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World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

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2016 Liver Transplantation: Global view

Incidence, risk factors and outcomes of *de novo* malignancies post liver transplantation

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Abstract

Liver transplantation (LT) is associated with a 2 to

7 fold higher, age and gender adjusted, risk of *de novo* malignancy. The overall incidence of *de novo* malignancy post LT ranges from 2.2% to 26%, and 5 and 10 years incidence rates are estimated at 10% to 14.6% and 20% to 32%, respectively. The main risk factors for *de novo* malignancy include immunosuppression with impaired immunosurveillance, and a number of patient factors which include; age, latent oncogenic viral infections, tobacco and alcohol use history, and underlying liver disease. The most common cancers after LT are non-melanoma skin cancers, accounting for approximately 37% of *de novo* malignancies, with a noted increase in the ratio of squamous to basal cell cancers. While these types of skin cancer do not impact patient survival, post-transplant lymphoproliferative disorders and solid organ cancer, accounting for 25% and 48% of malignancies, are associated with increased mortality. Patients developing these types of cancer are diagnosed at more advanced stages, and their cancers behave more aggressively compared with the general population. Patients undergoing LT for primary sclerosing cholangitis (particularly with inflammatory bowel disease) and alcoholic liver disease have high rates of malignancies compared with patients undergoing LT for other indications. These populations are at particular risk for gastrointestinal and aerodigestive cancers respectively. Counseling smoking cessation, skin protection from sun exposure and routine clinical follow-up are the current approach in practice. There are no standardized surveillance protocol, but available data suggests that regimented surveillance strategies are needed and capable of yielding cancer diagnosis at earlier stages with better resulting survival. Evidence-based strategies are needed to guide optimal surveillance and safe minimization of immunosuppression.

Key words: Liver transplant; Immunosuppression; Risk; Outcomes; Malignancy

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Core tip: The risk of new cancers is significantly increased after liver transplantation (LT), and is driven by patient factors, oncogenic viruses and lifelong immunosuppression. *De novo* malignancy is a major risk factor for mortality after LT, equaling the risk of cardiovascular disease or infectious diseases. The risk of *de novo* malignancies may be reduced by attention to patient risk factors and minimization of immunosuppression when possible. Ultimately rigorous surveillance is needed to allow for early diagnosis and attenuation of mortality risk.

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INTRODUCTION

Liver transplantation (LT) is the definitive therapy for decompensated end-stage liver disease regardless of etiology. During the past 2 decades, the outcomes of LT have steadily improved as a result of more widespread expertise, better surgical techniques and more effective and better tolerated immunosuppressive agents. The growing number of LT recipients and improving survival rates place particular importance on the factors that jeopardize long term survival. Inherent to this population is the need for lifelong immunosuppression, which is associated with some broad categories of risk for morbidity and mortality. These include infection, cardiovascular risks, renal injury and cancer. When studied in patients surviving the early post LT period, *de novo* malignancy emerges as the leading category of immunosuppression associated long term mortality risk, accounting for approximately 21% to 25% of deaths^[1,2]. This review summarizes current knowledge of *de novo* malignancy post LT including; epidemiology, pathogenesis, disease burden, clinical implications, preventive and surveillance considerations, while emphasizing risk factors and outcomes.

INCIDENCE

Multiple studies report widely varying incidence rates of *de novo* malignancy post LT, along with considerable variations in associated risks, cancer types and outcomes. The incidence of *de novo* malignancies in relatively large cohorts (subjectively defined as more than 150 patients) is summarized in Table 1, the last row of which contains the means of the respective variables. These include single center experiences^[3-7], registry based studies^[8-11], and the majority are retrospective with few exceptions^[12]. Variability in *de novo* malignancy

incidence rates reflect actual differences (based on differing cohort characteristics and risks) and artificial differences (based on differing methodologies and study design). The factors impacting actual differences in cancers types and their incidence may include age, gender, racial and geographical considerations, as well as the predominant underlying liver diseases and their associated comorbidities. Whereas artificial heterogeneity may be less apparent, yet could arise from variability in the: (1) definitions of *de novo* malignancy, *e.g.*, not all include non-melanoma skin cancers; (2) designated time threshold for of exclusion of cancers that are likely pre-existing before LT; (3) method of identification of malignancies, *e.g.*, in-center chart review vs utilization of cancer registries; (4) surveillance protocols and frequency of clinical follow up at study centers (critical for in-center reporting of cancer cases); (5) duration of follow up post LT since cancer incidence increases with time^[8,13]; and (6) in the case of standardized incidence ratio (SIR) calculations, the control population used and type of cancers captured by the respective registries. In this review, we have described incidence rates of cancers and as well as the SIR where possible, as it allows age and gender adjusted risk analysis. SIR is calculated as the ratio of observed incidence in a cohort to the expected incidence in the population (hence has no unit).

Cancer registry data used to calculate expected age and gender adjusted incidence rates for SIR estimation doesn't capture non-melanoma skin cancers (NMSCs). Therefore SIR analyses succinctly reflect the risk of more life-threatening types of cancer. Interestingly, purely registry based analyses yield higher SIR values for *de novo* malignancy post LT, ranging from 2.2 to 4.9^[10,14-16], than 1.4 to 3.1^[2,9,11,17,18] of single and multi-center studies. The reasons for this are unclear but could reflect differing approaches to immunosuppression given the reporting bias for higher transplant volume centers.

RISK FACTORS FOR *DE NOVO* MALIGNANCY

The risk factors for the development of *de novo* malignancy after LT are not fully understood, but it is likely that patient, transplant and environmental factors interact to shape that risk.

Immunosuppression related risk

Over the past few decades, a better understanding of the role of the immune system in preventing malignancy in immunocompetent individuals helped establish the concept of immunosurveillance^[19]. Transplant recipients receive lifelong immunosuppression with chronic impairment of immunosurveillance, which promotes proliferation and survival of malignant cellular clones. Though immunosuppressive drug dose intensity likely contributes to cancer risk, the evidence for this is

Table 1 Summary of study characteristics and reported incidence of de novo malignancy post liver transplantation in large series

Ref.	Year published	Country of study center	Study period	No. of liver transplant recipients	⁴ Duration of follow-up (yr)	⁴ Age at transplant in patients with de novo malignancy (yr)	Proportion of males with de novo malignancy	⁴ Interval to de novo malignancy (yr)	Overall incidence of de novo malignancy (number of patients)	5/10/15 and 20 yr incidence of de novo malignancy	Estimated overall risk relative to control population
Jonas <i>et al.</i> ^[28]	1997	Germany	1988-1994	458	4.2	46 ± 14	48%	3.6	7.2% (33)	14.6%/-/-/-	-
Jain <i>et al.</i> ^[3]	1998	United States	1996-2006	1000	6.5 ± 1	Approximately 56	77%	3	5.7% (57)	-	SIR calculated for specific cancer types
Kelly <i>et al.</i> ^[25]	1998	United Kingdom	1988-1996	888	-	Approximately 52	46%	2 ± 1.5	² 4.4% (29)	-	-
Galve <i>et al.</i> ^[16]	1999	Spain	1984-1997	1827	-	-	-	2.5 ± 1.8	3.8% (70)	-	-
Haagsma <i>et al.</i> ^[6]	2001	The Netherlands	1979-1996	174	5.1	Approximately 49	29%	5.9	12% (21)	6%/20%/55%/-	RR = 4.3 (95%CI: 2.4-7.1)
Sanchez <i>et al.</i> ^[5]	2002	United States	1985-1999	1421	5.5 ± 3.7	50 ± 12	55%	-	8.8% (125)	-	-
Saigal <i>et al.</i> ^[4]	2003	United Kingdom	1988-1999	1140	-	51.5	70%	3.8 ± 2.8	2.6% (30)	-	-
Beniloch <i>et al.</i> ^[60]	2004	Spain	1991-2001	772	4.3	50	59%	3.5	5.3% (41) ¹	-	-
Oo <i>et al.</i> ^[65]	2005	United Kingdom	1982-2004	1778	6.5	-	43%	-	7.9% (141)	-	SIR = 2.1 (95%CI: 1.7-2.2)
Herrero <i>et al.</i> ^[7]	2005	Spain	1990-2001	187	5.5	-	-	-	26% (49)	25%/39%/-/-	RR = 2.9 (95%CI: 1.6-5.0)
Yao <i>et al.</i> ^[60]	2006	United States	1988-2000	1043	6.7	53.2	52%	-	4.8% (50)	-	-
Aberg <i>et al.</i> ^[9]	2008	Finland	1982-2005	540	6.3	-	53%	5.1	6.7% (36) ³	5%/13%/-/-16%	³ SIR = 2.6 (95%CI: 1.8-3.5)
Jiang <i>et al.</i> ^[10]	2008	Canada	1983-1998	2034	-	-	53%	3.5 ± 2.8	5.5% (113) ¹	2%/8.6%/-/-	¹ SIR = 2.5 (95%CI: 2.1-3.0)
Watt <i>et al.</i> ^[12]	2009	United States	1990-1994	798	10	-	60% ¹	-	21.4% (171)	12%/22%/-/-	-
Finkenstedt <i>et al.</i> ^[18]	2009	Austria	1982-2007	779	4.1	-	-	4.4	12.3% (96)	10%/24%/32%/42%	¹ SIR = 1.9 (95%CI: 1.5-2.4)
Baccarani <i>et al.</i> ^[17]	2010	Italy	1991-2005	417	6.7	-	74%	4.2	10.3% (43) ¹	-	¹ SIR = 2.6 (95%CI: 1.9-3.6)
Tjon <i>et al.</i> ^[27]	2010	Denmark	198-2007	85	5	-	-	-	3% (50)	10%/19%/34%/-	SIR = 2.2 (95%CI: 1.6-2.8)
Park <i>et al.</i> ^[67]	2012	South Korea	1998-2008	1952	3.5 ± 2.8	56	79%	3.4 ± 2.4	2.3% (44) ¹	-	¹ RR = 7.7 for men and 7.3 for women
Chatrath <i>et al.</i> ^[21]	2013	United States	1997-2004	534	5.7 ± 3.2	53 ± 12	67%	4 ± 2.2	13.7% (73)	12%/25%/-/-	¹ SIR = 3.1 (95%CI: 2.9-3.2)
Wimmer <i>et al.</i> ^[24]	2013	Germany	1985-2007	609	5.2	53 ± 10	73%	5.7	11.5% (70)	10%/26%/35%/-	-
Ettorre <i>et al.</i> ^[11]	2014	Italy	1990-2008	1675	5.2	-	-	3.2	5.9% (98) ¹	-	¹ SIR = 1.4 (95%CI: 1.2-1.7)
Yu <i>et al.</i> ^[69]	2014	China	2005-2011	569	3.5 ± 2.2	-	76%	-	3.2% (17)	-	-
Antinucci <i>et al.</i> ^[17]	2015	Argentina	2006-2014	168	-	67 ± 7	75%	1.3	7.5% (12)	-	-
Sanaei <i>et al.</i> ^[68]	2015	Iran	1992-2012	1700	-	34 ± 10	63%	5.5	2.2% (38)	-	-
Overall means				940	5.5	52	61%	3.8	8.10%	11%/22%/39%/29%	3.0

