476 DOI: 10.4254/wjh.v7.i7.926]

54 **Delgado-Coello B**, Briones-Orta MA, Macias-Silva M, Mas-Oliva J. Cholesterol: recapitulation of its active role during liver regeneration. *Liver Int* 2011; **31**: 1271-1284 [PMID: 21745289 DOI: 10.1111/j.1478-3231.2011.02542.x]

55 **Jiang M**, Liu F, Xiong WJ, Zhong L, Xu W, Xu F, Liu YB. Combined MELD and blood lipid level in evaluating the prognosis of decompensated cirrhosis. *World J Gastroenterol* 2010; **16**: 1397-1401 [PMID: 20238407 DOI: 10.3748/wjg.v16.i11.1397]

56 **Ginanni Corradini S**, Siciliano M, Parlati L, Molinaro A, Cantafora A, Poli E, Mennini G, Melandro F, Vestri AR, Merli M, Bianco P, Corsi A, Toniutto P, Bitetto D, Falleti E, Attili AF, Berloco P, Rossi M. Recipient perioperative cholesterolaemia and graft cholesterol metabolism gene expression predict liver transplant outcome. *Liver Int* 2014; **34**: e290-e301 [PMID: 24256518 DOI: 10.1111/liv.12351]

57 **Goldstein JL**, Brown MS. The LDL receptor. *Arterioscler Thromb Vasc Biol* 2009; **29**: 431-438 [PMID: 19299327 DOI: 10.1161/ATVBAHA.108.179564]

58 **Nikkilä K**, Åberg F, Isoniemi H. Transmission of LDLR mutation from donor through liver transplantation resulting in hypercholesterolemia in the recipient. *Am J Transplant* 2014; **14**: 2898-2902 [PMID: 25231171 DOI: 10.1111/ajt.12961]

59 **Nordestgaard BG**, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, Wiklund O, Hegele RA, Raal FJ, Defesche JC, Wiegman A, Santos RD, Watts GF, Parhofer KG, Hovingh GK, Kovanen PT, Boileau C, Averna M, Borén J, Bruckert E, Catapano AL, Kuivenhoven JA, Pajukanta P, Ray K, Stalenhoef AF, Stroes E, Taskinen MR, Tybjaerg-Hansen A; European Atherosclerosis Society Consensus Panel. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J* 2013; **34**: 3478-3490a [PMID: 23956253 DOI: 10.1093/eurheartj.eht273]

60 **Najam O**, Ray KK. Familial hypercholesterolemia: a review of the natural history, diagnosis, and management. *Cardiol Ther* 2015; **4**: 25-38 [PMID: 25769531 DOI: 10.1007/s40119-015-0037-z]

61 **Sethi A**, Stravitz RT. Review article: medical management of the liver transplant recipient - a primer for non-transplant doctors. *Aliment Pharmacol Ther* 2007; **25**: 229-245 [PMID: 17217455 DOI: 10.1111/j.1365-2036.2006.03166.x]

62 **Ben-Ari Z**, Pappo O, Mor E. Intrahepatic cholestasis after liver transplantation. *Liver Transpl* 2003; **9**: 1005-1018 [PMID: 14526393 DOI: 10.1053/jlts.2003.50212]

63 **Tejera P**, Karalis D, Xiao G, Simon B, Amori R. Severe hypercholesterolemia and cholestatic liver disease after liver transplant: a case of lipoprotein X. The ICE/ENDO 2014 International Congress of Endocrinology and the 96th annual meeting of Endocrine Society in Chicago, June 21-24, 2014 Chicago, SUN-0851

64 **Yeh H**, Kitchens WH, Elias N, Kelsey PB, Markmann JF, Hertl M. Hyperlipidemia due to biliary stricture after living-donor liver transplantation. *Transplantation* 2011; **92**: e29-e30 [PMID: 21909017 DOI: 10.1097/TP.0b013e31822d095d]

65 **Madhwal S**, Atreja A, Albeldawi M, Lopez R, Post A, Costa MA. Is liver transplantation a risk for cardiovascular disease? A meta-analysis of observational studies. *Liver Transpl* 2012; **18**: 1140-1146 [PMID: 22821899 DOI: 10.1002/lt.23508]

66 **Chhatrala R**, Siddiqui MB, Stravitz RT, Driscoll C, Sanyal A, Sargeant C, Luketic V, Sharma A, Sterling R, Matherly S, Puri P, Siddiqui MS. Evolution of serum atherogenic risk in liver transplant recipients: Role of lipoproteins and metabolic and inflammatory markers. *Liver Transpl* 2015; **21**: 623-630 [PMID: 25762084 DOI: 10.1002/lt.24100]

67 **Chan DC**, Watts GF, Gan SK, Ooi EM, Barrett PH. Effect of ezetimibe on hepatic fat, inflammatory markers, and apolipoprotein B-100 kinetics in insulin-resistant obese subjects on a weight loss diet. *Diabetes Care* 2010; **33**: 1134-1139 [PMID: 20185740 DOI: 10.2337/dc09-1765]

68 **Åberg F**, Koljonen V, Nikkilä K, Boyd S, Arola J, Isoniemi H. Thiazolidinedione therapy versus lifestyle recommendation in the treatment of post-liver transplant graft steatosis. *Ann Transplant* 2014; **19**: 389-396 [PMID: 25105443 DOI: 10.12659/AOT.890664]

69 **Farnier M.** Ezetimibe/statin combination therapy to treat patients with type 2 diabetes. *Atheroscler Suppl* 2015; **17**: 2-8 [PMID: 25659869 DOI: 10.1016/S1567-5688(15)50002-0]

70 **Averna M.** The effect of ezetimibe on NALFD. *Atheroscler Suppl* 2015; **17**: 27-34 [PMID: 25659874 DOI: 10.1016/S1567-5688(15)50007-X]

P- Reviewer: Stieger B, Tiao MM, Zhao YL
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Li D

World Journal of Hepatology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v8.i22.933

World J Hepatol 2016 August 8; 8(22): 933-941
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Basic Study

Antifibrotic effects of ambrisentan, an endothelin-A receptor antagonist, in a non-alcoholic steatohepatitis mouse model

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Author contributions: Okamoto T and Koda M contributed equally to this work; Koda M designed the research; Okamoto T, Koda M, Miyoshi K, Onoyama T, Kishina M, Matono T, Sugihara T, Hosho K and Okano J performed the research; Okamoto T and Koda M analyzed the data; Okamoto T, Koda M, Isomoto H and Murawaki Y wrote the paper.

Institutional review board statement: All experiments were performed in accordance with the Animal Experimentation Guidelines of Tottori University (Yonago, Japan). The study was reviewed and approved by the ethics committee of Tottori University. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Tottori University (approval number; 14-Y-8).

Conflict-of-interest statement: All authors declare that they have no conflict of interest. All authors certify that this article is not under consideration for publication elsewhere. Publication is approved by all authors and by the responsible authorities where the work was carried out.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

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Received: February 24, 2016
Peer-review started: March 4, 2016
First decision: April 15, 2016
Revised: June 22, 2016
Accepted: July 11, 2016
Article in press: July 13, 2016
Published online: August 8, 2016

Abstract

AIM: To examine the effects of the endothelin type A receptor antagonist ambrisentan on hepatic steatosis and fibrosis in a steatohepatitis mouse model.

METHODS: Fatty liver shionogi (FLS) FLS-*ob/ob* mice (male, 12 wk old) received ambrisentan (2.5 mg/kg orally per day; n = 8) or water as a control (n = 5) for 4 wk. Factors were compared between the two groups, including steatosis, fibrosis, inflammation, and endothelin-related gene expression in the liver.

RESULTS: In the ambrisentan group, hepatic hydroxyproline content was significantly lower than in the control group (18.0 μg/g ± 6.1 μg/g *vs* 33.9 μg/g ± 13.5 μg/g liver, respectively, P = 0.014). Hepatic fibrosis estimated by Sirius red staining and areas positive

for α-smooth muscle actin, indicative of activated hepatic stellate cells, were also significantly lower in the ambrisentan group (0.46% ± 0.18% *vs* 1.11% ± 0.28%, respectively, $P = 0.0003$; and 0.12% ± 0.08% *vs* 0.25% ± 0.11%, respectively, $P = 0.047$). Moreover, hepatic RNA expression levels of procollagen-1 and tissue inhibitor of metalloproteinase-1 (TIMP-1) were significantly lower by 60% and 45%, respectively, in the ambrisentan group. Inflammation, steatosis, and endothelin-related mRNA expression in the liver were not significantly different between the groups.

CONCLUSION: Ambrisentan attenuated the progression of hepatic fibrosis by inhibiting hepatic stellate cell activation and reducing procollagen-1 and *TIMP-1* gene expression. Ambrisentan did not affect inflammation or steatosis.

Key words: Endothelin; Ambrisentan; Steatohepatitis; Hepatic stellate cell; Hepatic fibrosis; Oxidative stress; Hepatic hydroxyproline

Core tip: Endothelin (ET) can activate hepatic stellate cells, leading to the progression of hepatic fibrosis. Furthermore, ET-1 may increase the inflow of free fatty acids from the fat tissue into the liver and exacerbate hepatic steatosis. Therefore, ET-1 antagonism may be a novel target for steatohepatitis. The present study showed that ambrisentan, an ET type A receptor antagonist, attenuated hepatic fibrosis by inhibiting hepatic stellate cell activation, without affecting hepatic steatosis, in a non-alcoholic steatohepatitis mouse model.

Okamoto T, Koda M, Miyoshi K, Onoyama T, Kishina M, Matono T, Sugihara T, Hosho K, Okano J, Isomoto H, Murawaki Y. Antifibrotic effects of ambrisentan, an endothelin-A receptor antagonist, in a non-alcoholic steatohepatitis mouse model. *World J Hepatol* 2016; 8(22): 933-941 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i22/933.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i22.933

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is characterized by hepatic fat deposition, inflammation, and differing degrees of fibrosis[1]. In the pathophysiology of NASH, the deposition of fat in liver cells, which occurs in association with obesity and insulin resistance, is a benign process in most patients but is followed by inflammation and fibrosis in the liver in response to multiple insults, such as oxidative stress and various adipokines or cytokines acting in parallel[2]. In NASH, the serum endothelin-1 (ET-1) level is elevated and is correlated with hepatic fibrosis severity[3]. The development of hepatic fibrosis is mediated to a large extent by the activation of hepatic stellate cells (HSCs). ET-1 is released from sinusoidal endothelial cells and HSCs, which serves to activate the HSCs and accelerate collagen fiber synthesis in them[4]. Furthermore, ET-1 acts as a mediator and is elevated in conditions such as insulin resistance, hyperglycemia, oxidative stress, and endothelial cell dysfunction[5,6]. ET-1 also increases vascular superoxide production and promotes cell proliferation by inducing reactive oxygen species[7].

Ambrisentan is a selective ET type A receptor (ETAR) antagonist approved for the treatment of patients with pulmonary arterial hypertension[8]. ETAR antagonists improve liver fibrosis in cirrhotic rats[9], but their effects on NASH are unknown. Fatty liver shionogi (FLS)-*ob/ob* mice are characterized by hyperphagia, obesity, hyperlipidemia, and diabetes mellitus[10]. As described in our previous study using these mice[11], FLS-*ob/ob* mice are generated by transferring the *Lep*^{ob} gene into the FLS mouse genome, causing FLS mice to spontaneously develop chronic hepatic steatosis but not obesity. The resultant FLS-*ob/ob* mice show severe steatosis, hepatocellular ballooning, and advanced hepatic fibrosis histologically. They also display increased oxidative stress, elevated production of inflammatory and profibrotic cytokines, and increased apoptosis of hepatocytes, and eventually develop cirrhosis and liver tumors[12,13]. For these reasons, FLS-*ob/ob* are considered to be animal model the most closely represents human metabolic syndrome-related NASH. Against this background, this study investigated the therapeutic effects of ambrisentan on hepatic steatosis and fibrosis in NASH using FLS-*ob/ob* male mice.

MATERIALS AND METHODS

Animals

A total of 13 male FLS-*ob/ob* mice (age, 8 wk; body weight, 42.88 g ± 1.74 g) were obtained from Shionogi Research Laboratories (Shiga, Japan) and housed in a controlled environment (24 ℃ ± 2 ℃; 12:12-h light:Dark cycle). Mice were provided *ad libitum* water and a standard powdered diet (CE-2, 4.6% fat; CLEA Japan, Tokyo, Japan). To maintain dietary intake in both groups at an equal level, food consumption and body weight were monitored throughout observation. All experiments were performed in accordance with the Animal Experimentation Guidelines of Tottori University (Yonago, Japan). The study was reviewed and approved by the ethics committee of Tottori University. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Tottori University (approval number, 14-Y-8) and the animal protocol was designed to minimize pain and discomfort to the animals.

Administration of ambrisentan

At the age of 12 wk, male FLS-*ob/ob* mice were randomly assigned to the ambrisentan ($n = 8$) or control ($n = 5$) group. Intragastric gavage administration was carried out in conscious animals with an appropriately

sized gastric tube. Ambrisentan (2.5 mg/kg per day; ADooQ BioScience, Irvine, CA) was orally administered every afternoon for 4 wk as a bolus through a gastric tube. Water was administered to the control group. At week 4, animals were fasted for 4 h and tail vein blood was drawn and subjected to blood glucose determination. Animals were killed by pentobarbital anesthesia injection (Dainippon Sumitomo Pharma, Osaka, Japan) after 4 wk and blood was collected from the right ventricle. Plasma samples were frozen and stored at -80 ℃ Liver and visceral fat were then weighed, snap-frozen in liquid nitrogen, and stored at -80 ℃. Additional liver specimens were fixed in 10% buffered formalin (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and embedded in paraffin (Wako Pure Chemical Industries, Ltd.) for histological analysis.

Analysis of hepatic cholesterol and triglycerides

Snap-frozen liver samples (50 mg) were homogenized and extracted using chloroform-methanol (2:1 v/v; Wako Pure Chemical Industries, Ltd.). The organic phase was then dried and resuspended in 2-propanol containing 10% Triton X-100. Total cholesterol and triglyceride contents were measured with the Cholesterol E-test (Wako Pure Chemical Industries, Ltd.) and Triglyceride E-test (Wako Pure Chemical Industries, Ltd.), respectively.