¹Excluding non-melanoma skin cancers; ²Excluding post-transplant lymphoproliferative disorder; ³Excluding basal cell skin cancer; ⁴Median or mean ± SD. SIR: Standardized incidence ratio.

indirect. Comparative studies indicate a lower SIR for *de novo* malignancies in LT (2.2) recipients compared with heart (2.5) or lung (3.6) recipients who typically require higher intensity of immunosuppression^[16,20]. A higher rate of hepatocellular carcinoma recurrence has been described with higher trough levels of the calcineurin inhibitors (CNI), tacrolimus and cyclosporine, particularly in the early post LT period^[21,22]. Calcineurin inhibitors inhibit T-lymphocyte cell mediated immunity, and may also increase the risk of malignancy by increasing synthesis of growth factors, *e.g.*, transforming growth factor- β , interleukin-6 and vascular endothelial growth factor in tumor cells, and impair DNA repair, thereby enhancing tumor growth, metastasis and angiogenesis^[23]. The duration of immunosuppression also likely increases risk of malignancy, with increased incidence reported in recipients who were immunosuppressed before LT^[9].

The choice of immunosuppressive drug is a potentially modifiable cancer risk factor. Cyclosporine initially, and tacrolimus subsequently, have been and remain the mainstay of long term immunosuppression in LT over the last few decades. Even though some studies have shown higher carcinogenic risk with tacrolimus^[7,24], and others with cyclosporine based protocols^[2,25-27], there is no accepted cancer risk advantage for either agent^[3,28]. A more practical concern in choice of immunosuppressant relates to the class of mammalian target of rapamycin (mTOR) inhibitors, sirolimus and everolimus, though these agents are used mainly in renal sparing regimens. The putative

Table 2 A listing on known oncogenic viruses and the malignancies associated with them

Oncogenic virus	Associated malignancy
EBV	PTLD
Human papilloma virus	Cervical, skin, oropharynx, anal
Human T-cell lymphotropic virus type 1	Adult T cell leukemia
Kaposi's sarcoma-associated herpesvirus	KS, primary effusion lymphoma, castleman's disease
HBV	HCC
HCV	HCC, PTLD ¹

¹Role controversial. HCC: Hepatocellular carcinoma; EBV: Epstein-Barr virus; PTLD: Post transplant lymphoproliferative disorder; KS: Kaposi's sarcoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

anti-proliferative properties of mTOR inhibition include inhibition of cellular growth, proliferation, metabolism and angiogenesis^[29]. Though there is no prospective randomized controlled study data currently, a number of retrospective studies have described lower rates of hepatocellular carcinoma (HCC) recurrence^[30,31], and *de novo* malignancies^[32,33] with mTOR inhibitors post LT and renal transplantation^[34]. A meta-analysis of retrospective studies has shown that mTOR inhibitor, sirolimus, is of value in preventing recurrence and increasing survival in those transplanted for HCC^[35].

The post LT cancer risk related to anti-metabolites has been described for azathioprine in one study, with an odds ratio (OR = 3.8, 95%CI: 1.7-8.6, $P = 0.004$)^[36]. Whereas mycophenolate mofetil has been shown to have anti-tumor properties in animal studies^[37], and was associated with a trend towards lower risk of non-skin *de novo* malignancies post renal transplant in a large United States, and European/Canadian registry based study^[38]. In a recent study of solid organ transplant, mycophenolate mofetil use was associated with lower risk of proximal colon cancer^[39].

Immunosuppression induction with anti-lymphocyte antibodies or anti-thymocyte globulin was associated with increased of skin cancer in one study^[9], however that risk was not seen in larger series using anti-thymocyte globulin induction^[2,28]. Rejection episodes also did not alter the risk of malignancy in LT recipients^[5,6,12,40]. These data suggest that higher levels of immunosuppression in the short term do not increase the long term risk of cancer.

Immunosuppression also increases the cancer risk related to latent oncogenic virus infections (Table 2)^[41]. Oncogenic virus associated tumors may be more immunogenic than those related to other factors, and may regress once immunosuppression is stopped or minimized^[42]. This provides the rationale for a decrease in immunosuppression as the first line intervention for some virus related cancers, such post-transplant lymphoproliferative disorder (PTLD), particularly when associated with Epstein-barr virus (EBV) viremia^[43].

Recipient related factors

The association of specific patient factors with cancer risk are organized and elaborated on below.

Age: Advanced age is a well described risk factor for *de novo* malignancy^[2,7,8,12,44,45], although this is not a universal finding^[25,28]. This suggests that other factors may supersede age in cancer risk, though some caveats are notable with the extremes of age. For example the SIR for early PTLD was high (18.1) in pediatric LT recipients in one study^[9], with a similar observation in another study^[10]. In another study, LT recipients older than 60 had > 2 fold higher 5-year incidence of new cancers (> 40%) compared to younger LT recipients (< 20%), largely due to non-skin cancers, with significantly higher cancer related mortality^[46].

Gender and race: There is conflicting data on the relative risk of *de novo* malignancy according to gender, with slightly higher SIR of cancers in females in one registry study^[14], and in males in another^[45], limiting any meaningful conclusion. Although skin cancer risk would be expected to differ according to race, there is limited data of cancer risk in relation to race. Non-Caucasian race was associated with a higher hazard ratio (HR = 2.5, 95%CI: 1.3-4.3) for non-skin cancers in one study, but the small size of that subgroup was limiting^[2].

Indication for LT: Patients who receive LT for certain indications are more prone to some malignancies. Patients with primary sclerosing cholangitis (PSC) in a United States multicenter prospective observational study had the highest cumulative incidence of non-skin cancer of 5.5%, 10.4%, and 21.9% at 1, 5, and 10 years, respectively^[12]. Patients with PSC and inflammatory bowel disease (IBD) and an intact colon at the time of LT were at increased risk of gastrointestinal (colon) malignancy (HR = 2.34, 95%CI: 1.02-5.38)^[12], which may not be surprising given the association of PSC and IBD with colon cancer risk. However, patients with PSC also exhibited an increased risk for PTLD, skin malignancies and solid organ malignancies^[12]. A high cancer risk for LT recipients with PSC was also observed in an Italian study, though cancer types were not specified^[47]. The reasons for generalized cancer risk are unclear, but may reflect immunosuppression before LT, and possibly vitamin D deficiency which may promote malignancy^[48].

Alcohol use history and smoking: Many studies have described the carcinogenic properties of alcohol and smoking in immunocompetent individuals^[49,50]. Alcoholic liver disease (ALD) is associated with increased cancer risk post LT^[7,12,36,45,51-54]. Synergy between the carcinogenic effects of alcohol and smoking is well described^[55,56]. Smokers were more likely to have alcoholic liver disease than non-smokers (35% vs 13%,

Table 3 A summary of ranges of reported overall incidence rates and standardized incidence ratios of a number of cancer types following liver transplantation^[2-5,7-12,14-16,28,36,47,66,110,118-120]

	Incidence (%)	SIR
Skin cancers		
Represent 24%-54% of all cancers, average 37%		
Overall (non-melanoma)	0.9-11.6	2.1-70, average 24
Squamous cell cancer	0.6-15.3	Not reported
Basal cell cancer	0.6-10.6	Not reported
KS	0.2-1.4	128-144
Melanoma	0.2-3.9	4.4
Post-transplant lymphoproliferative disorders		
Represent 4%-57% of all cancers, average 25%		
Overall	0.5-2.9	3.9-21, average 12
Hodgkin's lymphoma	0.001-0.4	8.2-8.9
Non-Hodgkin's lymphoma	0.8-3.7	3.5-37.3
Solid organ cancers		
Represent 24%-75% of all cancers, average 48%		
Overall	1.4-7.5	1.4-3.1, average 2.3
Lip	1.8	14-24.8
Oropharyngeal	1.7-1.9	7-10
Lung	0.6-2.4	1.4-2.0
Stomach	0.2-0.7	0.5-3.7
Colorectal	0.5-1.1	1.4-4.9
Breast (in females)	0.2-0.6	0.6-1.6 ¹
Cervix (in females)	0.7-1.4	1.3-5.7
Prostate (in males)	0.2-1.8	0.6-1.6 ¹

¹The SIR was not found to be significantly higher for transplant recipients compared with the reference population. Of note, studies often reported either incidence rate or SIR, but rarely both values. SIR: Standardized incidence ratio; KS: Kaposi's sarcoma.

$P = 0.008$) in one study^[56], and patients transplanted for ALD were more likely to be smokers (82% vs 45%, $P = 0.001$) and smoked more cigarettes per day (27 ± 15 vs 16 ± 11 , $P = 0.001$) in another^[54]. A United Kingdom registry study reported a higher SIR (3.16) of *de novo* malignancy for ALD compared to all other LT indications (1.99)^[45]. In the immunocompetent population, there is evidence that the increased risk of cancer due to alcohol abuse could be reversed by abstinence^[57]. However, this effect may be delayed by a more than a decade^[58], with cancer risk carried through post LT.

History of cancer prior to LT: A history of cancer prior to LT was not associated with its recurrence after LT^[8,25]. However, LT for HCC has been associated with an increased risk of *de novo* malignancy^[7,44]. An increased incidence of non-skin cancers in patients with a history of non-liver cancer prior to LT (30.8% vs 8.3%, $P = 0.001$) has also been described, where it was additionally an independent predictor of non-skin *de novo* malignancies (HR = 2.5, 95%CI: 1.3-4.9, $P = 0.005$)^[2]. This association is supported by data from renal transplantation studies^[34,59,60]. Therefore, a prior history of cancer may reflect a patient's composite (genetic and epigenetic) risk of malignancy.

SITE SPECIFIC *DE NOVO* MALIGNANCIES

The risk of *de novo* malignancy is variable across a range of tumor types, as reported by cancer registry

studies. These cancers are commonly grouped according to three broad categories including; skin cancers, PTLD and solid organ cancers. The risks of specific tumors post LT are summarized in Table 3.

Skin cancers

Skin malignancy, typically NMSC, is the most common malignancy after LT^[2,7,9,12,40,61]. These include squamous cell cancer (SCC), basal cell cancer (BCC) and Kaposi's sarcoma (KS). Ultraviolet radiation is an important risk factor in the pathogenesis of skin malignancies, and exerts a field cancerization mutagenic effect in exposed areas of the skin^[62-65]. In a prospective study of LT recipients with comprehensive dermatology follow-up, only total pre transplant sun burden and skin characteristics were found to be the risk factors for NMSC^[66]. The relative risk of cutaneous malignancies in this cohort was found to be 20 fold higher than the general population. Conversely studies from Iran, South Korea and China described no to very low incidence rates of skin cancer, likely due to the prevalent skin types^[67-69]. In organ transplant recipients, SCC is more common than BCC, in contrast to the general population^[44,70]. Additionally, while SCC and BCC are easily surveyed and resected, SCC can behave more aggressively in LT recipients^[70,71]. In general though, LT recipients with SCC and BCC have similar survival to patients not developing *de novo* malignancies post LT^[2,40].

Immunosuppression with CNIs and azathioprine is a significant risk factor for NMSC^[72-76], but it is likely

the degree of immunosuppression that represents the main risk rather than the choice of agent^[62,77,78]. However, there is mounting evidence that mTOR inhibitors have protective effect against NMSC due to their aforementioned anti-proliferative properties^[72,77], especially in renal transplant recipients. In a randomized trial, converting renal transplant recipients with NMSC from CNI to sirolimus based immunosuppression was associated with a reduced risk of subsequent NMSC (relative risk 0.56, 95%CI: 0.32-0.98) and longer recurrence free interval (15 mo vs 7 mo, $P = 0.02$)^[79]. However, similar evidence in LT recipients is currently lacking.

Kaposi's sarcoma is related to human herpes virus-8 (HHV-8) and occurs only in immunocompromised individuals. The incidence of KS after LT reflects the prevalence of HHV-8 (also known as Kaposi's sarcoma-associated herpes virus), with high rates reported in the Mediterranean region^[80,81]. Not surprisingly the highest rates and SIR (commonly > 100) for KS post LT are reported in Italian transplant series^[11,17,47].

Post-transplant lymphoproliferative disorders

The term PTLD encompasses a broad spectrum of lymphoproliferative disorders observed in the immunocompromised solid organ transplant recipients. It is the second most common malignancy in LT recipients, and is notable in its wide age distribution, extending to the very young^[14]. The rate of PTLD is lower in the liver compared to other solid organ recipients^[82], likely due to lower immunosuppression levels needed to prevent liver allograft rejection, and possibly a smaller number of donor lymphocytes in the graft^[83]. The other factor driving PTLD risk is EBV infection, with associated PTLD generally occurring earlier, in the first 12 to 18 mo, after LT and involving younger patients^[82,84]. Infection with EBV and immunosuppression appear to play crucial roles in the pathogenesis of PTLD. EBV mismatch between donor and recipient of LT increases the risk of PTLD by 70 fold^[85,86]. Primary infection with EBV after LT also increases the risk significantly^[87]. Primary EBV or latent (of virus within B cells) infection can stimulate B cell proliferation and transformation^[88]. EBV associated PTLD occurs three times more frequently in pediatric patients^[87,89]. This is likely a reflection of the EBV negative status of pediatric recipient, whereas EBV infects 90% of the adults worldwide^[90].

Another important phenotype of PTLD develops later post LT in the absence of EBV infection involves older recipients and carries a worse prognosis^[82]. The pathogenesis of EBV negative PTLD is uncertain^[91], but some risk factors were described in a study of 480 adult LT recipient PTLD in France, where 16 developed PTLD^[92]. These were age above 50, LT for hepatitis C virus (HCV) or alcoholic cirrhosis, and the use of anti-lymphocyte antibodies such as muromonab, the latter reported by others^[82,87]. The use of anti-thymocyte globulins in LT for HCV cirrhosis augmented PTLD risk in another study (27% for HCV vs 6.4% for non-HCV

cases, $P = 0.08$)^[93]. When compared to lymphomas in the immunocompetent population, PTLD are more likely to exhibit extra-nodal involvement, high-grade and poor outcomes^[94]. Factors which confer a poor prognosis with PTLD are; high grade or stage at diagnosis^[43], T cell disease^[95], central nervous system and bone marrow involvement^[96,97], poor performance status^[98], higher number of extra-nodal sites^[98], and EBV negative disease^[43,85,99].

Solid organ cancers

Like PTLD, this category of *de novo* malignancy carries significant risk of mortality post LT, but is a term loosely used to group a wide range of tumor types and organ involved. Some characteristics of risk are evident in relation to subgroups of solid organ cancers, including aerodigestive, gastrointestinal, genitourinary and gynecologic systems.

Aerodigestive cancers

Aerodigestive cancers are associated with smoking and alcohol use, and arise from the tissues of the aerodigestive tract, which include the respiratory tract and the upper part of the digestive tract (including the lips, mouth, tongue, nose, throat, vocal cords, and part of the esophagus and windpipe). These are largely reported as head and neck cancers and lung cancer post LT.

A meta-analysis of studies examining head and neck cancer after LT found an overall SIR of 3.8 (95%CI: 2.7-4.9)^[100]. They develop at mean post LT intervals that range from 34 to 61 mo^[3-5,92,101]. Liver transplant recipients with a history of tobacco use and ALD are at high risk for developing head and neck cancers^[7,12,102], and in some studies only developed in patients with a history of ALD^[6,103].

In a large study encompassing all solid organ transplants in the United States, the SIR for lung cancer after LT was found to be 1.95 (95%CI: 1.74-2.19)^[14]. Lung cancer develops at mean post LT intervals ranging from 42 to 50 mo^[3,5,28,61,101]. The main risk factors for lung cancer, similar to the general population, in LT recipients was smoking^[2,7,12,54]. Those transplanted for ALD also had increased risk of lung cancer compared to those transplanted for other causes (4.3% vs 0.7%, $P < 0.001$), though tobacco use which prevalent in this population may confound these observations^[7,12,54]. Post LT lung cancer is commonly diagnosed in advanced stages^[3,5,54], suggesting the need for diligent surveillance programs in the high risk population (smokers and those transplanted for ALD). It remains unclear how long tobacco and alcohol related cancer risk persist following cessation.