Biochemical analysis

Blood samples were immediately separated by centrifugation at 2000 *g* for 15 min at 4 ℃ and stored at -80 ℃ until further use. Serum samples were analyzed to determine the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Measurement of hepatic hydroxyproline content

Hepatic tissue (400 mg wet weight) was hydrolyzed in 4 mL of 6 mol/L HCl at 105 ℃ overnight. The hydrolysate was then thoroughly evaporated under vacuum. The sediment was resuspended in distilled water, decolorized with activated charcoal, and filtered; the filtrate was then acidified to pH 5.0 and evaporated under vacuum. The sediment was resuspended in distilled water, mixed with 2 mL of isopropanol, and then incubated with 1 mL of 7% chloramine-T for 5 min at room temperature. After addition of Ehrlich's solution (2 mL; 1.76 g p-dimethylaminobenzaldehyde dissolved in 4.08 mL 60% perchloric acid and 95.5 mL of isopropanol), the mixture was incubated at 60 ℃ for 10 min. The absorbance of the cooled mixture was measured at 562 nm.

Measurement of hepatic fibrosis area

As in our previous study[11], formalin-fixed, paraffin-embedded liver sections (4-μm-thick) were stained with picrosirius red (Chroma-Gesellschaft Schmid GmbH and Co., Munster, Germany) and counterstained with fast green (Chroma-Gesellschaft Schmid GmbH and Co.). The areas of hepatic fibrosis were subsequently measured in 10 randomly selected fields in each specimen (magnification, × 400) using WinROOF ver.5.71 software and the Olympus BX51N-34 microscope.

Measurement of hepatic steatosis area

Following the staining of neutral lipids in frozen-fixed, cryostat-embedded liver sections (4-mm-thick) with oil red O (Sigma-Aldrich, St. Louis, MO), areas of hepatic steatosis were measured using WinROOF version 5.71 software (Mitani Corporation, Tokyo, Japan) in 10 randomly selected fields (magnification, × 400; Olympus BX51N-34; Olympus Corporation, Tokyo, Japan) per specimen[11].

Immunostaining for α-smooth muscle actin

Immunostaining for α-smooth muscle actin (α-SMA) was used for the detection and counting of activated HSCs. As described previously[11], α-SMA was detected by staining with mouse monoclonal anti-α-SMA antibody (cat. No. MS-113-R7; Thermo Fisher Scientific, Fremont, CA) without dilution. Goat anti-mouse Ig from the Histofine Mouse Stain kit (cat. No. 414322; Nichirei Biosciences, Inc., Tokyo, Japan) was used without dilution as the secondary antibody. HSCs activation was assessed by using WinROOF ver.5.71 software to measure the areas of α-SMA staining in 10 randomly selected fields (magnification × 400; Olympus BX51N-34) per specimen.

Analysis of inflammatory cell infiltration of hepatic tissue

F4/80, a mature mouse cell surface glycoprotein expressed at high levels on Kupffer cells, was immunohistochemically stained using a rat monoclonal anti-mouse F4/80 antibody (cat. No. ab6640; Abcam, Tokyo, Japan) diluted to 1:100 with 0.01 mol/L PBS according to the manufacturer's instructions. Goat anti-rat secondary antibody from the Histofine Simple Stain Mouse MAX-PO (Rat) kit (cat. No. 414311; Nichirei Biosciences, Inc.) was used without dilution. Immunopositive cells were analyzed in 10 intralobular ocular fields (magnification, × 400; Olympus BX41N-34) per specimen[11].

Analysis of oxidative stress

Immunohistochemical staining for 8-hydroxy-2-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, was used to assess oxidative stress[11]. A monoclonal mouse anti-8-OHdG antibody (cat. No. MOG-020P; Nikken SEIL, Shizuoka, Japan) diluted in 200 μL distilled water was used, following the manufacturer's instructions. Goat anti-mouse Ig from the Histofine Mouse Stain kit served as the secondary antibody without dilution. WinROOF ver.5.71 software was used to analyzed immunopositive cells using 10 intralobular ocular fields (magnification × 400; Olympus BX41N-34) per specimen, and values are expressed as the ratios (%) of fields. Also, 4-hydroxynonenal (4-HNE) was semi-quantified *via* immunohistochemical staining using a monoclonal mouse anti-4-HNE antibody (cat. no. MHN-020P; Nikken SEIL) diluted in 200 μL distilled water following the

manufacturer's instructions. Goat anti-mouse Ig from the Histofine Mouse Stain kit was used as the secondary antibody without dilution. Ten randomly selected fields (magnification, × 400) in each 4-HNE-stained specimen were classified into immunopositive grades 1, 2, 3 and 4 (0%-10%, 11%-20%, 21%-30%, and > 30%, respectively) and the mean values of 10 fields were calculated.

RNA extraction and reverse transcription-PCR analysis

As described previously[11], total RNA was extracted from homogenized hepatic tissue samples using the RNeasy Lipid Tissue Mini kit (Qiagen, Hilden, Germany). Absorbance at 260 nm was measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific), to determine RNA concentrations and RNA quality was confirmed by electrophoresis on ethidium bromide-stained 1% agarose gels. Total RNA (2 μg) was reverse transcribed in a final volume of 11.5 μL containing 4 μL of 5 × standard buffer, 2 μL of 0.1 mol/L dithiothreitol, 1 μL of SuperScript Ⅱ RNase H reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA), 2 μL of 10 mol/L MdNTP (Promega, Madison, WI), 1 μL of 50 pmol/μL Random Primer (Promega), 0.5 μL of 100 pmol/μL Oligo (dT)15 Primer (Promega), and 1 μL of 40 U/μL ribonuclease inhibitor (Wako Pure Chemical Industries, Ltd.). Mixtures were incubated at 37 ℃ for 60 min and 95 ℃ for 5 min, and were then cooled to 4 ℃ for 5 min using a MyCycler Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA).

Real-time PCR

Quantitative real-time PCR assays (7900HT Fast Real-time PCR system; Applied Biosystems, Carlsbad, CA) proceeded as described previously[11]. The assays were used a final volume of 10 mL containing 250 nmol/L Universal Probe-Library probe (Roche, Basel, Switzerland), 900 nmol/L forward primer, 900 nmol/L reverse primer, 5 mL EXPRESS qPCR Supermix with Premixed Rox (Invitrogen), and 2 mL cDNA. mRNA level of transforming growth factor-β1 (TGF-β1; GenBank: NM_011577), procollagen-type Ⅰ (GenBank: U08020), connective tissue growth factor (CTGF; GenBank: NM_010217), tumor necrosis factor-α (TNF-α; GenBank: NM_013693), monocyte chemoattractant protein-1 (MCP-1; GenBank: NM_100127112), tissue inhibitor of metalloproteinases-1 (TIMP-1; GenBank: NM_011593), peroxisome proliferator-activated receptor (PPAR-α; GenBank: NM_007988.3), sterol regulatory element-binding protein 1c (SREBP1c; GenBank: NM_011480), microsomal triglyceride transfer protein (MTP; GenBank: NM_008642), endothelin-1 (ET-1; GenBank: NM_010204), endothelin-converting enzyme (ECE; GenBank: NM_199307), endothelin-1 type A receptor (ET-1A; GenBank: NM_010332), and endothelin-1 type B receptor (ET-1B; GenBank: U32329) were assessed using the 7900HT Fast Real-Time PCR System with SDS2.3 software (Applied Biosystems) and with β-actin (GenBank: NM_007393) as an internal standard. Thermal cycle conditions were 95 ℃ for 20 s, followed by 45 cycles of 1 s at 95 ℃ and 20 s at 60 ℃. The relative mRNA expression levels were calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Differences between groups were statistically analyzed using unpaired Student's *t*-tests. All statistical analysis was performed using StatFlex ver.6.0 for Windows software (Artech Co. Ltd., Osaka, Japan). All data are expressed as means ± SD, with *P* values less than 0.05 considered to indicate significant differences.

RESULTS

Characteristics of FLS-ob/ob mice

As shown in Table 1, the two groups of mice did not differ in terms of food consumption, bodyweight, liver weight, liver-to-bodyweight ratio, visceral fat weight, or levels of serum AST and ALT. There was no difference in hepatic histology with hematoxylin-eosin staining between the two groups (Figure 1A and B).

Effects of ambrisentan on hepatic steatosis

To assess the effects of ambrisentan on lipid metabolism, we determined the hepatic steatosis area, hepatic lipid contents, and gene expression of hepatic lipogenesis, lipolysis, and lipid transporter genes. Oil red O staining showed no differences in area of hepatic steatosis between the groups (ambrisentan *vs* control; 15.0% ± 6.0% *vs* 17.0% ± 7.7%; *P* = 0.614; Figure 1C-E). Steatosis-related mRNA expression levels (PPAR-α, SREBP-1c, FAS, and MTP) were not different between the two groups (Table 2). Hepatic total cholesterol and triglyceride contents also revealed no differences between the two groups (Table 1). These findings suggested that ambrisentan did not affect lipid metabolism and accumulation in the liver of FLS-*ob/ob* mice.

Effects of ambrisentan on hepatic fibrosis

To assess whether ambrisentan attenuated hepatic fibrosis, we determined the antifibrotic effects of ambrisentan in the FLS-*ob/ob* mice. Sirius red staining showed that the area of fibrosis was decreased by ambrisentan compared with the control (0.46% ± 0.18% *vs* 1.11% ± 0.28%, respectively, *P* = 0.0003; Figure 1F-H). Hepatic hydroxyproline (Hyp) content was significantly reduced by ambrisentan compared with the control (18.0 μg/g ± 6.1 μg/g liver *vs* 33.9 μg/g ± 13.5 μg/g liver, respectively, *P* = 0.014; Figure 1I). Moreover, the area of positive α-SMA immunostaining was significantly reduced by ambrisentan (0.12% ± 0.08% *vs* 0.25% ± 0.11%, respectively *P* = 0.047; Figure 1J-M).

In relation to extracellular matrix metabolism in the liver, as shown in Table 2, ambrisentan reduced the mRNA expression levels of procollagen-1 by 60% and TIMP-1 by 45% but the mRNA expression of TGF-β1 and CTGF did not differ between the two groups.

Table 1 Effects of ambrisentan administration on various parameters in fatty liver shionogi-*ob/ob* mice

Parameters	Control group (n = 5)	Ambrisentan group (n = 8)	P value
Body weight (g)	47.3 ± 3.6	47.0 ± 4.6	0.27
Liver weight (g)	5.4 ± 1.2	5.1 ± 1.1	0.75
Liver/body weight ratio	0.11 ± 0.02	0.11 ± 0.01	0.68
Visceral fat weight (g)	2.5 ± 0.3	2.7 ± 0.3	0.32
Weekly dietary intake (g)	31.7 ± 9.3	29.4 ± 9.0	0.66
Serum AST (U/L)	143 ± 20	155 ± 43	0.59
Serum ALT (U/L)	120 ± 52	151 ± 65	0.38
Hepatic cholesterol (mg/dL)	24.5 ± 1.56	27.2 ± 2.58	0.06
Hepatic triglyceride (mg/dL)	1152 ± 500	929 ± 210	0.28

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

Effects of ambrisentan on the inflammatory reaction in the liver

The process of hepatic fibrosis is driven primarily by inflammation in response to liver damage. There were fewer F4/80-positive cells in the ambrisentan group than in the control group, but not significantly so (6.5 ± 3.9 *vs* 15.2 ± 11.5, respectively, P = 0.055; Figure 2A-C). Levels of inflammation-related mRNA (TNF-α and MCP-1) did not differ between the two groups (Table 2).

Effects of ambrisentan on oxidative stress

Oxidative stress is involved in the development of NASH. We determined oxidative stress by two methods: 8-OHdG as an index of DNA damage and 4-HNE as an index of lipid peroxidation. Ambrisentan did not affect the ratio of 8-OHdG-positive cells in the liver compared with the control (73.8% ± 12.4% *vs* 78.2% ± 11.5%, respectively, P = 0.538; Figure 2D-F) and did not alter the immunostaining grade for liver 4-HNE (2.36 ± 0.37 *vs* 2.35 ± 0.41, respectively, P = 0.958; Figure 2G-I).

Effects of ambrisentan on ET-related mRNA in the liver

Finally, we measured ET-related gene expression in FLS-*ob/ob* mice. The levels of ET-related mRNAs (ET-1, ECE, ETAR, and ETBR) were not different between the two groups (Table 2).

Table 2 Hepatic mRNA expression levels of various genes in the control and ambrisentan groups

mRNA	Control group (n = 5)	Ambrisentan group (n = 8)	P value
Procollagen-1	1.76 ± 0.58	1.06 ± 0.43	0.024
TGF-β1	1.60 ± 0.80	1.14 ± 0.17	0.13
CTGF	1.43 ± 0.49	1.52 ± 0.40	0.36
TIMP-1	2.98 ± 1.58	1.34 ± 0.61	0.02
TNF-α	2.37 ± 2.65	2.37 ± 3.02	1
MCP-1	10.20 ± 10.06	8.14 ± 8.90	0.39
SREBP1c	0.69 ± 0.19	0.80 ± 0.17	0.29
FAS	0.76 ± 0.34	0.87 ± 0.46	0.67
PPAR-α	0.81 ± 0.16	0.98 ± 0.27	0.24
MTP	0.95 ± 0.09	0.99 ± 0.09	0.45
ET-1	1.40 ± 0.57	1.47 ± 0.50	0.82
ECE	1.02 ± 0.13	1.23 ± 0.23	0.09
ETAR	3.74 ± 3.35	2.55 ± 1.56	0.4
ETBR	2.07 ± 0.76	1.87 ± 0.49	0.59

TGF: Transforming growth factor; CTGF: Connective tissue growth factor; TIMP: Tissue inhibitor of metalloproteinase; TNF: Tumor necrosis factor; MCP: Monocyte chemoattractant protein; SREBP: Sterol regulatory element-binding protein; FAS: Fatty acid synthase; PPAR: Peroxisome proliferator-activated receptor; MTP: Microsomal triglyceride transfer protein; ET: Endothelin; ECE: Endothelin-converting enzyme; ETAR: Endothelin type A receptor; ETBR: Endothelin type B receptor.