Gastrointestinal cancers

The most common gastrointestinal cancer seen in solid organ transplant recipients is colon cancer^[14]. The SIR for colon cancer in LT recipients ranges from 1.4 to as high 27.3 in subsets of high risk patients with PSC^[16,17,45].

Patients receiving LT for PSC are at particularly high risk for colon cancer, due to the association with IBD^[12,45,104-106]. In the study by Watt *et al.*^[12] PSC alone (HR = 1.9, $P = 0.12$) was not a risk factor for gastrointestinal malignancy, whereas patients with PSC, IBD and intact colons had a significant cancer risk (HR = 3.51, 95%CI: 1.48-8.36, $P = 0.005$). Colon cancer was more common in LT recipients with ulcerative colitis (SIR = 27.3 vs 3.5), than those without it, particularly in patients older than 40 (SIR = 4.8 vs 1 in younger patients)^[45]. Longer duration of IBD and more extensive colonic involvement increase the risk for colorectal cancer in LT recipients with PSC^[104-106]. Colorectal cancer develops at a younger age in LT recipients compared with the general population, and has a worse prognosis^[107,108]. A relatively high incidence of colon and stomach cancer have been reported in a South Korean study^[67], with otherwise relatively low (2.2%-2.3%) *de novo* malignancy incidence rates reported in East Asian studies^[67,69].

Genitourinary and gynecologic cancers

Registry studies indicate an increased SIR of some (cervical, vulvar, bladder and kidney) but not all genitourinary or gender-specific (breast, prostate, uterine, ovarian) cancers following solid organ transplant^[10,14-17,45]. In the largest of these, there was a slightly lower SIR for breast and prostate cancer in transplant recipients^[14]. Cervical cancer risk was significantly elevated in one series (SIR = 30.7)^[17], and other human papilloma virus related cancers (vulvar, vaginal, anal, penile) all appear to have higher SIR (range 2.4-7.6) relative to the general population^[14]. Bladder cancer risk is increased in a number of studies, with a range of SIR value from 1.5 to 2.4^[14-16], and were noted to develop late (10 years) post LT in one cohort^[47].

SURVIVAL AFTER *DE NOVO* NON-SKIN CANCERS

In a comparison of patients from a solid organ transplant cancer registry with a general population from the Surveillance, Epidemiology, and End Results database, transplant patients were more likely to be diagnosed with American Joint Commission on Cancer stage > 2 cancers, and worse cancer-specific survival^[109]. The relative risk of cancer-related mortality compared to the general population was 2.9 (95%CI: 1.59-5.11)^[7]. In a large single center study *de novo* malignancy, excluding NMSC, was a leading category of mortality risk (14.2%), along with infections (15%), disease recurrence (13%) and cardiovascular (9%) complications^[2]. Patient survival rates at 1.3 and 5 years after diagnosis of *de novo* malignancy were 55%, 36%, and 27% compared with 100%, 100% and 67% for patients with only NMSC, $P = 0.001$, respectively^[2]. Similarly, *de novo* malignancy excluding NMSC was associated with an increased risk of mortality [HR = 4.9 (95%CI: 1.67-14.2), $P = 0.003$] in another large series^[40], and probability of death after

diagnosis was 40% at 1 year, and 55% at 5 years, respectively^[12].

There is considerable variability in reported survival after PTLD, with median survival as low as 2 mo (95%CI: 0.3-3.5 mo) in one study^[36], likely as a result of heterogeneity in risk characteristics of PTLD^[95]. Longer median survival intervals (27 mo to 35 mo) are noted in other LT series^[12,14], with reported 1 and 5 year survival rates of 56% and 46%, respectively^[82]. Pediatric LT recipients with PTLD appear to have better outcomes, with median survival of 8.2 years and reported 10 years survival rates of 59%^[85,96], and no reported mortality in some series^[94]. Advanced stage, Burkitt or Burkitt-like PTLD, and c-myc translocations indicated poor prognosis and short survival in pediatric PTLD^[96].

The reported site-specific cancer survival rates for the aforementioned solid organ cancer categories are: Oropharyngeal cancer 1 and 5 year survival of 43% to 78% and 56% respectively, lung cancer 1 and 5 year survival of 41% to 43% and 16% respectively, gastrointestinal cancers 1 and 5 year survival of 67% to 80% and 52% respectively, and genitourinary cancers 1 and 5 year survival of 79%-100% and 71% respectively^[3,12].

SURVEILLANCE

The increased risk and mortality associated with *de novo* malignancies underlines the need for surveillance strategies to detect tumors at earlier stages, allow more effective treatments, and improve survival. However, there are no standardized surveillance protocols for LT recipients at present. Routine follow up visits alone were only capable of detecting 12% of the non-skin cancers in one series, and annual visits resulted in identifying half of all malignancies in another^[8,9]. Poor compliance with surveillance protocols was also cited as a limitation in a study where active surveillance identified only 3 of 28 non-skin cancers^[7]. These data further highlight the need for regimented surveillance strategies in this regard.

In a compelling study, the incidence and outcome of *de novo* malignancy were compared before and after institution of an intensified surveillance protocol which included: Annual chest and abdominal computerized tomography (CT), urological, gynecological (pap smear and mammography) and dermatological examination, and colonoscopies every 5 years^[18]. With a historical surveillance program consisting of annual chest radiographs and abdominal ultrasounds serving as the reference comparator, the detection rate for *de novo* malignancies increased from 4.9% to 13% with intensified surveillance ($P = 0.001$), fewer tumors were diagnosed at stage III or IV (46% vs 75%), and median survival following a diagnosis of non-skin cancer increased from 1.2 to 3.3 years ($P = 0.001$)^[18].

At another center, a similarly multifaceted surveillance protocol that included: (1) urinalysis, chest radiographs and abdominal ultrasounds performed every

6 mo in the first year post LT and annually thereafter; (2) mammography every two years; (3) colonoscopy every 7-10 years if no adenomas were detected; and (4) in patients with smoking history, an annual otolaryngological evaluation and low dose CT of the chest after 2006^[110]. Patients that were diagnosed with *de novo* malignancy through active surveillance had better survival (all were alive after 25 mo of follow up) compared with patients diagnosed with symptomatic disease or incidentally (median survival of 13.5 mo) ($P = 0.002$)^[110]. The use of annual low dose chest CT in LT recipients with more than 10 pack years of cumulative smoking history led to a diagnosis of early stage lung cancer in 12% of patients^[111].

Additionally, special populations amongst LT recipient and the specialized surveillance strategies that are or may be warranted for them include those with: (1) underlying PSC and IBD, or IBD alone of more than 8-10 years duration with annual surveillance colonoscopy; (2) a history of human papilloma virus infection with annual pap smear in females, and annual genital and anal pap/scraping in both genders; and (3) patients from the Mediterranean region with testing of HHV-8 titers due to increased prevalence and association with risk of KS^[112].

PREVENTATIVE MEASURES

Smoking is a major risk factor for cancer, especially nasopharyngeal cancers and lung cancer, as well cardiovascular disease related mortality^[56], and smoking cessation should be counseled as early as possible. Regular application of broad spectrum sunscreen (SPF > 50, with high-UVA absorption) over sun-exposed areas in solid organ transplant recipients, in conjunction with counseling of excessive sun exposure avoidance, reduced the risk of actinic keratosis, invasive SCC and BCC from developing in a prospective case control study in solid organ transplant recipients^[113]. Protective clothing has also been shown to protect against UV radiation^[114]. The minimization of immunosuppression without risking graft rejection is limited by the lack of accurate markers of over or under immunosuppression, but would likely to attenuate the risk of *de novo* malignancy in LT recipient. There is also insufficient evidence to guide the routine use of mTOR inhibitors in at risk patients, but those studies are ongoing^[115-120].

CONCLUSION

Liver transplant recipients are at increased risk of cancer when compared to the general population, and the most commonly encountered cancers are NMSC, PTLT, and aerodigestive. They are due mainly due to the effects of immunosuppression and latent oncogenic viruses prevalent in the population. Important risk factors for development of *de novo* malignancy include age, degree of immunosuppression, history of smoking and alcohol abuse and transplantation for PSC and ALD. *De novo* malignancies, excluding NMSC, represent a major risk

category for post LT mortality. There are no standardized surveillance protocols for *de novo* malignancy post LT, but available evidence supports adoption of some consistent surveillance strategies. Minimization of immunosuppression and attention and counseling related to other risk factors in LT recipients may reduce an individual's risk of developing cancers post LT, but more evidence is needed to optimize care.

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Hepatitis C virus and neurological damage

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Abstract

Chronic hepatitis C virus (HCV) infection exhibits a wide range of extrahepatic complications, affecting various organs in the human body. Numerous HCV patients suffer neurological manifestations, ranging from cognitive impairment to peripheral neuropathy. Overexpression of the host immune response leads to the production of immune complexes, cryoglobulins, as well as auto-antibodies, which is a major pathogenic mechanism responsible for nervous system dysfunction. Alternatively circulating inflammatory cytokines and chemokines and HCV replication in neurons is another factor that severely affects the nervous system. Furthermore, HCV infection causes both sensory and motor peripheral neuropathy in the mixed cryoglobulinemia as well as known as an important risk aspect for stroke. These extrahepatic manifestations are the reason behind underlying hepatic encephalopathy and chronic liver disease. The brain is an apt location for HCV replication, where the HCV virus may directly wield neurotoxicity. Other mechanisms that takes place by chronic HCV infection due the pathogenesis of neuropsychiatric disorders includes derangement of metabolic pathways of infected cells, autoimmune disorders, systemic or cerebral inflammation and alterations in neurotransmitter circuits. HCV and its pathogenic role is suggested by enhancement of psychiatric and neurological symptoms in patients attaining a sustained virologic response followed by treatment with interferon; however, further studies are required to fully assess the impact of HCV infection and its specific antiviral targets associated with neuropsychiatric disorders.

Key words: Hepatitis C virus; Neuro disorders; Blood brain barrier; Nervous system; Inflammation

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Core tip: There is high prevalence rate of neuropsychiatric ailments with respect to patients infected with chronic hepatitis C virus (HCV). Brain inflammatory disorders, cerebrovascular disease peripheral neuropathy, psychiatric disturbs and cognitive symptoms are the complex clinical signs which occurs when infected by chronic HCV infection. HCV prompts psychiatric and neurological symptoms through numerous pathways with imprecise mechanisms, which includes neurotransmitter and metabolic pathway imbalance, immune-mediated responses and direct brain neurotoxicity inflammation. Awareness of HCV-associated neuropsychiatric disorders and its pathogenic mechanisms is vital to understand the clinical manifestations and to introduce an applicable treatment.

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INTRODUCTION

Hepatitis C virus (HCV) with a prevalence rate of 2.8% globally, affects around 185 million people^[1]. Targeting mainly liver parenchymal cells (hepatocytes), HCV causes severe hepatitis, cirrhosis that could lead to hepatocellular carcinoma if left unattended. It may affect other organs too^[2]. The association of hepatitis with insulin resistance and diabetes type 2 is well documented. Nevertheless it has also been correlated to various other organs like eye, gut, kidney, thyroid and cardiovascular complications leading to rheumatic diseases, neuropathology, and dermatological complications^[3-8]. Hence, it could be considered a systemic complication, due to its ability to use cellular machinery for replication regardless of the organ^[9]. How HCV is correlated with extrahepatic complications is poorly understood. Nevertheless, chronic infections are characterized by hepatic and systemic inflammation through activation of several signaling pathways and through their effect of release of various cytokines and augmented oxidative stress^[10]. HCV infection could directly or indirectly cause systemic inflammations by inducing immunological response to the disease and metabolic imbalance.

The correlation of HCV disease covers a wide spectrum of clinical manifestations therefore it necessitates the importance of understanding the disease in this context, clinically, to overcome these manifestations in a more robust way. It is reported that chronic HCV infection

has been associated with neurological as well as psychiatric conditions in upto about 50% of the cases^[11]. Major HCV related neurological ailments comprise of autoimmune disorders, cerebrovascular events, myelitis, encephalopathy, encephalomyelitis, and cognitive impairment; psychiatric disorders include, anxiety depression, and fatigue^[12,13]. All the aforementioned complications that are manifested by HCV infection, regardless of the severity of disease^[14]. There seems to be a lack of knowledge about how hepatitis is linked to numerous complications though it's certain that the brain can be a suitable site for viral replication^[15]. Nevertheless sequence analysis of HCV residing in liver and brain show variability suggesting an evolutionary path that a virus may embark on to be able to infest the central nervous system (CNS)^[14,16].

CNS INVOLVEMENT IN HCV INFECTION

HCV infection has been correlated to numerous neurological disorders ranging from meningeal to encephalic inflammation and leukoencephalitis^[17,18].

Clinically a wide range of complications have been associated with HCV infection, ranging from encephalomyelitis to loss of neurons though sphincter impairment, spastic quadriparesis and sensory dominate the clinically know complication^[17]. Finding the viral genome in brain during postmortem signifies the correlation of disease with neurological pathology. HCV related transverse myelitis and neuronal malfunction has been well documented^[19-22].

Severe demyelination in addition to the infiltration of parenchyma and perivascular T cells has been linked to HCV infection by examining spinal cord biopsy. The commencement of the disease is indicated by acute partial transverse and myelopathy transverse myelitis, or else by sensory ataxia or spastic paraplegia. Recurrence and multi-segmental spinal involvement are commonly reported. A patient with no sign of virus but positive for anti-HCV antibodies postulates an immune mediated response leading to neurological complications.

Chronic HCV has also been associated with severe encephalomyelitis^[23,24]. Magnetic resonance imaging (MRI) reports point toward CNS injuries in the cerebral and cerebellar white matter. Clinically, dysfunctional psychomotors, consciousness, hemianopsia, urinary retention, hemiparesis and other neurological defects are documented. HCV has been proposed to prompt demyelination *via* an immune-mediated response. These findings proposed that in cases with acute disseminated encephalomyelitis the likelihood of HCV infection increases^[11].

NEUROPHYSIOLOGICAL SYMPTOMS

Around 50% HCV infection patients complain of neuropsychiatric symptoms, brain fog, fatigue, and also show quality of life impairment upto some extent, regardless liver disease severity^[25]. During the onset of the disease

HCV patients report complications like, fatigue, malaise, maintaining attention and forgetfulness. In a study on 37 HCV infected patients without other complications by McAndrews *et al.*^[26], verbal learning impairment and lack of attention were observed. A correlation of cognitive impairment and fatigue with HCV infection was observed in half of the patients observed in a study conducted by Weissenborn *et al.*^[27], comparing neuropsychological functioning of HCV positive patients with normal liver function; though in another study by Montagnese *et al.*^[28], an exceptionally high incidence of fast (β -dominated) electroencephalograms was documented.

REPLICATION OF HCV IN BRAIN

HCV, though primarily infecting the liver, is frequently associated with CNS abnormalities^[27]. Neurocognitive defects in chronic HCV infection independent of hepatic encephalopathy is increasingly reported in several studies^[10,26,29]. It is however unclear if the CNS itself supports the viral replication. A recent study has shown the expression of HCV receptors in the brain microvascular endothelial cells. Interestingly, the microvascular endothelia are the only cells in the neuronal pool to bear the receptors for HCV^[30]. Microvascular endothelial cells, that form integral components of blood brain barrier (BBB), are thus assumed to play critical role in the transit of HCV into CNS^[30].

Quantification of HCV RNA in the brain, liver and plasma have shown a 1000-10000 fold lower load in brain compared to the liver. The HCV RNA was detected in a minimum of one region of the brain of four HCV infected subjects, independent of human immunodeficiency virus (HIV) co-infection status. The viral RNA quantities from the brain and liver - however significantly varied between clinical samples, which may be due to a higher postmortem interval resulting in the degradation of RNA in some sample^[30]. The E1 and 5' untranslated region sequences of HCV also varies between the liver, brain and plasma, further reinforcing the hypothesis of HCV replication and involvement in the brain^[31-33].

Visualizing the hepatocytes expressing HCV antigen is difficult due to the low cellular viral^[34,35]. Based on the relatively low HCV RNA content in brain to the liver, detection of HCV antigen in the brain is extremely challenging, and existing imaging methodologies are not sensitive enough to detect the cells of CNS that are infected by the virus^[29].

Prior studies have shown the presence of HCV RNA in microglia and astrocytes that were also isolated by laser capture microdissection^[36,37]. Another study has shown that two independently derived brain endothelial cell lines (hCMEC/D3 and HBMEC) facilitate the entry and replication of the virus. Antibodies specific for CD81, SR-BI, and claudin-1 inhibited the infection, demonstrating a common receptor dependent entry pathway for hepatocytes and hepatoma-derived cell lines^[30,38,39]. All these studies have shown that the viral entry may not

be limited to hepatocytes. mRNA and protein profile database have shown the expression of CD81, SR-BI, and claudin-1 in epithelial and endothelial cells derived from various tissues^[40,41] strongly suggesting that HCV infection may be supported by extrahepatically^[29]. Besides, the entry of HCV into the brain endothelial cells, its replication has also been observed. The HCV infected hCMEC/D3 cells release lower level of virus that can potentially infect hepatoma cells, thereby spreading infection which was CD81 dependent.

Studies have also shown that ApoE plays important role in the infection of brain endothelial cell^[42,43]. This is evident by the neutralization of HCV infection in hCME/D3 cells by ApoE antibodies, while only partially neutralized Huh 7 further, underlining its role in exacerbation of infection in the hCME/D3 cells^[30].

The tight junction between endothelial cells forming the BBB restricts the exchange of substances between the blood and CNS. Moreover, the receptor-mediated efflux transport systems further restrict the entry of hydrophilic molecules into the brain^[44]. The presence of multidrug resistance proteins such as the P glycoprotein in the BBB provide a protective niche for the replication of virus by restricting the access of antiviral drugs in patients treated for HCV infection^[45]. Studies have also shown the inhibition of HCV replication through antiviral agents targeting NS3 protease and NS5B polymerase enzymes *in vitro*^[30].

Disruption of BBB result in enhanced access of pathogens such as the HIV and west Nile virus into the CNS^[46,47]. Infection of hCMEC/D3 is associated with enhanced HCV RNA and antigen expression as confirmed by TUNEL staining with increased permeability to FD-70 a paracellular permeability marker.