DISCUSSION

This study had two important findings. First, ambrisentan did not affect lipid metabolism. Second, it significantly attenuated the progression of hepatic fibrosis. Thus, ET-1 antagonism reduced hepatic fibrosis without improving hepatic steatosis. Ambrisentan did not reduce body weight, blood glucose levels, or hepatic steatosis compared with the control group. ET-1 is reported to increase lipolysis in human and bovine adipocytes[14]. Therefore, ET-1 may increase the inflow of free fatty acids from the fat tissue into the liver and exacerbate hepatic steatosis. ET-1 reduced the cholesterol efflux in macrophages, resulting in exacerbation of lipid accumulation in macrophages[15]. However, the present study showed that ambrisentan did not affect lipid accumulation in hepatocytes or the contents of hepatic cholesterol and triglyceride. Furthermore, the expression levels of lipid metabolism-related genes-such as SREBP-1c and FAS, which are involved in hepatic lipogenesis[16], PPAR-α, which is involved in β-oxidation of fatty acids, and MTP, which transports triglyceride to very low-density lipoprotein-were not affected by ambrisentan. From these findings, our *in vivo* experiments using FLS-*ob/ob* mice indicated that ETAR antagonism was not involved in hepatic lipid metabolism. Hyperleptinemia is reported to regulate the sensitivity of ET-1 for steatosis in NASH cirrhotic rats[16]. Because the FLS-*ob/ob* mice used in our study are leptin deficient[12], FLS-*ob/ob* mice may have low sensitivity for ET-1 in steatosis, and ET-1 may be less involved in hepatic steatosis in these mice.

Second, we investigated the effect of ambrisentan on hepatic fibrosis. The present study showed that ETAR antagonism reduced the hepatic Hyp content and the area of hepatic fibrosis through the inhibition of HSC activation. Several studies have implicated ET-1 in fibrogenesis of the kidney, cardiovascular system, and liver[2,9,17,18]. HSCs express ETAR and ET type B receptors. ET-1 is secreted from HSCs and acts in HSCs and other cells in an autocrine and paracrine manner. Our previous *in vitro* experiments showed that ET-1 increased fibrogenic gene expression *via* ETAR[17]. Furthermore, Cho *et al*[19] reported that an oral ETAR antagonist attenuated collagen synthesis in rat liver fibrosis due to cholestasis. The present study confirmed that the ETAR antagonist also inhibited hepatic fibrosis in a mouse NASH model. HSCs are activated by several factors and stimulants and produce extracellular matrix proteins. Rocky *et al*[9] and Pinzani *et al*[20] showed that ET-1 increased DNA synthesis and cell growth *via* ETAR in cultured HSCs.

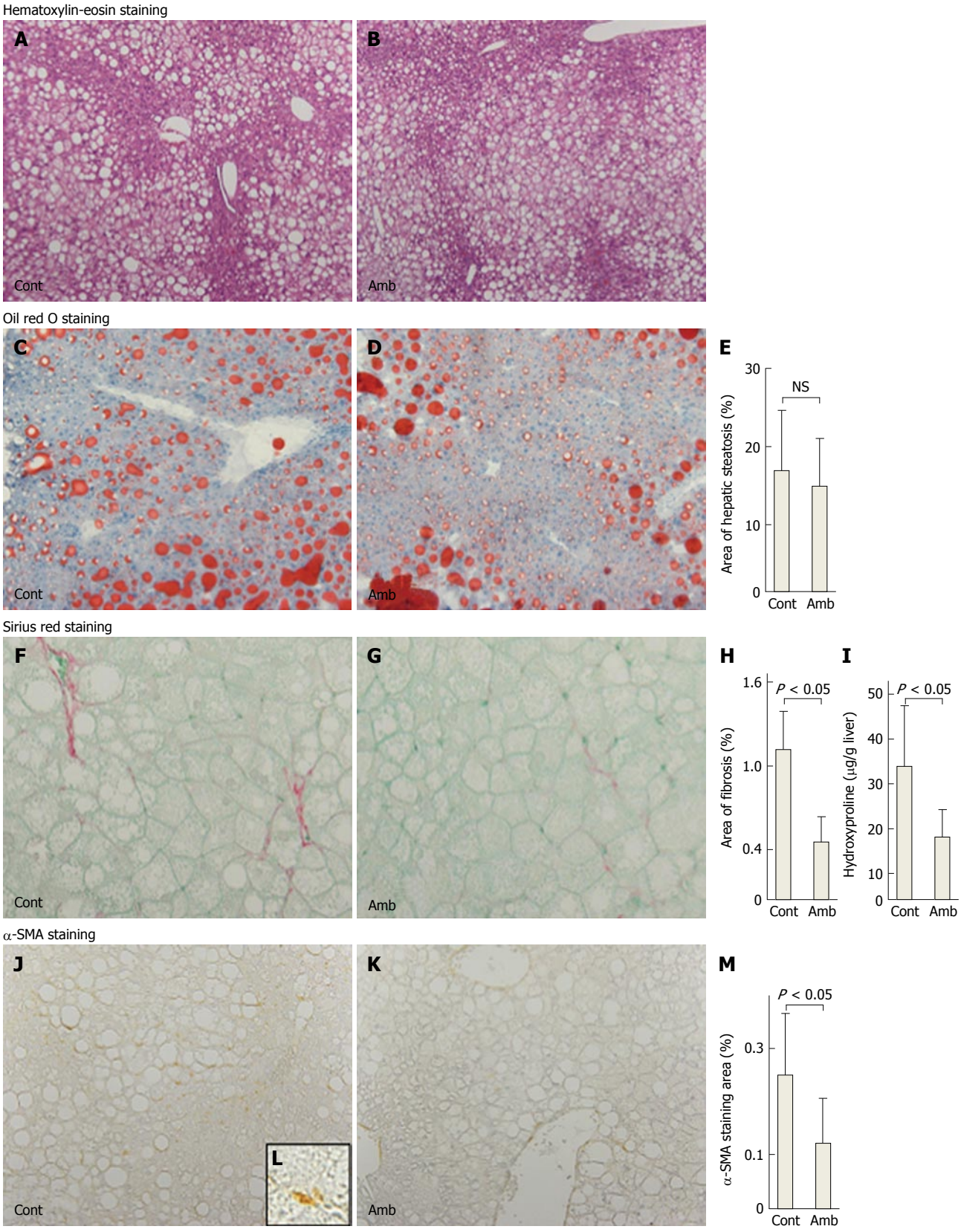

Figure 1 Histological analyses of liver tissues. Representative images of hematoxylin-eosin staining (magnification, × 100) in the (A) control and (B) ambrisentan groups; representative images of oil red O staining (magnification, × 100) in the (C) control and (D) ambrisentan groups; E: The proportion (%) of the hepatic steatosis area stained with oil red O was measured using image analysis. Hepatic fibrosis was determined by Sirius red staining. Representative images of Sirius red staining (magnification, × 400) of the (F) control and (G) ambrisentan groups; the proportion (%) of the hepatic fibrosis area stained with Sirius red was measured using image analysis ($P < 0.01$); H: The area of fibrosis was significantly decreased in the ambrisentan group compared with the control group; I: Comparison of hepatic hydroxyproline content between groups; representative images of α-SMA immunostaining (magnification, × 400) in the (J) control and (K) ambrisentan groups; L: Shows a higher magnification (× 1000) of an α-SMA-positive cell (arrow); M: Quantitation of an area of α-SMA immunostaining measured by image analysis ($P < 0.05$). The area of α-SMA immunostaining was significantly reduced in the ambrisentan group compared with the control. α-SMA: α-smooth muscle actin.

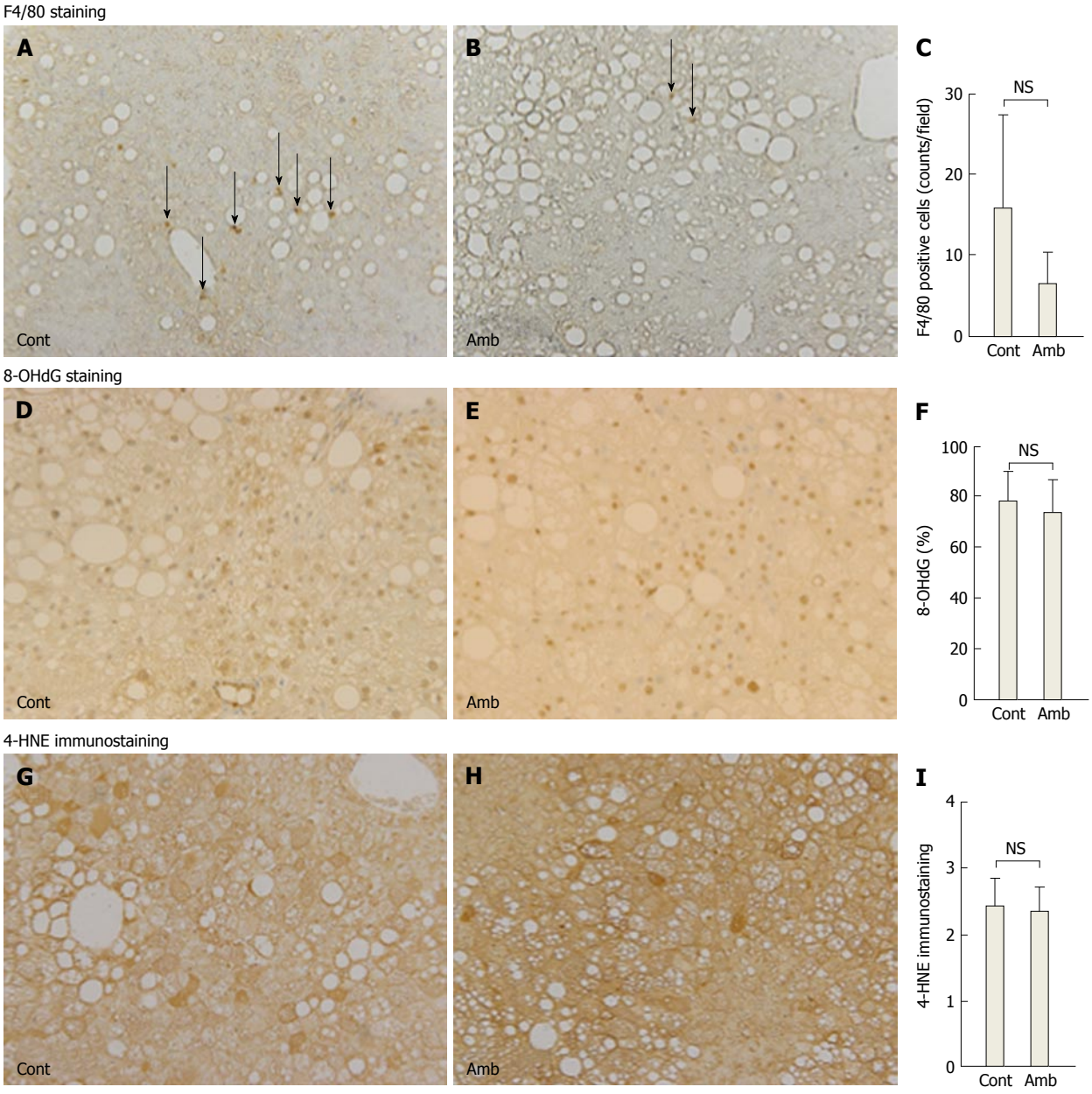

Figure 2 F4/80 immunostaining. Representative images of F4/80 immunostaining (magnification, × 400) of Kupffer cells in the (A) control and (B) ambrisentan groups; C: Numbers of immunopositive F4/80 cells (arrows) in both groups; representative immunostaining for 8-OHdG (magnification, × 400) in the (D) control and (E) ambrisentan groups; F: Comparison of 8-OHdG-immunopositive cells between the groups; immunostaining for 4-HNE (magnification, × 400) in the (G) control and (H) ambrisentan groups; I: Comparison of 4-HNE-immunopositive cells between the groups. 4-HNE: 4-hydroxynonenal.

Our study showed that ETAR antagonism reduced HSC activation. Therefore, in the NASH model, ET-1 is involved in the activation of HSCs *via* ETAR. HSCs are activated by cytokines, oxidative stress, and inflammation. However, ambrisentan did not affect oxidative stress, as assessed by 8-OHdG and 4-HNE, or the inflammatory reaction, as assessed by *TNF-α* and *MCP-1* gene expression or F4/80-positive cells. Therefore, ET-1 may directly activate HSCs.

ET-1 stimulates extracellular matrix protein production by HSCs. In an HSC culture study, ET-1 increased the production of procollagen-1 and TGF-β1 *via* ETAR[17]. However, although the present study indicated that ETAR antagonism attenuated the gene expression of procollagen-1, it did not influence the gene expression of TGF-β1 and CTGF, which is downstream of TGF-β1. This discrepancy may be attributable to the model of liver injury. A previous report[9] showed that ET antagonism reduced TGF-β1 mRNA levels in the carbon tetrachloride model, but its levels were not altered in cholestatic-induced liver injury. Such data showed that the effects of ET-1 antagonism in TGF-β1 may depend on the liver injury model. Therefore, ET-1 might not play a major role in TGF-β1 expression in mild liver injury models such as cholestasis or steatohepatitis.

The present study showed that ETAR antagonism reduced TIMP-1 gene expression. TIMP-1 is a high-affinity inhibitor of many matrix metalloproteinases and suppresses matrix degradation, resulting in the progression of liver

fibrosis. ET-1 is reported to increase TIMP-1 mRNA in fibroblasts[21]. In our study, ETAR antagonism attenuated TIMP-1 expression and might improve hepatic fibrosis by increasing fibrolysis. From these results, it appears that ambrisentan improved hepatic fibrosis by inhibiting HSC activation and suppressing procollagen-1 and *TIMP-1* gene expression.

The present study has some limitations. First, it involved a small number of mice and a relatively short duration of ambrisentan treatment. We included only 8 ambrisentan-treated mice and 5 controls and the study duration was only 4 wk. Therefore, examination of a larger number of mice and a longer administration period is required to validate these results. Second, our experiments did not include non-NASH mice arms because we could not obtain DS mice, the original wild-type of FLS-*ob/ob* mice. Therefore, further study is needed using another NASH mouse model.

In conclusion, ambrisentan attenuated the progression of hepatic fibrosis by suppressing the activation of HSCs and reducing procollagen-1 and TIMP-1 expression.

COMMENTS

Background

In non-alcoholic steatohepatitis (NASH), the serum endothelin-1 (ET-1) level is elevated and is correlated with hepatic fibrosis severity. The development of hepatic fibrosis is mediated to a large extent by the activation of hepatic stellate cells (HSCs). ET-1 serves to activate the HSCs and accelerates collagen fiber synthesis in them. Furthermore, ET-1 acts as a mediator and is elevated in conditions such as insulin resistance, hyperglycemia, oxidative stress, and endothelial cell dysfunction.

Research frontiers

Ambrisentan, a selective ET type A receptor (ETAR) antagonist improves liver fibrosis in cirrhotic rats, but their effects on NASH are unknown. ET-1 may become a novel target for the treatment of NASH.

Applications

The present study has shown ambrisentan improved hepatic fibrosis by inhibiting HSC activation and suppressing procollagen-1 and tissue inhibitor of metalloproteinase-1 (*TIMP-1*) gene expression, but did not affect hepatic steatosis. The combination therapy of ambrisentan with other drugs for lipid accumulation may be more effective for NASH.

Terminology

NASH: Nonalcoholic steatohepatitis is characterized by hepatic fat deposition, inflammation, and differing degrees of fibrosis.

Peer-review

This is an interesting study. The authors report that "ambrisentan" attenuates the progression of hepatic fibrosis by inhibiting the activation of HSCs and reducing procollagen-1 and *TIMP-1* gene expression. According to them it did not affect inflammation and steatosis. No doubt these results are interesting.

REFERENCES

1 **Pascale A**, Pais R, Ratziu V. An overview of nonalcoholic steatohepatitis: past, present and future directions. *J Gastrointestin Liver Dis* 2010; **19**: 415-423 [PMID: 21188334]

2 **Tilg H**, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 2010; **52**: 1836-1846 [PMID: 21038418 DOI: 10.1002/hep.24001]

3 **Degertekin B**, Ozenirler S, Elbeg S, Akyol G. The serum endothelin-1 level in steatosis and NASH, and its relation with severity of liver fibrosis. *Dig Dis Sci* 2007; **52**: 2622-2628 [PMID: 17429733 DOI: 10.1007/s10620-006-9147-8]

4 **Mallat A**, Préaux AM, Serradeil-Le Gal C, Raufaste D, Gallois C, Brenner DA, Bradham C, Maclouf J, Iourgenko V, Fouassier L, Dhumeaux D, Mavier P, Lotersztajn S. Growth inhibitory properties of endothelin-1 in activated human hepatic stellate cells: a cyclic adenosine monophosphate-mediated pathway. Inhibition of both extracellular signal-regulated kinase and c-Jun kinase and upregulation of endothelin B receptors. *J Clin Invest* 1996; **98**: 2771-2778 [PMID: 8981923 DOI: 10.1172/JCI119103]

5 **Ottosson-Seeberger A**, Lundberg JM, Alvestrand A, Ahlborg G. Exogenous endothelin-1 causes peripheral insulin resistance in healthy humans. *Acta Physiol Scand* 1997; **161**: 211-220 [PMID: 9366964 DOI: 10.1046/j.1365-201X.1997.00212.x]

6 **Shaw SG**, Boden PJ. Insulin resistance, obesity and the metabolic syndrome. Is there a therapeutic role for endothelin-1 antagonists? *Curr Vasc Pharmacol* 2005; **3**: 359-363 [PMID: 16248779 DOI: 10.2174/157016105774329471]

7 **Wedgwood S**, Dettman RW, Black SM. ET-1 stimulates pulmonary arterial smooth muscle cell proliferation via induction of reactive oxygen species. *Am J Physiol Lung Cell Mol Physiol* 2001; **281**: L1058-L1067 [PMID: 11597896 DOI: 10.1161/CIRCULATIONAHA.107.742510]

8 **Galiè N**, Olschewski H, Oudiz RJ, Torres F, Frost A, Ghofrani HA, Badesch DB, McGoon MD, McLaughlin VV, Roecker EB, Gerber MJ, Dufton C, Wiens BL, Rubin LJ. Ambrisentan for the treatment of pulmonary arterial hypertension: results of the ambrisentan in pulmonary arterial hypertension, randomized, double-blind, placebo-controlled, multicenter, efficacy (ARIES) study 1 and 2. *Circulation* 2008; **117**: 3010-3019 [PMID: 18506008]

9 **Rockey DC**, Chung JJ. Endothelin antagonism in experimental hepatic fibrosis. Implications for endothelin in the pathogenesis of wound healing. *J Clin Invest* 1996; **98**: 1381-1388 [PMID: 8823303 DOI: 10.1172/JCI118925]

10 **Soga M**, Kishimoto Y, kawaguchi J, Nakai Y, Kawamura Y, Inagaki S, Katoh K, Oohara T, Makino S, Oshima I. The FLS mouse: a new inbred strain with spontaneous fatty liver. *Lab Anim Sci* 1999; **49**: 269-275 [PMID: 10403441]

11 **Kishina M,** Koda M, Kato J, Tokunaga S, Matono T, Sugihara T, Ueki M, Murawaki Y. Therapeutic effects of the direct renin inhibitor, aliskiren, on non-alcoholic steatohepatitis in fatty liver Shionogi ob/ob male mice. *Hepatol Res* 2014; **44**: 888-896 [PMID: 23777387 DOI: 10.1111/hepr.12186]

12 **Soga M**, Hashimoto S, Kishimoto Y, Hirasawa T, Makino S, Inagaki S. Insulin resistance, steatohepatitis, and hepatocellular carcinoma in a new congenic strain of Fatty Liver Shionogi (FLS) mice with the Lep(ob) gene. *Exp Anim* 2010; **59**: 407-419 [PMID: 20660987 DOI: 10.1538/expanim.59.407]

13 **Sugihara T**, Koda M, Kishina M, Kato J, Tokunaga S, Matono T, Ueki M, Murawaki Y. Fatty liver Shionogi-ob/ob mouse: A new candidate for a non-alcoholic steatohepatitis model. *Hepatol Res* 2013; **43**: 547-556 [PMID: 23057725 DOI: 10.1111/j.1872-034X.2012.01101.x]

14 **Eriksson AK**, van Harmelen V, Stenson BM, Aström G, Wåhlén K, Laurencikiene J, Rydén M. Endothelin-1 stimulates human adipocyte lipolysis through the ET A receptor. *Int J Obes* (Lond) 2009; **33**: 67-74 [PMID: 18982011 DOI: 10.1038/ijo.2008.212]

15 **Lin CY**, Lee TS, Chen CC, Chang CA, Lin YJ, Hsu YP, Ho LT. Endothelin-1 exacerbates lipid accumulation by increasing the protein degradation of the ATP-binding cassette transporter G1 in macrophages. *J Cell Physiol* 2011; **226**: 2198-2205 [PMID: 21520072 DOI: 10.1002/jcp.22556]

16 **Yang YY**, Tsai TH, Huang YT, Lee TY, Chan CC, Lee KC, Lin HC. Hepatic endothelin-1 and endocannabinoids-dependent effects of hyperleptinemia in nonalcoholic steatohepatitis-cirrhotic rats. *Hepatology* 2012; **55**: 1540-1550 [PMID: 22183953 DOI: 10.1002/hep.25534]

17 **Koda M**, Bauer M, Krebs A, Hahn EG, Schuppan D, Murawaki Y. Endothelin-1 enhances fibrogenic gene expression, but does not

promote DNA synthesis or apoptosis in hepatic stellate cells. *Comp Hepatol* 2006; **5**: 5 [DOI: 10.1186/1476-5926-5-5]

18 **Arthur MJ**, Mann DA, Iredale JP. Tissue inhibitors of metalloproteinases, hepatic stellate cells and liver fibrosis. *J Gastroenterol Hepatol* 1998; **13** Suppl: S33-S38 [PMID: 9792032]

19 **Cho JJ**, Hocher B, Herbst H, Jia JD, Ruehl M, Hahn EG, Riecken EO, Schuppan D. An oral endothelin-A receptor antagonist blocks collagen synthesis and deposition in advanced rat liver fibrosis. *Gastroenterology* 2000; **118**: 1169-1178 [PMID: 10833492 DOI: 10.1016/S0016-5085(00)70370-2]

20 **Pinzani M**, Milani S, De Franco R, Grappone C, Caligiuri A, Gentilini A, Tosti-Guerra C, Maggi M, Failli P, Ruocco C, Gentilini P. Endothelin 1 is overexpressed in human cirrhotic liver and exerts multiple effects on activated hepatic stellate cells. *Gastroenterology* 1996; **110**: 534-548 [PMID: 8566602 DOI: 10.1053/gast.1996.v110.pm8566602]

21 **Knowles JP**, Shi-Wen X, Haque SU, Bhalla A, Dashwood MR, Yang S, Taylor I, Winslet MC, Abraham DJ, Loizidou M. Endothelin-1 stimulates colon cancer adjacent fibroblasts. *Int J Cancer* 2012; **130**: 1264-1272 [PMID: 21445967]

P- Reviewer: Murdaca G, Ohkoshi S, Sanal MG, Tasci I
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Li D

World Journal of Hepatology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v8.i22.942

World J Hepatol 2016 August 8; 8(22): 942-948
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

Living donor liver transplantation for high model for end-stage liver disease score: What have we learned?

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Author contributions: Dabbous H, Sakr M, Abdelhakam S, Montasser I and El-Meteini M designed the research; Dabbous H and El-Meteini M contributed equally to the work; Dabbous H, Bahaa M, Said H and El-Meteini M performed the research; Dabbous H, Abdelhakam S, Montasser I and El-Meteini M contributed analytic tools; Dabbous H, Sakr M, Abdelhakam S, Montasser I, Bahaa M and El-Meteini M analyzed the data; Dabbous H, Abdelhakam S, Montasser I, Said H and El-Meteini M wrote the paper.

Institutional review board statement: This study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University Institutional Review Board.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous data that were obtained after each patient agreed to management by written consent.

Conflict-of-interest statement: None of the authors have any conflicts of interests and no financial disclosure.

Data sharing statement: The technical appendix, statistical code, and dataset are available from the corresponding author at saratropical@yahoo.com. The participants gave informed consent for the data sharing.

Manuscript source: Invited manuscript

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Received: March 15, 2016
Peer-review started: March 18, 2016
First decision: April 19, 2016
Revised: May 12, 2016
Accepted: July 11, 2016
Article in press: July 13, 2016
Published online: August 8, 2016

Abstract

AIM: To assess the impact of model for end-stage liver disease (MELD) score on patient survival and morbidity post living donor liver transplantation (LDLT).

METHODS: A retrospective study was performed on 80 adult patients who had LDLT from 2011-2013. Nine patients were excluded and 71 patients were divided into two groups; Group 1 included 38 patients with a MELD score < 20, and Group 2 included 33 patients with a MELD score > 20. Comparison between both groups was done regarding operative time, intra-operative blood requirement, intensive care unit (ICU) and hospital stay, infection, and patient survival.

RESULTS: Eleven patients died (15.5%); 3/38 (7.9%)

patients in Group 1 and 8/33 (24.2%) in Group 2 with significant difference (P = 0.02). Mean operative time, duration of hospital stay, and ICU stay were similar in both groups. Mean volume of blood transfusion and cell saver re-transfusion were 8 ± 4 units and 1668 ± 202 mL, respectively, in Group 1 in comparison to 10 ± 6 units and 1910 ± 679 mL, respectively, in Group 2 with no significant difference (P = 0.09 and 0.167, respectively). The rates of infection and systemic complications (renal, respiratory, cardiovascular and neurological complications) were similar in both groups.

CONCLUSION: A MELD score > 20 may predict mortality after LDLT.

Key words: Living donor liver transplantation; Model for end-stage liver disease score; Morbidity; Mortality; Infection

Core tip: We assessed the impact of model for end-stage liver disease (MELD) score on patient survival and morbidity after living donor liver transplantation (LDLT). A total of 71 patients were included and divided into two groups: Group 1 had 38 patients with a MELD score < 20 and Group 2 had 33 patients with a MELD score > 20. We compared between both groups regarding operative time, intra-operative blood requirement, duration of intensive care unit and hospital stay, infection, and patient survival. We found that a MELD score > 20 could predict mortality after LDLT.

Dabbous H, Sakr M, Abdelhakam S, Montasser I, Bahaa M, Said H, El-Meteini M. Living donor liver transplantation for high model for end-stage liver disease score: What have we learned? *World J Hepatol* 2016; 8(22): 942-948 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i22/942.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i22.942

INTRODUCTION

Orthotopic liver transplantation (OLT) is now considered an established treatment option for patients with end-stage liver diseases (ESLD). However, the increasing scarcity of grafts in comparison to the number of waiting patients, as well as the high procedure cost, lead to difficult decisions about how to distribute such scarce organs[1,2]. This highlights the need to identify patients who are likely to have good outcome following liver transplantation[3,4]. The Child-Turcotte-Pugh (CTP) score was originally developed for assessing the outcome of patients with liver cirrhosis and portal hypertension and was extended to stratify patients on the waiting list for liver transplantation[5]. The use of CTP in prioritizing potential liver transplant recipients is limited by several factors. Ascites and hepatic encephalopathy are subjective variables and are affected by medical treatment; also CTP score lacks renal function assessment which strongly affects prognosis in cirrhotic patients[6]. The model for end-stage liver disease (MELD) was first described by Malinchoc *et al*[7] as a mathematical model for predicting postoperative three-month survival for patients who underwent transjugular intrahepatic porto-systemic shunt. The MELD score was then validated as a predictor of mortality for a wide variety of liver diseases[8], including cirrhotic patients awaiting liver transplantation[9]. Afterwards, MELD score was incorporated as a clear and objective system based on easily measurable laboratory parameters to reduce mortality among patients on the waiting list[10,11]. The ideal allocation system should allocate livers to candidates who are most likely to die without transplantation, and also to those who have a high probability of survival after OLT[12]. In February 2002, the United Network for Organ Sharing introduced a new allocation policy for cadaveric liver transplants based on the MELD score[13]. This new policy stratified patients according to the risk of death while they are on the waiting list[14]. The impact of the MELD score on postoperative mortality is still elusive.

The aim of this retrospective study was to assess the impact of the MELD score on patient survival and morbidity post living donor liver transplantation (LDLT).