In conclusion, it was observed that the brain microvascular endothelium, expressing the major viral receptors essentially contribute to the CNS infection of HCV. Specific brain endothelial cell lines have been identified to support the entry and replication of HCV in the brain that may be controlled by antibodies specific for HCV receptor-such as interferon and antiviral agents.

Low level release of virus by HCV-infected hCMEC/D3 cells with cytotoxic properties supports a model in which the BBB provides - an ideal extrahepatic environment for infection, implying a direct role of HCV to induce neuropathology^[30].

MECHANISMS CONTRIBUTING TO NEUROLOGICAL DYSFUNCTION

HCV could lead to various CNS complications ranging from cerebrovascular events to autoimmune syndromes. Acute cerebrovascular events which includes transient ischemic attacks, ischemic stroke and lacunar syndromes have been reported in patients suffering with HCV^[13,48,49]. Occurrence of occlusive vasculopathy as well the vasculitis is also the well-known events^[50,51]. Isolated CNS vasculitis could lead to the narrowing of

cerebral arteries^[52]. In few of the patients, the CNS ischemic changes might be possible in the setting of an anti-phospholipid associated syndrome or it might be associated with the anti-neutrophil cytoplasmic antibodies^[53]. A recent study has shown the HCV-metabolic syndrome association with an evidence that HCV infection is a great risk factor for an enhanced thickness in the carotid wall and plaque formation, thus is a major contributory factor of cerebrovascular mortality specifically in the patients who have higher levels of HCV-RNA^[54].

Encephalopathic syndromes that have been clinically characterized by confusion, altered consciousness, cognitive impairment, dysphagia, and dysarthria are linked with the diffuse involvement of white matter in HCV patients with cryoglobulins and/or circulating anticardiolipin antibodies. The patients suffering with these syndromes have also shown small lesions in the sub-cortical regions and periventricular white matter. Additionally, alterations in severe and diffuse infra and supratentorial white matter that could cause vasculitis have been observed in patients with coincidental systemic vasculitis. Another study has shown that a CNS vasculitis-induced ischemic damage in a patient that also suffering with mixed cryoglobulins (MC), peripheral neuropathy, and relapsing multiinfarct encephalopathy^[55]. The neuropathological examination of this patient has shown multiple ischemic lesions (0.5-3 mm in diameter) in the white matter of cerebral hemispheres, cerebellum, parenchymal infiltration, and an accumulation of the lymphocytes around small vessels. Further study has also shown the incidence of vasculitis-induced ischemic changes in a patient that was suffering with chronic HCV, MC, and sensory neuropathy^[56].

Besides the encephalopathic syndromes, cognitive decline that has been clinically characterized by an impaired attention, visual constructive, and spatial functions have been associated with an enhanced occurrence of periventricular white matter high intensity signals (WMHISs) on T2-weighted MRI^[56]. The patients have shown a relationship between CG level and number of impaired cognitive functions whereas no correlation was observed with systemic manifestations of CG, including peripheral neuropathy. A variation in the WMHIS has shown vessel disease that could lead to chronic hypo-perfusion of white matter and local alterations of blood-brain barrier^[57]. Spectrum of CNS syndromes encountered in HCV patients is not restricted to the foregoing vasculitic and vasculopathic forms but also causes inflammatory disorders such as an acute encephalitis, meningoradiculitis and encephalomyelitis. Studies have also shown the patients suffering with leuko-encephalitis and perivascular T-cell infiltration in association with HCV genome^[18] or fatal progressive encephalomyelitic syndromes^[17]. Another study has shown a patient suffering with an acute disseminated encephalomyelitis, an autoimmune post-infectious CNS disease that has been developed after HCV-infection

which supports the role of cellular immune-mediated mechanisms in CNS complications of HCV infection^[24].

Most of the patients with chronic HCV-infection complain of fatigue, poor memory and impaired concentration. Fatigue, mood alterations and cognitive dysfunction has shown a disturbed social and physical activity of the patients. Few of the HCV patients with severe fatigue also complain of sleep disturbances, restless leg syndrome, muscle and joint pain, and depression. A recent study of 53 HCV-positive patients with neuropsychiatric has shown an increase choline and myo-inositol concentrations in the basal ganglia and white matter, and an increase in the concentration of creatinine, N-acetyl-aspartate (NAA), and N-acetyl-aspartyl-glutamate in basal ganglia^[58], these findings are consistent with the HCV-induced chronic cellular inflammation. Another study revealed an increased ratio of choline/creatine (Cho/Cr) in the basal ganglia as well as the frontal white matter of HCV infected patients through magnetic resonance spectroscopy^[59]. Further findings have shown^[60] a lower level of NAA/Cr ratio in the frontal grey matter of HCV-patients without any change in the Cho/Cr ratio. Both findings have suggested the occurrence of an increased cell membrane turnover and reduced neuronal function^[27]. Use of ondansetron which is a competitive antagonist of serotonin receptors has ameliorated fatigue in HCV infected patient. Also, the placebo controlled randomized study of thirty six HCV infected patients have shown an improved fatigue and depression scores with ondansetron^[61]. These studies have shown an important role of serotonergic pathway dysfunction that causes fatigue, reduced level of serum tryptophan, and a reduced synthesis of serotonin^[62,63]. Moreover, findings of fifteen HCV infected patients reporting neuropsychiatric symptoms was carried out through different neuropsychological tests including 18F-fluoro-desoxy-glucose, serotonin, and positron emission tomography. The results have shown significant decrease in striatal and midbrain dopamine availability and decrease metabolism in limbic, parietal, frontal, and temporal cortices. These findings further confirmed significant role for defective dopaminergic transmission in causing cognitive impairment in the HCV^[64]. The HCV infection has also been linked with myopathy and a few cases of non-inflammatory and inflammatory myopathies were reported. The clinical features of HCV associated myopathies ranging from progressive weakness to relapsing forms, mild increase in muscle enzymes, and moderate weakness. In non-inflammatory myopathies, pathological features include vacuolar changes^[65] and necrotizing myopathy^[66] with slow or progressive proximal weakness, and selective atrophy of type 2 fibers in relapsing myopathy. Study has showed the oxidative mitochondrial damage in a patient with severe ptosis, generalized weakness, diplopia and respiratory involvement, and ultra-structural changes of mitochondrial shape, and cristae^[67]. Additional findings have showed that HCV promotes tumor necrosis factor -

mediated apoptosis in myocytes^[68].

CRYOGLOBULINEMIA

Cryoglobulins are immunoglobulins in nature. They are able to clump together at temperatures below 37 °C^[69,70] causing organ damage chiefly through two main pathways, vascular sludging (Hyperviscosity syndrome, associated with type I cryoglobulinaemia) or immune-mediated (Vasculitis, associated with mixed cryoglobulinemia)^[70]. Causes of cryoglobulinaemia range from, infections, autoimmune disorders and malignancy though the main culprit is HCV^[70]. Our understanding of cryoglobulinaemia advocate successive antiviral therapy in conjunction with targeted therapy rather than following a monotherapeutic^[71,72].

While our understanding of the disease progression still has loop holes, chronic immune stimulation/lymph proliferation due to increased cryoglobulins production, formation of complex by cryoglobulins or their antigens and their inadequate clearance are considered to be three main causative factors of cryoglobulinemia.

TYPES OF CRYOGLOBULINEMIA

Type I cryoglobulinemia is characterized by monoclonal globulins produced during lymphoproliferative disease. These Igs precipitate during exposure to cold, leading to inflammatory vasculitis and vessel obstruction.

Types II and III cryoglobulinemia is associated with increased production of cryoglobulins by proliferative B-cells clones^[72,73]. Chronic HCV infection may trigger hyperactivation of B-cells causing infection *via* CD81, a cell surface protein^[74,75].

HCV IN CRYOGLOBULINEMIA

Detection of HCV in 1989^[76] significantly changed the course of scientific research from fundamental to HCV-oriented cryoglobulinaemia^[77]. Ferri *et al*^[78] detected circulating HCV-RNA in almost 90% of subjects with mixed cryoglobulinemia, though subsequent analyses by other groups discovered wider ecological. HBV is found to be associated with mixed cryoglobulinemia whereas HCV is primarily correlated with type II cryoglobulinaemia^[79]. HIV infected patients have low percentage (7%-17%) of cryoglobulinemia but rises to almost 65% in patients coinfecting with HCV^[78] that can be reduced by anti-retroviral therapies^[79]. Apart from viral infections the disease has also been associated with a wide range of other infectious.

HCV infection is an important model to understand the mechanisms that lie behind cryoglobulinaemic etiology. HCV lympho-tropism is the first step in B-cell hyper proliferation, regardless of cryoglobulinaemia^[78,80]. E2 an HCV envelope protein interacts with the major extracellular loop of tetraspanin CD81, a signaling protein expressed by hepatocytes, B and T lymphocytes^[74]. This interaction purportedly triggers prolonged

B-cell stimulation^[81]. B-cell clones are found in peripheral blood, bone marrow, and liver HCV patients, predominantly those with type II cryoglobulinaemia. These B-cell clones produce monoclonal IgM with an idiotype, WA that works as a cross-linker, this binds to immunoglobulins directed towards HCV core protein. Precipitates from HCV-related cryoglobulinaemia patients comprise of HCV core proteins and RNA, this means the immune system forms cryoglobulins during chronic HCV infection^[82].

PERIPHERAL NEUROPATHY

Peripheral neuropathy (PN) is a complication secondary to large number of common diseases such as diabetes, thyroid disorders, renal failure, vitamin deficiency and treatments, including viral infection. Degradation of sensory or motor axons commonly occurs which disrupts the effective communication between the central and peripheral nervous system^[83]. Clinically, PN manifests itself as motor impairment largely resulting in weakness, sensory defects such as numbness, paresthesia, hyperalgesia/allodynia and pain or more severe autonomic dysfunction leading to organ failures. Patients may present with multiple symptoms of varying severity, making it highly heterogeneous, which in turn depends on the underlying trigger^[84]. Degeneration of axon, vascular occlusion and inflammation^[84] with perivascular trafficking of mononuclear cells are essential pathologic features of the debilitating condition^[85]. Demyelization and absence of axonal fascicular differentiation is also reported.

Forty percent to seventy-five percent patients positive for HCV, present with symptomatic PN, being more prominent with HCV associated cryoglobulinemia (CG)^[86]. Although presence of serum cryoglobulins is a prognostic marker of severe manifestation of PN, symptoms may be reported even in its absence, underlying a direct role of the virus in precipitating the disease^[87]. Yoon *et al*^[88] reported 43.5% prevalence of PN in the absence of CG based on clinical and electrophysiological examination of HCV infected patients.

Involvement of peripheral nervous system in HCV infection is variable depending upon age, duration of infection, CG and other comorbidities^[12,88]. Twenty-six percent to eighty-six percent HCV patients positive for CG present with clinical/electrophysiological PN. Pathogenesis of HCV associated PN is indirect and mostly inflammatory, as the virus itself does not invade the nerve and muscle tissues. Mechanisms proposed to explain the neurologic manifestation of the virus include the vascular deposition of HCV RNA containing CG, direct viral invasion and perivascular inflammation^[89].

HCV associated neurologic impairments range from sensory axonopathy to mononeuritis multiplex. Sensory or motor impairment of one or more distal nerves is most frequent, that tends to become symmetric causing loss of sensation and weakness^[90]. Prevalence of sensory and motor neuropathy with HCV was found to be

9% and 10% respectively^[91]. Sensory predominant symmetrical polyneuropathy involves perivascular infiltration of lymphocytes and monocytes of small sized vessels. Mononeuritis multiplex, involving one or two non-contiguous nerves, has a more systemic effect and involves inflammation of medium sized vessels with myriad of inflammatory cells accompanied with vascular necrosis that is asymmetric^[92]. Asymmetrical sensory neuropathies may be large or small fiber. Demyelinating polyneuropathy and polyradiculoneuropathy is less frequently encountered with HCV infection. Pure motor neuropathies and autonomic neuropathies are rare in HCV^[12].

Abd El-Kader *et al.*^[93] estimated the prevalence of PN in patients with HCV related liver disorders based on complete neurologic examination and nerve conduction study^[93]. Of the 50 subjects included in the study, 22% had sensory abnormalities, 18% had motor impairment while 10% had a combination of both. Furthermore, PN was found unrelated to serum vitamin B12 levels and severity of disease. Distal sensory loss of pain and reflexes may observe on neurologic examination in otherwise asymptomatic patients.

Biasiotta *et al.*^[90] characterized the clinical and neurological features of HCV related neuropathies. Sixty-eight percent (47 out of 69) of patients were diagnosed with peripheral neuropathy with 45 exhibiting a predominantly sensory, distal symmetric polyneuropathy while 2 showing mononeuropathy multiplex. Thermal pain sensitivity was specifically linked to pure small fiber neuropathy while sensory abnormalities observed in both mononeuropathy multiplex and mixed fiber, distal symmetric polyneuropathy.

PN can be considered a manifestation of HCV induced CG^[86]. Abnormal immunoglobulin, reversibly precipitating at low temperatures, *i.e.*, 4 degrees, with marked rheumatoid factor activity are produced by B cells, form immune complexes that obstruct vessels and trigger vascular inflammation^[90]. IgG and IgM are primarily implicated to precipitate HCV associated PN either by their direct binding to myelin inducing an erosive immune attack or as lymphocytic irritant within the vasa nervorum resulting in vasculitis. In one study antibodies against myelin associated glycoprotein were identified to induce the immune trigger^[93]. Such demyelinating association is rare with an occurrence of 5 per 10000. Anti MAG neuropathy is clinically characterized with sensory ataxia with motor involvement and hand tremor intention involving large nerve fibers^[94].

PSYCHIATRIC DISORDERS IN HCV-INFECTED PATIENTS

HCV like HIV is among the few known infections that cause psychiatric disorders^[95]. Illicit drug injection (IDU) is a major factor for HCV infection^[96]. IDU is common among patients who have personality disorders besides alcoholism, illicit sexual behavior and mood

disorders^[97-99]. Alcohol is predominantly associated with increased prevalence of anti-HCV antibody. Synergy between alcohol and HCV aggravates liver disease and lessens the effectiveness of interferon (IFN) treatment. IFN α is increasingly being used to treat HCV because of its effectiveness though it can induce a variable number of psychiatric disorders likes acute confusional state, depressive and agitated manic episode^[100]. Up to 70% of HCV patients treated with IFN may develop depression^[96]. This could be attributed to numerous pathophysiological complication that are associated with IFN treatment including distorted monoamine metabolism^[96], increase in apoptosis, BDNF reduction, and altered hypothalamus-pituitary-adrenal axis function^[100]. In one study observing the neurological implication of IFN in conjunction with ribavirin treatment the authors found 1/4 of all the patients developed major depressive disorder (MDD). Higher interleukin 6 concentrations in serum, history of psychiatric condition, depression and low educational level considerably increases the incidence of MDD during antiviral therapy^[101]. Symptoms of neuro-vegetative depression start to occur early during treatment though cognitive symptoms start in a span of 4 wk of IFN treatment^[96]. Depression, anxiety, and cognitive impairments can be treated through serotonergic antidepressants, while neuro-vegetative symptoms like loss of appetite, fatigue, sexual impairment, and psychosomatic symptoms are not much responsive to treatment by SSRIs^[102]. Hence serotonin-norepinephrine reuptake inhibitors, bupropion, methylphenidate or modafinil are used to address neuro-vegetative disorders^[100]. Confusional state induced by IFN α is associated with psychomotor retardation, disorientation, Parkinsonism, and psychosis in addition to induction of manic disorder. In case of acute mania mood stabilization and antidepressants are administered^[100]. HCV patients with MDD or bipolar symptoms are more prone to psychiatric disorders as compared to patients with no psychiatric illnesses during treatment with IFN^[96]. It's not just IFN but HCV itself might be associated with mood disorder. This can be in part linked to factors such as high-risk behavior, stigma and drug abuse. Nevertheless, evidence suggests the association some HCV genotypes such as 3a are related to risk of depression^[103]. Neuronal invasion by HCV is another factor that could lead to mental distortion^[103-105].

ANTIVIRAL TREATMENT

HCV related CG is clinically challenging. Use of immunosuppressive agents such as glucocorticoids and cytotoxic drugs is not recommended to manage CG induced severe neuropathic pain, because of the ensuing viral infection. Interestingly, antiviral agents considerably improve symptoms, underlining pathogenic role of virus in precipitating the secondary symptoms. Targeting the underlying viral infection is thus a reasonable strategy to treat HCV associated CG symptoms, although the response produced would be slow. Additionally, anti-

ral therapy suppresses B cell proliferation in the bone marrow, thereby controlling CG in more than one way. However, achieving a sustained virologic response is critical for the success of these therapies^[86]. Immunosuppression prior to induction of antiviral therapy can be considered in patients with severe symptoms to obtain a reasonable and timely therapeutic response. Ideally, a combination of immunosuppressive and an anti-viral agent is highly desirable that can directly act on the proliferating B cells while simultaneously wiping out the etiologic trigger, *i.e.*, the virus^[106].

IFN α is a cytokine produced by cells that primarily modulates the immune response during viral infections^[83]. Interferon in combination with other drugs is an essential component of HCV therapy. Therapeutic utility of IFN lies in their ability to decrease virus replication rate, inhibit lymphocyte proliferation, Ig synthesis with enhanced immune complex competency and macrophage activity^[86].

IFN α monotherapy, although active against virus, was associated with heightened autoimmunity^[107]. Thus, the IFN α itself is assumed to brew the pathogenic inflammatory environment for neuropathy *via* immune mediated myelin degradation and vessel occlusion causing nerve ischemia, in the absence of CG. Despite its increased high autoimmune titer, IFN α forms the core of HCV therapy. Peg IFN α -ribavirin is clinically, virological, immunologically superior to IFN α -ribavirin and is recommended in mild to severe cryovas with HCV. Moreover, it was associated with shorter duration of therapy, less frequent side effects and deaths producing, sustained virologic response in 60% patients^[84]. Therapeutic success of the combination varies between 48%-88% depending upon the HCV genotype. Reduction of neuropathic pain in HCV positive patients was observed from 65.2% to 22.1% after peg IFN and ribavirin therapy^[108]. Chronic inflammatory demyelinating polyneuropathy, reported in a minority of HCV infected population, was significantly corrected with IFN α and ribavirin therapy, although a few studies have classified it as a side effect of IFN α ^[109]. In that case, intravenous Ig administration and plasma exchange were effective for management of PN. The efficacy of ribavirin in reducing PN is attributed to its viral clearance, decreasing inflammation, circulating cryoglobulins and anti MAG antibodies^[83].