MATERIALS AND METHODS

Between January 2011 and January 2013, 80 adult patients with ESLD had received LDLT at the Ain Shams Center for Organ Transplant, Cairo, Egypt. Nine patients were excluded: Three had small-for-size grafts; one recipient had a combined organ (liver and kidney) transplant and 5 recipients had incomplete follow-up records. The remaining 71 transplants were included in this retrospective study. Seventy patients had LDLT with a right liver graft, and one patient had a left liver graft. The graft recipient weight ratio was between 0.8 and 1.7. The immunosuppressive regimen included cyclosporine or tacrolimus, mycophenolate mofetil (MMF), and corticosteroids in all patients except those transplanted for hepatocellular carcinoma (HCC). In patients transplanted for HCC, the regimen included calcineurin inhibitor and steroids only. Trough levels of cyclosporine were maintained between 200 and 300 ng/mL. Trough levels of tacrolimus were maintained between 8 and 12 ng/mL. Rapid withdrawal of corticosteroids within three months was routine in all patients (all transplanted for hepatitis C virus). In cases of acute rejection, the first-line therapy consisted of optimization of the maintenance level of immunosuppression. If there was no response, then MMF or rapamycin were added to the patient's regimen, if not already being taken. In some cases, a shift from cyclosporine to tacrolimus was beneficial. A small dose of steroids was used if all other measures failed.

The seventy one patients included in this study were

Table 1 Demographic data, Child classification, and cold and warm ischemia time among the studied groups *n* (%)

Variable	MELD < 20 (*n* = 38)	MELD > 20 (*n* = 33)
Age (yr) (mean ± SD)	47.8 ± 7.8	46.2 ± 7.9
Sex		
Male	34 (89.5	32 (97)
Female	4 (10.5)	1 (3)
Diagnosis		
ESLD	27 (71.1)	26 (78.8)
HCC	3 (7.9)	0
ESLD + HCC	8 (21)	7 (21.2)
Child-Turcotte-Pugh		
A	0	0
B	3 (7.9)	0
C	35 (92.1)	33 (100)
Cold ischemia time (min)	47 ± 23	42 ± 30
Warm ischemia time (min)	54.4 ± 20.2	53.7 ± 16.9

ESLD: End-stage liver disease; HCC: Hepatocellular carcinoma; MELD: Model for end-stage liver disease.

divided into two groups. Group 1 included 38 patients with a MELD score less than 20, and Group 2 included 33 patients with a MELD score more than 20.

The MELD score was calculated using laboratory results collected immediately before liver transplantation with no adjustments for malignancy. We calculated the MELD score using the following formula: MELD = [0.957 × ln (creatinine mg/dL) + 0.378 × ln (bilirubin mg/dL) + 1.12 × ln (INR) + 0.643 × 10^8]. We reported the age, sex of the recipient, diagnosis, indication for liver transplantation, modified CTP score as well as cold and warm ischemia time. The diagnosis of chronic liver disease was confirmed by histopathology of the explanted liver. The modified CTP score was calculated and each patient was categorized as A, B, or C. Operative data (including operative time and intra-operative blood transfusion) and early post-operative outcomes [including intensive care unit (ICU) stay, hospital stay, incidence of infection and other morbidities including renal impairment, cardiovascular, respiratory and neurological complications] were compared between the two groups. Overall patient survival was also compared between the two groups. Survival was calculated using the date of transplant to either 5 years post-transplant or to the end-point of this study in January 2016.

Statistical analysis

Categorical data were presented as numbers and percentages. Quantitative data were presented as the mean, standard deviations, ranges, median and interquartile ranges. For qualitative data, the comparison between the two groups was performed by using the χ^2 test and Fisher exact test. For quantitative data, the comparison between the two groups was performed using an independent *t*-test for parametric data and a Mann-Whitney test for non-parametric data. The Kaplan-Meier survival analysis was used to assess the overall survival of both groups. The confidence interval was set to 95%, and the margin of error that was accepted was set to 5%. All data were analyzed using SPSS version 17. A *P*-value more than 0.05 was considered to indicate a non-significant (NS) difference between the two groups; a *P*-value less than 0.05 was considered to be statistically significant (S).

The statistical methods of this study were reviewed by Ahmed Mukhtar, Department of Anesthesia and Critical Care, Diploma of Medical Biostatistics, Faculty of Medicine, Cairo University, Cairo, Egypt.

RESULTS

This retrospective study included 71 patients classified into two groups according to their preoperative MELD score. Demographic data, Child classification, and cold and warm ischemia time were comparable between both groups (Table 1).

MELD score and survival

Overall patient survival was compared between both groups from the date of transplant to 5 years post-transplant or to the end-point of this study in January 2016. Eleven patients (15.5%) died during this study: Three patients out of 38 (7.9%) in Group 1 with a MELD less than 20 and 8 patients out of 33 (24.2%) in Group 2 with a MELD more than 20.

The 1, 3 and 5-year survival rates in Group 1 were 94.7%, 94.7% and 92.1% respectively, in comparison to 81.8%, 81.8% and 75.8% respectively in Group 2 with statistically significant difference between both groups ($P = 0.02$). Mortality occurred mainly in the early postoperative period in ICU because of respiratory failure due to weak respiratory muscles with poor weaning capability from mechanical ventilation (two patients in Group 1 and six patients in Group 2).

Figure 1 shows the Kaplan-Meier curve for overall survival of both groups, where Group 1 patients had a statistically significant higher overall survival rate compared to Group 2 patients.

MELD score and hospital stay

In this study, there was no statistically significant difference observed among the two groups with regard to mean hospital and ICU stay. In Group 1, the mean hospital stay was 30 ± 14 d in comparison to 29 ± 18 d in Group 2 ($P = 0.937$). The mean ICU stay in Group 1 was 7 ± 3 d, while in Group 2, it was 9 ± 4 d ($P = 0.315$).

MELD score and operative data

There was no statistically significant difference between the groups with respect to operative time, blood loss, and intra-operative blood transfusion (cell saver, blood product). The mean operative time in Group 1 was 11.1 ± 2 h (with a range of 7-15 h), and in Group 2, it was 10.6 ± 1.4 h (with a range of 9-14 h), ($P = 0.292$). The mean volume of blood transfusion and cell saver re-transfusion were 8 ± 4 units and 1668 ± 202 mL, respectively, in Group 1 in comparison to 10 ± 6 units and

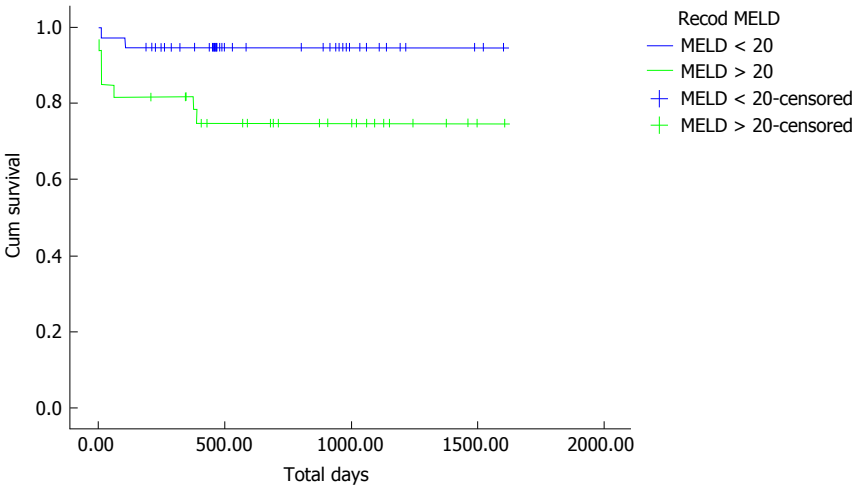

Figure 1 Kaplan-Meier curve for overall survival of both groups. The Group 1 patients that had a MELD score < 20 had higher overall survival rates than the Group 2 patients that had a MELD score > 20. MELD: Model for end-stage liver disease; Cum survival: Cumulative survival.

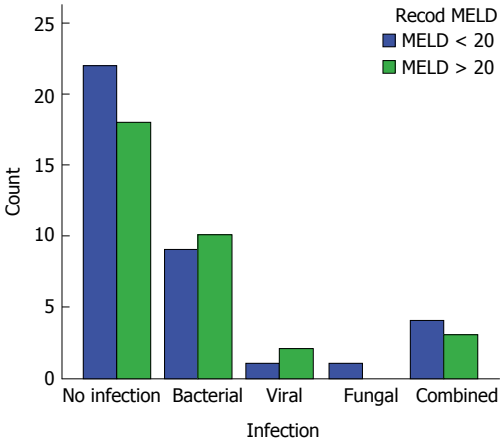

Figure 2 Infection rates in both groups. MELD: Model for end-stage liver disease.

1910 ± 679 mL, respectively, in Group 2 (P = 0.09 and 0.167).

MELD score and postoperative complications

Infection: The overall incidence of infection in this study was 42.3% (30 out of 71 patients). In Group 1, the incidence of infection was 39.5% (15/38 patients). Bacterial infection was the most common representing 23.6% of the patients, while viral infection [cytomegalovirus (CMV)] was detected in 2.6%, fungal in 2.6% and combined infection in 10.5%. In Group 2, the incidence of infection was 45.5% (15/33 patients). Bacterial infection was the most common type of infection, representing 30.3%, while viral infection (CMV) was detected in 6%, fungal in 0% and combined infection in 9.1%. No statistically significant difference was detected between the groups regarding infection rates (P = 0.79) (Figure 2).

Systemic complications: There were no significant differences observed between groups with regard to the incidence of systemic complications including renal, respiratory, cardiovascular, and neurological complications (34.2% and 45.5% in Groups 1 and 2, respectively, P = 0.869).

Renal impairment was the most common complication in both group (10.5% in Group 1 and 15.2% in Group 2), followed by cardiovascular complications (13.2% in Group 1 and 12.1% in Group 2) consisting of mainly hypertension in most patients and arrhythmia in 2 patients. Neurological complications occurred in 2.6% and in 3% of the patients in Groups 1 and 2, respectively. Respiratory complications (basal atelectasis, pleural effusion, adult respiratory distress syndrome and respiratory infection) occurred in 7.9% of the patients in Group 1 compared to 15.2% in Group 2. Two patients in Group 1 (5.3%) and 2 patients in Group 2 (6.1%) had a combined respiratory and other system complications (Figure 3).

DISCUSSION

The large imbalance between patient demand and donated organs is a pressing problem in LDLT. The best solution to this problem is still a matter of debate. Unfortunately, prioritizing extremely sick patients makes it likely that patients who are not as sick will be forced to wait until getting worse and their chances for success become also diminished[15]. Patients who are very sick may have worse post-transplant outcomes than healthier patients[16]. Thus, the optimal system would offer grafts to those who are sufficiently sick to justify the transplantation but not too sick to benefit from it[17]. The urgency of need should be optimized with the likelihood of satisfactory postoperative outcomes so as to avoid "ineffective transplantation"[18].

An accurate prognostic model could also help potential transplant recipients and their families make decisions by providing them with information on the patient's survival probability post-transplantation[19,20]. The MELD score was achieved to help prioritizing prospective liver allograft recipients. Its accuracy to predict short-term mortality

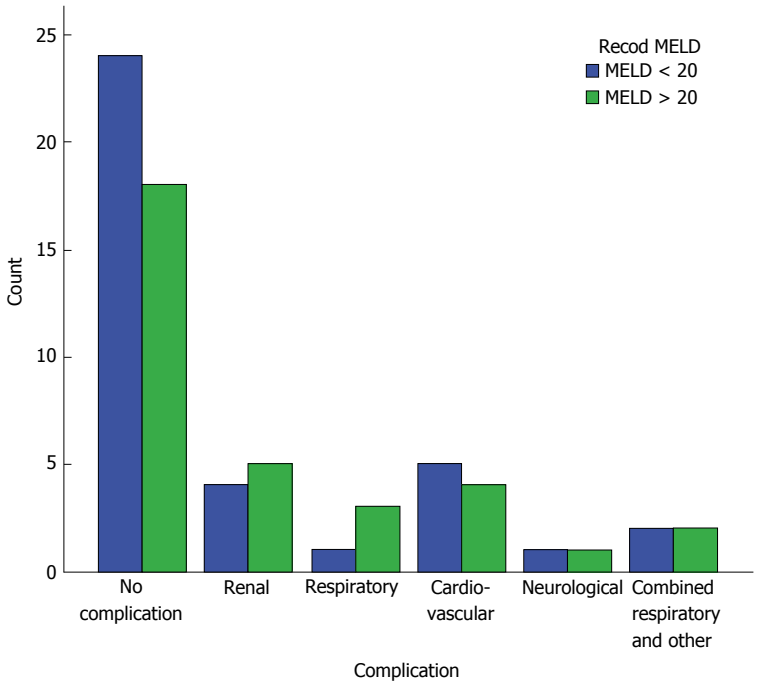

Figure 3 Incidence of systemic complications in both groups. MELD: Model for end-stage liver disease.

among patients with end-stage liver disease has been largely established[21]. However, an ideal selection system should incorporate predictions for survival while the patient is on the waiting list as well as following transplantation. The development of a model that may predict post-transplant outcomes based on pre-transplant variables is difficult because of variation in surgical skills and chance events that occur in the perioperative period. In addition to other factors such as graft rejection, biliary and vascular complications which are independent of pre-transplant events. Although it seems reasonable that pre-transplant variables which constitute the MELD score may influence the immediate post-transplant phase, their ability to predict long term outcome appears less likely. Recently, several investigators examined the predictive value of MELD for post-transplantation outcome, but with conflicting results and limited follow-up period; thus, a clear consensus has not emerged yet[22,23].

In a systematic review about the performance of MELD score in the setting of liver transplantation, Cholongitas *et al*[9] concluded that the MELD is not a good predictor for short-term mortality following liver transplantation, and further studies were needed to assess its long term performance. Additionally, Batista *et al*[24] demonstrated that the preoperative MELD score showed low overall accuracy for predicting survival after liver transplantation; similar to what was described in other Brazilian studies. On the other hand, worse survival rates in recipients with higher MELD scores has been reported by some authors[25-27]. The current study confirms the relation between MELD score and post liver transplantation survival. The incidence of mortality was 7.9% in patients with a MELD score less than 20 compared to 24.2% in patients with a MELD greater than 20, with significant difference between both groups (P = 0.02).

Our study shows no significant impact of MELD score on the duration of hospital and ICU stay; these findings are comparable with those of Poon *et al*[28], while many studies such as Foxton *et al*[29], demonstrated that liver transplantation of patients with higher MELD scores resulted in an increased ICU and hospital stay as well as increased need for renal replacement therapy. Additionally, Buchanan *et al*[30] showed that patients in the highest MELD group had a longer ICU stay than those in the lower MELD group (P = 0.008). Lee *et al*[31] and Massicotte *et al*[32] concluded that the MELD score did not predict blood loss or blood product requirements during liver transplantation, while others such as Feng *et al*[33] demonstrated that massive blood transfusion during liver transplantation can be predicted by preoperative MELD score. In our study, no definite relation was detected between MELD score and intra-operative blood loss or requirements of blood transfusion.

In the current study, the incidence of infection was comparable between both groups with no significant difference between a MELD score that was less or more than 20. This conclusion is the same finding of Li *et al*[34] in which a univariate analysis of risk factors for post-operative bacterial and fungal infections showed no statistically significant difference in regards to the MELD score. However, in the study of Selzner *et al*[35], high MELD score recipients had more frequent postoperative pneumonia in comparison to those with low MELD (P = 0.003), while no differences were observed in rates of biliary complications or overall infections.

In conclusion, a MELD score more than 20 can predict poor overall survival post LDLT. No significant relation was found between MELD score and intra-operative blood

loss or blood requirement, hospital and ICU stay, or post LDLT morbidity.

COMMENTS

Background

Orthotopic liver transplantation has become an established treatment approach for patients with end-stage liver disease, but the growing scarcity of grafts compared to numbers of waiting patients, and the high cost of this procedure, make it difficult to make decisions about how to distribute such scarce organs. The impact of the model for end-stage liver disease (MELD) score on postoperative mortality and morbidity following liver transplantation is not well-established yet.

Research frontiers

The authors assessed the impact of MELD score on patient survival and morbidity post living donor liver transplantation (LDLT) in the current retrospective study that was performed on 71 adult patients who had LDLT from 2011-2013. They were divided into two groups: Group 1 included 38 patients with a MELD score < 20, and Group 2 included 33 patients with a MELD score > 20. They found that MELD score > 20 can predict poor overall survival post LDLT. No significant relation was found between MELD score and intra-operative blood loss or blood requirement, hospital and intensive care unit stay, or post LDLT morbidity.

Innovations and breakthroughs

This is the first Egyptian study that addresses the impact of MELD score on patient survival and morbidity post living donor liver transplantation.

Applications

The findings of this study may represent a future strategy that may help prioritize prospective liver allograft recipients and predict post-transplantation outcome.

Terminology

The MELD score is a mathematical model based on easily measurable laboratory tests. It is calculated immediately prior to liver transplantation through the following formula: MELD = [0.957 × ln (creatinine mg/dL) + 0.378 × ln (bilirubin mg/dL) + 1.12 × ln (INR) + 0.643 × 10^{8}].

Peer-review

The authors have done a retrospective study on impact of MELD score on patient survival and morbidity after living donor liver transplantation. The data may be useful for liver transplantation.

REFERENCES

1 **Merion RM**, Schaubel DE, Dykstra DM, Freeman RB, Port FK, Wolfe RA. The survival benefit of liver transplantation. *Am J Transplant* 2005; **5**: 307-313 [PMID: 15643990 DOI: 10.1111/j.1600-6143.2004.00703.x]

2 **Biggins SW**. Beyond the numbers: rational and ethical application of outcome models for organ allocation in liver transplantation. *Liver Transpl* 2007; **13**: 1080-1083 [PMID: 17663407 DOI: 10.1002/lt.21210]

3 **Schaubel DE**, Sima CS, Goodrich NP, Feng S, Merion RM. The survival benefit of deceased donor liver transplantation as a function of candidate disease severity and donor quality. *Am J Transplant* 2008; **8**: 419-425 [PMID: 18190658 DOI: 10.1111/j.1600-6143.2007.02086.x]

4 **Schaubel DE**, Guidinger MK, Biggins SW, Kalbfleisch JD, Pomfret EA, Sharma P, Merion RM. Survival benefit-based deceased-donor liver allocation. *Am J Transplant* 2009; **9**: 970-981 [PMID: 19341419 DOI: 10.1111/j.1600-6143.2009.02571.x]

5 **Christensen E**. Prognostic models including the Child-Pugh, MELD and Mayo risk scores--where are we and where should we go? *J Hepatol* 2004; **41**: 344-350 [PMID: 15288486 DOI: 10.1016/j.jhep.2004.06.005]

6 **Durand F**, Valla D. Assessment of the prognosis of cirrhosis: Child-Pugh versus MELD. *J Hepatol* 2005; **42** Suppl: S100-S107 [PMID: 15777564 DOI: 10.1016/j.jhep.2004.11.015]

7 **Malinchoc M**, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871 [PMID: 10733541 DOI: 10.1053/he.2000.5852]

8 **Boursier J**, Cesbron E, Tropet AL, Pilette C. Comparison and improvement of MELD and Child-Pugh score accuracies for the prediction of 6-month mortality in cirrhotic patients. *J Clin Gastroenterol* 2009; **43**: 580-585 [PMID: 19197195 DOI: 10.1097/MCG.0b013e3181889468]

9 **Cholongitas E**, Marelli L, Shusang V, Senzolo M, Rolles K, Patch D, Burroughs AK. A systematic review of the performance of the model for end-stage liver disease (MELD) in the setting of liver transplantation. *Liver Transpl* 2006; **12**: 1049-1061 [PMID: 16799946 DOI: 10.1002/lt.20824]

10 **Tenório AL**, Macedo FI, Miranda LE, Fernandes JL, da Silva CM, Neto OL, Lacerda CM. Survival on waiting list for liver transplantation before and after introduction of the model for end-stage liver disease score. *Transplant Proc* 2010; **42**: 407-411 [PMID: 20304152 DOI: 10.1016/j.transproceed.2010.01.005]

11 **Freeman RB**, Wiesner RH, Edwards E, Harper A, Merion R, Wolfe R. Results of the first year of the new liver allocation plan. *Liver Transpl* 2004; **10**: 7-15 [PMID: 14755772 DOI: 10.1002/lt.20024]

12 **Ghobrial RM**, Gornbein J, Steadman R, Danino N, Markmann JF, Holt C, Anselmo D, Amersi F, Chen P, Farmer DG, Han S, Derazo F, Saab S, Goldstein LI, McDiarmid SV, Busuttil RW. Pretransplant model to predict posttransplant survival in liver transplant patients. *Ann Surg* 2002; **236**: 315-322; discussion 322-323 [PMID: 12192318]

13 **Martin AP**, Bartels M, Hauss J, Fangmann J. Overview of the MELD score and the UNOS adult liver allocation system. *Transplant Proc* 2007; **39**: 3169-3174 [PMID: 18089345 DOI: 10.1016/j.transproceed.2007.04.025]

14 **Ravaioli M**, Grazi GL, Ballardini G, Cavrini G, Ercolani G, Cescon M, Zanello M, Cucchetti A, Tuci F, Del Gaudio M, Varotti G, Vetrone G, Trevisani F, Bolondi L, Pinna AD. Liver transplantation with the Meld system: a prospective study from a single European center. *Am J Transplant* 2006; **6**: 1572-1577 [PMID: 16827857 DOI: 10.1111/j.1600-6143.2006.01354.x]

15 **UNOS**. Rationale for Objectives of Equitable Organ Allocation. [accessed 2011 Aug 15]. Available from: URL: http//www.unos.org/resources/bioethics.asp?index = 10

16 **Neuberger J**, Gimson A, Davies M, Akyol M, O'Grady J, Burroughs A, Hudson M. Selection of patients for liver transplantation and allocation of donated livers in the UK. *Gut* 2008; **57**: 252-257 [PMID: 17895356]

17 **Dawwas MF**, Gimson AE. Candidate selection and organ allocation in liver transplantation. *Semin Liver Dis* 2009; **29**: 40-52 [PMID: 19235658 DOI: 10.1055/s-0029-1192054]

18 **Zhang M**, Yin F, Chen B, Li YP, Yan LN, Wen TF, Li B. Pretransplant prediction of posttransplant survival for liver recipients with benign end-stage liver diseases: a nonlinear model. *PLoS One* 2012; **7**: e31256 [PMID: 22396731 DOI: 10.1371/journal.pone.0031256]

19 **Jacob M**, Lewsey JD, Sharpin C, Gimson A, Rela M, van der Meulen JH. Systematic review and validation of prognostic models in liver transplantation. *Liver Transpl* 2005; **11**: 814-825 [PMID: 15973726 DOI: 10.1002/lt.20456]

20 **Lewsey JD**, Dawwas M, Copley LP, Gimson A, Van der Meulen JH. Developing a prognostic model for 90-day mortality after liver transplantation based on pretransplant recipient factors. *Transplantation* 2006; **82**: 898-907 [PMID: 17038904]

21 **Kamath PS**, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: 11172350 DOI: 10.1053/jhep.2001.22172]

22 **Brown RS**, Kumar KS, Russo MW, Kinkhabwala M, Rudow DL, Harren P, Lobritto S, Emond JC. Model for end-stage liver disease

and Child-Turcotte-Pugh score as predictors of pretransplantation disease severity, posttransplantation outcome, and resource utilization in United Network for Organ Sharing status 2A patients. *Liver Transpl* 2002; **8**: 278-284 [PMID: 11910574 DOI: 10.1053/jlts.2002.31340]

23 **Jacob M**, Copley LP, Lewsey JD, Gimson A, Toogood GJ, Rela M, van der Meulen JH. Pretransplant MELD score and post liver transplantation survival in the UK and Ireland. *Liver Transpl* 2004; **10**: 903-907 [PMID: 15237375 DOI: 10.1002/lt.20169]

24 **Batista TP**, Sabat BD, Melo PS, Miranda LE, Fonseca-Neto OC, Amorim AG, Lacerda CM. Employment of MELD score for the prediction of survival after liver transplantation. *Rev Col Bras Cir* 2012; **39**: 105-111 [PMID: 22664516 DOI: 10.1590/S0100-69912012000200005]

25 **Brandão A**, Fuchs SC, Gleisner AL, Marroni C, Zanotelli ML, Cantisani G. MELD and other predictors of survival after liver transplantation. *Clin Transplant* 2009; **23**: 220-227 [PMID: 19210688 DOI: 10.1111/j.1399-0012.2008.00943.x]

26 **Monteiro F**, Coria SA, Boni R, Pereira LA. Model for end-stage liver disease: impact of the new deceased donor liver allocation policy in São Paulo, Brazil. *Transplant Proc* 2009; **41**: 226-228 [PMID: 19249520 DOI: 10.1016/j.transproceed.2008.09.059]

27 **Yoo HY**, Thuluvath PJ. Short-term postliver transplant survival after the introduction of MELD scores for organ allocation in the United States. *Liver Int* 2005; **25**: 536-541 [PMID: 15910490 DOI: 10.1111/j.1478-3231.2005.01011.x]

28 **Poon KS**, Chen TH, Jeng LB, Yang HR, Li PC, Lee CC, Yeh CC, Lai HC, Su WP, Peng CY, Chen YF, Ho YJ, Tsai PP. A high model for end-stage liver disease score should not be considered a contraindication to living donor liver transplantation. *Transplant Proc* 2012; **44**: 316-319 [PMID: 22410005 DOI: 10.1016/j.transproceed.2012.02.006]

29 **Foxton MR**, Al-Freah MA, Portal AJ, Sizer E, Bernal W, Auzinger G, Rela M, Wendon JA, Heaton ND, O'Grady JG, Heneghan MA. Increased model for end-stage liver disease score at the time of liver transplant results in prolonged hospitalization and overall intensive care unit costs. *Liver Transpl* 2010; **16**: 668-677 [PMID: 20440776 DOI: 10.1002/lt.22027]

30 **Buchanan P**, Dzebisashvili N, Lentine KL, Axelrod DA, Schnitzler MA, Salvalaggio PR. Liver transplantation cost in the model for end-stage liver disease era: looking beyond the transplant admission. *Liver Transpl* 2009; **15**: 1270-1277 [PMID: 19790155 DOI: 10.1002/lt.21802]

31 **Lee J**, Chung MY. Does the model for end-stage liver disease score predict transfusion amount, acid-base imbalance, haemodynamic and oxidative abnormalities during living donor liver transplantation? *J Int Med Res* 2011; **39**: 1773-1782 [PMID: 22117978]

32 **Massicotte L**, Beaulieu D, Roy JD, Marleau D, Vandenbroucke F, Dagenais M, Lapointe R, Roy A. MELD score and blood product requirements during liver transplantation: no link. *Transplantation* 2009; **87**: 1689-1694 [PMID: 19502961 DOI: 10.1097/TP.0b013e3181a5e5f1]

33 **Feng ZY**, Jin XD, Chen YZ. [Predictors of massive blood transfusion in liver transplantation for patients with benign end-stage liver disease]. *Zhonghua Yi Xue Za Zhi* 2008; **88**: 3040-3044 [PMID: 19192401]

34 **Li C**, Wen TF, Mi K, Wang C, Yan LN, Li B. Analysis of infections in the first 3-month after living donor liver transplantation. *World J Gastroenterol* 2012; **18**: 1975-1980 [PMID: 22563180 DOI: 10.3748/wjg.v18.i16.1975]

35 **Selzner M**, Kashfi A, Cattral MS, Selzner N, McGilvray ID, Greig PD, Levy GA, Renner EL, Grant DR. Live donor liver transplantation in high MELD score recipients. *Ann Surg* 2010; **251**: 153-157 [PMID: 19858705 DOI: 10.1097/SLA.0b013e3181bc9c6a]

P- Reviewer: He JY, Julie NL, Srivastava M
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DOI: 10.4254/wjh.v8.i22.949

World J Hepatol 2016 August 8; 8(22): 949-956
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

Boceprevir or telaprevir in hepatitis C virus chronic infection: The Italian real life experience

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Author contributions: All authors of CLEO study group equally contributed to conception and design of the study, acquisition of data, review the draft, and approved the final version.

Institutional review board statement: The study was reviewed and approved by the CLEO Governing Board.

Informed consent statement: All study participants, or their legal representative, provided verbal informed consent prior to study enrolment as decided by the CLEO Governing Board and according to the local rules.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest regarding this study.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

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Received: February 23, 2016
Peer-review started: February 24, 2016
First decision: April 15, 2016
Revised: June 23, 2016
Accepted: July 20, 2016
Article in press: July 22, 2016
Published online: August 8, 2016

Abstract

AIM: To check the safety and efficacy of boceprevir/telaprevir with peginterferon/ribavirin for hepatitis C virus (HCV) genotype 1 in the real-world settings.

METHODS: This study was a non-randomized, observational, prospective, multicenter. This study involved 47 centers in Italy. A database was prepared for the homogenous collection of the data, was used by all of the centers for data collection, and was updated continuously. All of the patients enrolled in this study were older than 18 years of age and were diagnosed with chronic infection due to HCV genotype 1. The HCV RNA testing was performed using COBAS-TaqMan2.0 (Roche, LLQ 25 IU/mL).

RESULTS: All consecutively treated patients were included. Forty-seven centers enrolled 834 patients as follows: Male 64%; median age 57 (range 18-78), of whom 18.3% were over 65; mean body mass index 25.6 (range 16-39); genotype 1b (79.4%); diagnosis of cirrhosis (38.2%); and fibrosis F3/4 (71.2%). The following drugs were used: Telaprevir (66.2%) and PEG-IFN-alpha2a (67.6%). Patients were naïve (24.4%), relapsers (30.5%), partial responders (14.8%) and null responders (30.3%). Overall, adverse events (AEs) occurred in 617 patients (73.9%) during the treatment. Anemia was the most frequent AE (52.9% of cases), especially in cirrhotic. The therapy was stopped for 14.6% of the patients because of adverse events or virological failure (15%). Sustained virological response was achieved in 62.7% of the cases, but was 43.8% in cirrhotic patients over 65 years of age.

CONCLUSION: In everyday practice, triple therapy is safe but has moderate efficacy, especially for patients over 65 years of age, with advanced fibrosis, non-responders to peginterferon + ribavirin.

Key words: Boceprevir; Telaprevir; Chronic hepatitis; Antiviral therapy; Peg-interferon; Ribavirin

Core tip: This study describes the role of antiviral therapy for chronic hepatitis C virus infections in everyday practice. Boceprevir or telaprevir, in combination with pegylated interferon and ribavirin, were used in this multicenter study organized by the Italian Association of Hospital Hepatologists (CLEO). A total of 834 patients were enrolled with this first available combination of direct-acting antiviral drugs. The data on the efficacies were quite similar to those produced by the registration studies; however, in the real world experience, patients were older and had more advanced liver disease. In this category of patients, the sustained virological response was less than 50%.

CLEO Study Group; Ascione A, Adinolfi LE, Amoroso P, Andriulli A, Armignacco O, Ascione T, Babudieri S, Barbarini G, Brogna M, Cesario F, Citro V, Claar E, Cozzolongo R, D'Adamo G, D'Amico E, Dattolo P, De Luca M, De Maria V, De Siena M, De Vita G, Di Giacomo A, De Marco R, De Stefano G, De Stefano G, Di Salvo S, Di Sarno R, Farella N, Felicioni L, Fimiani B, Fontanella L, Foti G, Furlan C, Giancotti F, Giolitto G, Gravina T, Guerrera B, Gulminetti R, Iacobellis A, Imparato M, Iodice A, Iovinella V, Izzi A, Liberti A, Leo P, Lettieri G, Luppino I, Marrone A, Mazzoni E, Messina V, Monarca R, Narciso V, Nosotti L, Pellicelli AM, Perrella A, Piai G, Picardi A, Pierri P, Pietromatera G, Resta F, Rinaldi L, Romano M, Rossini A, Russello M, Russo G, Sacco R, Sangiovanni V, Schiano A, Sciambra A, Scifo G, Simeone F, Sullo A, Tarquini P, Tundo P, Vallone A. Boceprevir or telaprevir in hepatitis C virus chronic infection: The Italian real life experience. *World J Hepatol* 2016; 8(22): 949-956 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i22/949.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i22.949

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is one of the main causes of liver cirrhosis, end-stage liver disease, hepatocellular carcinoma (HCC) and liver transplantation worldwide. Pegylated interferon-alpha (P) and ribavirin (R) have been the backbone of HCV treatment for more than a decade. In 2011, the approval of telaprevir (TVR) and boceprevir (BOC), two protease inhibitors (PI), opened the first generation of direct antiviral agents (DAAs) for the treatment of genotype 1 HCV infection.

In many randomized studies, triple therapy (the combination of P plus R with PI, such as TVR or BOC) is demonstrated to be more effective than P plus R alone, with an increased likelihood of sustained virological response (SVR) of more than 30%, when compared with the dual therapy (P + R), reaching 68%-75% of naive patients and 29%-83% of the experienced patients depending on the previous response to P + R[1-4]. The increase in SVR is associated with more side effects, and some of them, such as anemia and rash, were frequently causes of the withdrawal from treatment. However, as is well known, in the registered trials, the number of difficult-to-treat patients is rather small (cirrhotic, elderly, null responders to previous treatments and patients with comorbidities). However, even with restricted criteria for enrollment in phase 3 studies, a number of patients had

to stop the triple therapy due to either viral failure or adverse events (12%-15%).

TVR/BOC, approved for reimbursement in Italy in December 2012, have been used since January 2013. Since then, the group of the Association of Hospital Hepatologists (CLEO DAAs Study Group) was deeply involved in using these drugs, and the Governing Board of the Association decided to collect data from the Hospital centers belonging to the CLEO. The aim of our study was to determine what happens in everyday practice in terms of safety and efficacy using the triple therapy.

MATERIALS AND METHODS

Study design

This study was a non-randomized, observational, prospective, multicenter. This study involved 47 centers in Italy. A database was prepared for the homogenous collection of the data, was used by all of the centers for data collection, and was updated continuously.

Subjects

All of the patients enrolled in this study were older than 18 years of age, were diagnosed with chronic infection due to HCV genotype 1, and were consecutively seen in at least one of the centers between January 2013 and June 2014. No distinction was made between naive and previously treated patients. With regard to age, patients were divided into the following three groups: (1) less than 50; (2) between 50 and 65; and (3) over the age of 65. In this manner, we tried to avoid the division into only two categories (under 65 and over 65), which is presented in many papers and flattens the differences. Hepatitis B virus/human immunodeficiency virus positive patients or patients suffering from chronic liver disease due to other etiologies were excluded.

Treatment

Each center made the choice between TVR or BOC and Peg-IFN-alpha2a or Peg-IFN-alpha2b; patients were also treated with ribavirin (dose depending on the type of P chosen). The drugs were administered according to the manufacturer's instructions. TVR was administered with P + R for 12 wk followed by 36 wk of P + R; while patients treated with BOC received 4 wk of P + R (lead-in phase) followed by 44 wk of BOC + P + R. Patients treated with BOC or TVR had to respect the stopping rule concerning the kinetics of the viral load as follows: BOC patients with an HCV-RNA at week 12 greater than or equal to 100 IU/mL or detectable at 24 wk had to stop the therapy, while TVR patients with an HCV-RNA greater than 1000 IU/mL at week 4 or 12 or detectable at week 24 had to stop the treatment. They were classified as non-responders because of the virological failure.

Methods

Fibrosis was evaluated by a liver biopsy or by measuring the liver stiffness according to the manufacturer's instructions (Fibroscan®, Echosens, Paris, France). The results were expressed in kilopascal (kPa), and the cut-off values according to the literature were as follows: F1 was defined by a liver stiffness < 7.0 kPa; F2 was defined by a liver stiffness between 7.1-9.5; F3 was defined by a liver stiffness between 9.6-12.4; F4 (cirrhotic patients) was defined by liver stiffness values of up to 12.5 kPa[5]. Patients, according to their response to the previous treatment, were categorized as naive (never treated with antiviral drugs); relapsers (patients who were HCV RNA negative at the end of treatment and HCV RNA positive during the follow-up); partial responders (those with a reduction of HCV RNA during the treatment, but never become HCV RNA negative); and null responders (patients without any change in HCV RNA during the treatment and thereafter)[6].

AEs were graded by the investigators, according to the NIH grading system (CTCAE version 4.0). Hematological disorders, mainly anemia, were managed by reducing the ribavirin dose, giving erythropoietin, and/or with a blood transfusion, at the discretion of the physicians of each center. Hepatic decompensation during the therapy was defined by the new onset of one of the following clinical manifestations: Ascites, variceal hemorrhage, hepatic encephalopathy and onset of HCC.

A quantification of the HCV-RNA level was performed at baseline, 4 wk, 8 wk, 12 wk, the end of treatment, and 12 wk after the end of treatment. The HCV-RNA level was detected using real-time polymerase chain reaction (COBAS® TaqMan® HCV Test v2.0, Roche Diagnostics, Basel, Switzerland) with a lower limit of detection of 25 IU/mL. SVR was defined as HCV-RNA below the level of quantification 12 wk after the end of treatment.

Statistical analysis

All consecutively treated patients were included; data were analyzed according to the intention-to-treat principle. A preliminary descriptive analysis of the main demographic, virological and clinical baseline variables [gender, age, body mass index (BMI), HCV genotype, HCV RNA level, fibrosis grade, IL-28B, type of response to previous antiviral therapy, biochemical laboratory tests, concomitant diseases, side effects, and virological response during, at the end, and 12 wk after the end of therapy] of the entire population under investigation was carried out. Statistics measurements were as follows: Mean and standard deviation, mean standard error and 95%CI, median and range (when appropriate). At a later stage, univariate analysis and one-way ANOVA were conducted to verify the relationships between each independent variable and the dependent variable (SVR12). A χ^2 test for categorical variables and a *t*-test or Mann-Whitney test (when appropriate) for quantitative variables was used. A two-tailed *P*-value < 0.05 was considered to indicate statistical significance. Then, we looked for multicollinearity between those independent variables that statistically associated with SVR12. Finally, a multivariable logistic-regression analysis (step-

Table 1 Baseline characteristics of 834 patients enrolled

Age	Median 57 (range 18-78); age > 65: 18.3%
Sex	Male 64%, female 36%
BMI	Mean 25.6 (± SD) = 3.2 (range 16-39)
Genotype (%)	
1a	19.2
1b	79.4
1	1.4
HCV-RNA	
HCV-RNA $\leqslant 10^6$	42%
HCV-RNA > 10^6	58%
IL 28B (%)[1]	
TT	21.1
CT	65.4
CC	13.5
Fibrosis (%)	
F1	7.7
F2	21.1
F3	33
F4	38.2
Cirrhosis (CTP%)	
A5	70.8
A6	23.1
B7	4.5
B8	1.6
Previous treatment (%)	
Naive	24.4
Relapser	30.5
Partial responder	14.8
Null responder	30.3
Comorbidity (%)	
Diabetes mellitus	11.5
Alcohol	12.1

[1]Available on 513 patients (61.5%). BMI: Body mass index; HCV: Hepatitis C virus; IL: Interleukin; CTP: Child-Turcotte-Pugh classification; SD: Standard deviation.

wise selection procedure) was conducted to assess the relationship between the SVR and the pre-specified demographic and baseline clinical characteristics.

We have not carried out a statistical analysis comparing the two treatments. The reasons are as follows: (1) as already mentioned, this comparison was not one of the purposes of the study; and (2) each center not only chose BOC or TVR in its absolute discretion but also the type of pegylated interferon. This aspect would determine the division into the four groups with a very different dimension and would not provide acceptable results. Moreover, other studies similar to ours did not make any comparative analysis between the two treatments because of the same reasons[7,8].

All statistical analyses were performed using the software package SPSS for Windows (Rel SPSS 15.0; SPSS Chicago, IL, United States).

RESULTS

Eight hundred and thirty-four Caucasian patients observed in the 47 participating centers from January 2013 to June 2014 were enrolled, of whom 12.1% were also alcohol abusers, and 11.5% were affected by type 2 diabetes.

The two treatments (BOC/TVR) were analyzed together. The characteristics of the patients are reported in Table 1.

The majority of our patients were affected by genotype 1b (79.4%) and cirrhosis (38.2%). Among these 319 cirrhotic patients, 70.8% had a Child-Turcotte-Pugh Score of A5, 23.1% had A6; while 4.5% were B7 and 1.6% were B8. According to the response to previous treatments, 24.4% were naive, 30.5% were relapsers, 14.8% were partial responders and 30.3% were null-responders. According to the fibrosis grade, 7.7% of patients were F1, 21.1% were F2, 33.0% were F3 and 38.2% were F4.

HCV genotype 1b (79.4%) infections were more frequent than HCV 1a (19.2%), but the HCV genotype was not defined as 1b or 1a in 1.4% of the cases. As expected, in this population of relapsers and non-responders to prior antiviral therapy, only 13.5% of the patients had an IL-28B genotype CC. However, not all of the centers had this test available, but it was carried out on 61.5% of treated patients. Each center decided the choice of therapy, with the following percentage: TVR 66.2%, BOC 33.8%, Peg-IFN alpha2a 67.6% and Peg-IFN alpha2b 32.4%.

Overall, 70.4% of the patients completed a full course of therapy, while the treatment was stopped due to virological failure in 15% of the cases and for adverse events in 14.6%.

The overall SVR rate was 62.7% (95%CI: 59.1-66.3), while 70.1% of the patients had undetectable HCV-RNA levels at the end of triple therapy with a rate of relapse of 7.3% (Table 2). According to age, SVR was observed in 67.4% of patients < 50 years, 63.1% of the patients whose ages ranged from 50 to 65, and 55.3% of patients > 65 years ($P = 0.037$). SVR was observed in 65.7% of the naive patients, 73.7% of relapsers, 67.2% of partial responders and 55.1% of the null responders ($P = 0.012$). Only 53.4% of the cirrhotic patients had an SVR *vs* the 72.7% of patients with fibrosis F1 ($P = 0.003$), 73.4% with F2 ($P = 0.0001$), and 63.3% with F3 ($P = 0.013$); the lower rate of SVR of 43.8% was observed in cirrhotic patients over 65 years of age ($P = 0.0001$). When we compared the SVR observed in the categories F0/1/2 and 3 (68.1%) *vs* F4 (53.4%), there was a statistically significant difference ($P = 0.0001$). As for the relationship between SVR and the IL28B, the CC (70%), CT (57.5%), and TT (45.7%) groups, there was a statistically significant difference ($P = 0.029$) in favor of the CC group. Alcohol did not affect the percentage of SVR, while type 2 diabetes was statistically associated with SVR (OR = 0.55; 95%CI: 0.34-0.87, $P = 0.006$). The univariate analysis showed that six factors were independently associated with SVR. These factors were as follows: (1) a relapse after P + R treatment; (2) the stage of fibrosis; (3) age; (4) gender; (5) diabetes; and (6) the IL-28B status; while BMI, HCV-RNA at baseline, biochemistry at baseline and genotype subtype were not associated with SVR. The multivariate analysis with logistical regression revealed that only fibrosis F0/F1/F2 stages, IL-28B-CC and the absence of diabetes are independently associated

Table 2 Percentage of sustained virological response according to demographics and clinical characteristics

RVR[1]	66.5%
HCV-RNA negative at EOT	70.1%
Relapse[2]	7.3%
SVR 12[3]	62.7%
Age	
< 50 yr	67.4%
50-65 yr	63.1%
> 65 yr	55.3%
Previous treatment	
Naive	65.7%
Relapser	73.7%
Partial responder	67.2%
Null responder	55.1%
Fibrosis (%)	
F1	72.7%
F2	73.4%
F3	63.3%
F4	53.4%
F4 > 65 yr	43.8%

[1]HCV-RNA negative at week 4; [2]Those who achieved EOT but had HCV-RNA positive at week 12; [3]HCV-RNA negative 12 wk after the EOT. RVR: Rapid virological response; EOT: End of treatment; SVR: Sustained virological response; HCV: Hepatitis C virus.

with SVR ($P < 0.05$). The odds ratios for fibrosis stages F0/F1/F2 and F3 *vs* F4 (the reference category) were 2.3 (95%CI: 1.3-3.8; $P = 0.002$) and 1.5 (95%CI: 0.9-2.3; $P = 0.096$), respectively. The OR for IL28B-CC and IL-28B-CT *vs* IL-28B-TT (the reference category) were 3.2 (95%CI: 1.5-6.7; $P = 0.003$) and 1.5 (95%CI: 0.9-2.4; $P = 0.11$), respectively. As for diabetes, the odds ratio was 1.8 (95%CI: 0.9-3.5; $P = 0.075$).

Safety

Overall, AEs occurred in 617 patients (73.9%) during the treatment (Table 3). A total of 122 (14.6%) of the patients suspended the therapy due to AEs. In general, females stopped the treatment more often than males (16% *vs* 11%; $P = 0.043$). With increasing age, there was a statistically significant increase in AEs (9.4% *vs* 12.6% *vs* 18.4%; $P = 0.040$). There was no statistically significant difference in relation to subtype (1b 13.7% *vs* 9.3% 1a; $P = 0.18$); nor was there a statistically significant difference in relation to the histological diagnosis ($P = 0.58$) even if the F4 class showed the highest percentage (13.8%) of AEs compared to the other classes as follows: F3 (12.9%), F2 (9.8%), F1 (11.7%) and F0 (0.6%, four patients only in this group).

Anemia was the most frequent AE (52.9% of cases), especially in cirrhotic as already described[9], followed by asthenia (39.6%), neutro-thrombocytopenia (29.6%), rash/itching (23.2%), dysgeusia (8.6%), psychiatric disorders (6.7%), anorectal discomfort (5.9%) and others (14.9%). Among this last group, we recorded the following: Gastrointestinal disorders (23 cases), pulmonary infections (9), ascites (3), pancreatitis (2), thrombosis of retina (2), and new onset of cancer as follows: Hepatocellular carcinoma (1), breast (1), and kidney (1). Anemia was observed regardless of the DAA used, while rash was more frequently observed in the TVR treated patients. The main AEs that led to treatment discontinuation were rash (29.8%) and anemia (23.4%). There were no fatalities as the included patients had cirrhosis, but not as advanced as in the French study[8] where the 2.2% of the patients died.

Table 3 Adverse events (%) and treatment discontinuation

Adverse events (73.9%)	
Anemia	52.9
Asthenia	39.6
Neutro/thrombopenia	29.6
Dysgeusia	8.6
Psychiatric disorders	6.7
Anorectal symptoms	5.9
Others (see text)	14.9
Treatment discontinuation (122 cases; 14.6%)	Number of cases
Rash/Itch	36 (29.5%)
Anemia	28 (22.9%)
Asthenia	18 (14.7%)
Psychiatric disorders	6 (5%)
Pancytopenia	3 (2.5%)
Neutro/thrombopenia	3 (2.5%)
Others (see text)	28 (22.9%)

DISCUSSION

This study, conducted in 47 hospital centers in Italy, enrolled 834 patients consecutively seen in clinical settings. Because there was no selection of the cases, all of the patients seen and judged to be treatable by each center were included. For this reason, we can safely assume that this study mirrors what happens in real life. This is the main reason of the need for studies that monitor the safety after registration of the authorization of the prescription of new drugs. It is at this stage that many older patients with morbidity, concurrently taking other medications, are enrolled. Observational studies, such as those already published and our own, serve not only to validate the results of pivotal trials but also to provide information on safety and predictors of response that helps to more appropriately use the new drugs. Some aspects should be underlined, such as the age of the patients (18.3% more than 65), the percentage of advanced liver disease (Fibrosis score F3 plus F4 = 70.9%) and the high percentage (75.6%) of patients previously treated with P + R. It is quite remarkable that the percentage of patients with compensated cirrhosis was 37.1%; while in the registration studies, this group of difficult-to-treat patients did not exceed 15%.

When we analyzed the differences between the major registration studies conducted using TVR/BOC and our findings, the first observation was that the AEs causing discontinuation of drugs were different from those reported in the phase 3 trials, where these percentages ranged between 8%-15%. The true strength of "real life" studies is the inclusion of patients who visit the clinics in every day practice and represent HCV-related disease at every stage. The only weakness is that they are not

randomized, and specialized centers in different parts of the country are involved, which favors a certain degree of heterogeneity. However, this aspect is also present in the pivotal studies in which many centers participate, often scattered in different countries. Analyzing other studies similar to ours, the percentages of drug discontinuation varies from a minimum of 8% to a maximum of 38%[7-10]. However, it is difficult to entirely blame DDAs for some AEs, as in addition to BOC and TVR, there were two drugs, including P and R, with AEs well known for many years, especially anemia, itching, and nervousness.

In this study, among the AEs causing withdrawal from treatment, rash (29.5%) was the most frequent, although we did not observe DRESS syndrome or toxic epidermal necrolysis.

Rash was detected in both treatment groups, although it was more frequent in patients treated with TVR. Anemia was the second most important AE leading to discontinuation of therapy. In 11% of the patients, it was necessary to perform blood transfusions, while in 25%, epoetin was administered. Other cases were simply treated with a dose reduction of ribavirin. As for the AEs not causing withdrawal from therapy, we did not find remarkable differences with the pivotal trials (Table 3).

The SVR at 12 wk after the end of treatment was achieved by 62.7%, more than that achieved by the other similar studies. The high number of patients with cirrhosis and the presence of older patients explain the results, such as SVR, which was a percentage lower than that obtained from the pivotal studies. In naive patients, the results were similar to those previously obtained by partial responders, while those who had the best performance (SVR = 73.7%) were those who had a relapse at the end of the previous treatments. Similar data for this category of patients were achieved by the other studies[9,11,12] for experienced patients. Null responder patients to previous treatments had an SVR of 55.1%, better than that reported in other similar studies, whereas in one study[10], the SVR was less than 20%. The most relevant finding of this study was the negative correlation between the SVR and fibrosis grade. This result has been recently confirmed[13]. In fact, as reported in Table 3, the worst result (SVR = 43.8%) was achieved in patients with cirrhosis, who were older than 65 years of age. Indeed, these categories of patients (elderly, with cirrhosis and with many failures to previous treatments) represent the majority of patients requiring treatment today. Multivariate analyses showed that the most important factors linked to SVR were the grade of fibrosis, IL-28B-CC and not being diabetic.

In conclusion, the treatment with first generation PI (BOC/TVR) plus P + R is quite safe, but its efficacy is limited, especially for elderly cirrhotic patients. This information is very useful as DDA IFN-free drugs may change the antiviral therapy options for HCV, and there is no doubt that in many countries, these drugs will only be selectively available due to cost. Therefore, real life studies on "old" less expensive DDAs could be very useful for establishing drug delivery policies in relation to the resources available in each country.

ACKNOWLEDGMENTS

The CLEO Study Group thanks Michele Imparato, MD, for the data management, Massimo De Luca, MD, for the statistical analysis and Antonio Ascione, MD, for writing the draft.

COMMENTS

Background

Protease inhibitors (boceprevir or telaprevir) in combination with pegylated interferons and ribavirin are the first direct antiviral therapy for chronic infections with hepatitis C virus (HCV) genotype 1. They were introduced in 2011 and since then have been a step forward in the development of this therapy. In Italy, these therapies were introduced in 2013 and the Italian Association of Hospital hepatologists (CLEO) has begun, among the members of the association, the data collection.

Research frontiers

This study represents one of the few real-life studies with high number of cases, published in the international field and the only one regarding the Italian patients. Compared to the registration studies, the collection of data from patients who are treated every day provides valuable data to validate in clinical practice this treatment.

Innovations and breakthroughs

Therefore, the present study tested in practice the first two innovative drugs in chronic infections with HCV therapy that were expected at least for ten years. With their arrival in the therapeutic baggage of hepatologists, the authors have obtained results certainly better than the performance of conventional therapy with interferon and ribavirin alone, which has represented the standard of care for about fifteen years.

Applications

The data generated from this study show that these drugs have an acceptable safety profile but their effectiveness, especially in cirrhotic patients and with over 65 years of age, is quite modest. Their greater efficacy is obtained in patients with non-advanced liver damage. The new drugs, which are currently on the market for hepatitis C, are more active than the triple therapy, but their cost is extremely high. Therefore, these studies are of great social importance because, in countries that do not have an economy that allows the purchase of these drugs, the triple therapy can be offered with excellent results, choosing carefully the categories of patients to be treated.

Terminology

The letter "F" expresses the degree of fibrosis in the liver. In this study this aspect was defined by liver biopsy or by the Fibroscan tool, which, in a non-invasive way, is able to define the degree of rigidity and, therefore, the actual degree of fibrosis in the liver. The physical principle is that the higher the number in kilopascals, the higher the degree of fibrosis.

Peer-review

This topic of study is very topical and important. The authors' concept and ideas for this investigation is very note worthwhile and studies of real life experiences are most useful for the field.

REFERENCES

1 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl*

J Med 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]

2 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]

3 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]

4 **Zeuzem S**, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; **364**: 2417-2428 [PMID: 21696308 DOI: 10.1056/NEJMoa1013086]

5 **Ziol M**, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Lédinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48-54 [PMID: 15690481]

6 **Wedemeyer H**, Jensen DM, Godofsky E, Mani N, Pawlotsky JM, Miller V. Recommendations for standardized nomenclature and definitions of viral response in trials of hepatitis C virus investigational agents. *Hepatology* 2012; **56**: 2398-2403 [PMID: 22707382 DOI: 10.1002/hep.25888]

7 **Gordon SC**, Muir AJ, Lim JK, Pearlman B, Argo CK, Ramani A, Maliakkal B, Alam I, Stewart TG, Vainorius M, Peter J, Nelson DR, Fried MW, Reddy KR. Safety profile of boceprevir and telaprevir in chronic hepatitis C: real world experience from HCV-TARGET. *J Hepatol* 2015; **62**: 286-293 [PMID: 25218788 DOI: 10.1016/j.jhep.2014.08.052]

8 **Hézode C**, Fontaine H, Dorival C, Zoulim F, Larrey D, Canva V, De Ledinghen V, Poynard T, Samuel D, Bourliere M, Alric L, Raabe JJ, Zarski JP, Marcellin P, Riachi G, Bernard PH, Loustaud-Ratti V, Chazouilleres O, Abergel A, Guyader D, Metivier S, Tran A, Di Martino V, Causse X, Dao T, Lucidarme D, Portal I, Cacoub P, Gournay J, Grando-Lemaire V, Hillon P, Attali P, Fontanges T, Rosa I, Petrov-Sanchez V, Barthe Y, Pawlotsky JM, Pol S, Carrat F, Bronowicki JP. Effectiveness of telaprevir or boceprevir in treatment-experienced patients with HCV genotype 1 infection and cirrhosis. *Gastroenterology* 2014; **147**: 132-142.e4 [PMID: 24704719 DOI: 10.1053/j.gastro.2014.03.051]

9 **Bruno S**, Vierling JM, Esteban R, Nyberg LM, Tanno H, Goodman Z, Poordad F, Bacon B, Gottesdiener K, Pedicone LD, Albrecht JK, Brass CA, Thompson S, Burroughs MH. Efficacy and safety of boceprevir plus peginterferon-ribavirin in patients with HCV G1 infection and advanced fibrosis/cirrhosis. *J Hepatol* 2013; **58**: 479-487 [PMID: 23183529 DOI: 10.1016/j.jhep.2012.11.020]

10 **Colombo M**, Strasser S, Moreno C, Abrao Ferreira P, Urbanek P, Fernández I, Abdurakmonov D, Streinu-Cercel A, Verheyen A, Iraqi W, DeMasi R, Hill A, Lonjon-Domanec I, Wedemeyer H. Sustained virological response with telaprevir in 1,078 patients with advanced hepatitis C: the international telaprevir access program. *J Hepatol* 2014; **61**: 976-983 [PMID: 24946280 DOI: 10.1016/j.jhep.2014.06.005]

11 **Bonnet D**, Guivarch M, Bérard E, Combis JM, Remy AJ, Glibert A, Payen JL, Metivier S, Barange K, Desmorat H, Palacin A, Nicot F, Abravanel F, Alric L. Telaprevir- and boceprevir-based tritherapies in real practice for F3-F4 pretreated hepatitis C virus patients. *World J Hepatol* 2014; **6**: 660-669 [PMID: 25276282 DOI: 10.4254/wjh.v6.i9.660]

12 **Perry CM**. Telaprevir: a review of its use in the management of genotype 1 chronic hepatitis C. *Drugs* 2012; **72**: 619-641 [PMID: 22439668 DOI: 10.2165/11208370-000000000-00000]

13 **Ferenci P**, Caruntu FA, Lengyel G, Messinger D, Bakalos G, Flisiak R. Boceprevir Plus Peginterferon Alfa-2a/Ribavirin in Treatment-Naïve Hepatitis C Virus Genotype 1 Patients: International Phase IIIb/IV TriCo Trial. *Infect Dis Ther* 2016; **5**: 113-124 [PMID: 27228998 DOI: 10.1007/s40121-016-0110-5]

P- Reviewer: Abd El-Wahab EW, Li J, Lawless MW, Tang ZH
S- Editor: Gong XM **L- Editor**: A **E- Editor**: Li D

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