In patients not responding to antiviral therapy, addition of protease inhibitors telaprevir/boceprevir significantly enhanced the clinical outcome. Potency of the triple antiviral therapy was comprehensively described by Saadoun *et al.*^[110]. In a cohort with genotype 1 HCV and CG. Patients randomly received either telaprevir/boceprevir with peg IFN α /ribavirin for 48 wk and were followed up to 6 mo after treatment. Of the 56.6% patients included in the study with peripheral neuropathy, 47% showed improvement after treatment as clinically assessed by the neuropathy total symptom score. The study further highlighted the clinic considerations for the success of the triple antiviral

therapy.

Successes of these trials have driven the direct use antiviral agents to reduce cryoglobulinemia and related symptoms associated with HCV. The NS3/4A inhibitor simeprevir and the NS5B inhibitor sofosbuvir have recently been approved, for their nearly absolute sustained virologic response 95% with minimum toxicities^[106]. The magnified therapeutic response is due to their shortened courses of combination IFN free therapy.

Rituximab, a CD 20 monoclonal antibody, directly acts by arresting cryoglobulins production and its subsequent pathogenic cascade^[108]. Rituximab monotherapy is thus highly relevant in treating cryovas emergencies such as neuropathic pain^[111]. However its sole efficacy in reducing PN has not been satisfactorily assessed. In one study, 36% patients showed a subsidy in peripheral neuropath with rituximab administration.

Studies have reported higher efficacy and safety of rituximab against the conventional immunosuppressive agent's, *i.e.*, glucocorticoids, azathioprine and cyclophosphamide to treat cryovas. An early clinical remission of cryovas with rituximab therapy was reported in patients with HCV, who did not show improvement with previous antiviral treatment^[111-113]. Patients with liver cirrhosis, not eligible for antiviral treatment, also showed improvement with rituximab therapy, with enhanced protidosynthetic and ascites activity of the liver.

Rituximab monotherapy effectively alleviated MC symptoms in about 71.4%-84% HCV patients^[111,112]. Rituximab given in combination peg IFN α -ribavirin was evaluated in patients with severe HCV-cryo, resistant to IFN α combination therapy. Of the 16 patients enrolled, 15 showed marked clinical improvement with 10 complete responders. Efficacy and safety of peg IFN α /ribavirin with and without rituximab was evaluated in two separate studies. Rituximab with the antiviral regimen produced earlier clinical remission^[114-116].

Recently, low dose of interleukin 2 (IL2) has emerged as a promising approach based on the presence of defective regulatory T cells in HCV-cryo. CD4⁺ CD25⁺ Fox P3⁺ T cells are assumed to be responsible for disease refractoriness, after the complete resolution of HCV and vasculitis^[117,118]. These are regulatory cells that control the autoimmune response of the body and their deficiency during viral infection account for the expansion of holistic B cells. Efficacy of low dose IL2 in patients with refractory HCV-cryo was assessed in a prospective open labeled phase I / II a trial wherein IL2 increased the percentage of the regulatory T cells while decreasing the B cells^[119-121].

DISCUSSION

Antiviral treatment should be the first line treatment for managing mild to moderate vascular and neurologic symptoms. Symptoms usually recede with an optimum sustained virologic response. Rituximab therapy should be opted for patients with severe exacerbations of secondary symptoms. Plasmapheresis may be required

before placing patients on the antiviral therapy. Patients on IFN therapy should be monitored as IFN therapy may aggravate the symptoms. Corticosteroids may be used for temporary relief of minor inflammatory pain. Immunosuppressant should be the last resort opted in patients not responding to antiviral treatment/refractory disease.

CONCLUSION

Age and duration of HCV infection are the major clinical determinants of PN. Furthermore, it was found that duration of HCV infection and not the presence of cryoglobulins, was related to PN. Clinically, Neuropathic pain with HCV should be approached with a multimodal approach, with the prime objective being reducing the viral load, that automatically resolves secondary symptoms. Treatment should be strategized based on the severity of the disease and patients response. Addition of steroids, tricyclic antidepressants, local anesthetics and opioids may be required to the standard antiviral therapy, in case of acute pain attacks. Persistence or relapse of neurologic symptoms despite viral clearance may be indicative of other seeding conditions.

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Antiviral therapy for hepatitis C: Has anything changed for pregnant/lactating women?

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Abstract

Hepatitis C virus (HCV) affects about 3% of the world's

population, with the highest prevalence in individuals under 40. The prevalence in pregnant women varies with geographical distribution (highest in developing countries). Prevalence also increases in sub-populations of women at high risk for blood-transmitted infections. HCV infection in pregnancy represents a non-negligible problem. However, most of the past antiviral regimens cannot be routinely offered to pregnant or breastfeeding women because of their side effects. We briefly reviewed the issue of treatment of HCV infection in pregnant/breastfeeding women focusing on the effects of the new direct-acting antivirals on fertility, pregnancy and lactation in animal studies and on the potential risk for humans based on the pharmacokinetic properties of each drug. Currently, all new therapy regimens are contraindicated in this setting because of lack of sufficient safety information and adequate measures of contraception are still routinely recommended for female patients of childbearing potential.

Key words: Hepatitis C virus infection; Breastfeeding woman; Antiviral therapy; Pregnancy category; Direct-acting antivirals

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Core tip: Until recently, the only drugs available for the treatment of hepatitis C virus infection had a well-documented teratogenic effect limiting their use in childbearing women. Recently, new generation drugs, designated the direct-acting antivirals have been approved. There are no studies available describing their effects on pregnant and lactating women. We here will try to analyze their pharmacokinetic properties and data from animal studies to try to predict their potential use pregnancy.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health problem that affects more than 150 million people (about 3% of the world's population), most of whom are unaware of their infection^[1,2]. The prevalence of HCV infection is between 0.5%-2% in most European countries and in the United States, in which 5-10 million and almost 4 million people, respectively, are affected, most of whom are in the fourth decade of life^[1,3-5]. Differently, the prevalence of HCV infection exceeds 10% in some developing countries (especially in Africa, Asia and South America)^[1]. One of the modes of HCV transmission is vertical transmission. Rates of vertical transmission of HCV infection range between 2%-10%^[4]. Although HCV infection acquired at birth may resolve spontaneously, about 25000 to 50000 of children become chronically infected^[4-7]. The prevalence of HCV infection in children is very low in Europe and the United States (0.05%-0.36%)^[8], and increases to between 1.8% and 5.8% in Egypt (which has the highest prevalence of pediatric HCV infection), Sub-Saharan Africa, Mongolia and the Amazon Basin^[8]. Consequently, birth to an infected mother is one of most frequent routes of infection, and is comparable to injection drug use, unsafe medical practices and high-risk sexual practices^[1].

Acute HCV infection, asymptomatic in most cases, can progress to chronic hepatitis in more than half the patients. Chronic hepatitis C is associated with progression to fibrosis, which leads to liver cirrhosis in about 10% to 20% of patients within 20-30 years. Lastly, from about 1%-5% of cirrhotic patients can develop hepatocellular carcinoma each year^[2]. Currently, HCV-related liver cirrhosis is the major cause of liver transplantation in developed countries^[1,2]. Notwithstanding the decline in the number of cases of acute HCV infection, the burden of liver cirrhosis, hepatocellular carcinoma and HCV-related death remains high due to the existence of a reservoir of infected patients^[1,3].

The epidemiology of hepatitis C infection might change radically in the next few years thanks to antiviral therapies that result in viral clearance in terms of sustained virologic response (SVR), namely undetectable HCV RNA 12 wk (SVR12) or SVR24 after treatment completion. HCV infection is cured in more than 99% of patients who achieve an SVR. Generally, liver disease can be cured only in non-cirrhotic patients^[2].

The effect of maternal viremia on vertical transmission and on the rate of spontaneous resolution of the acquired infection among newborns is not well defined. However, mothers with undetectable plasma HCV RNA levels rarely transmit HCV by the vertical

route^[4]. Therefore, it seems reasonable to assume that treatment to decrease viremia in pregnant women with chronic HCV may result in lower rates of vertical HCV transmission.

Until 2011, the standard-of-care therapy for HCV infection, which was based on the association of pegylated interferon (PEG-IFN) and Ribavirin, resulted in an SVR in only 40%-80% of patients depending on HCV genotype (lower for genotypes 1 and 4 than for genotypes 2 and 3)^[2]. In 2011, new antiviral drugs, namely, direct-acting antivirals (DAAs), became available. These drugs act mainly by targeting the non-structural HCV proteases NS3-4A and NS5A or by inhibiting RNA-dependent RNA polymerase, and are thus referred to as protease inhibitors and inhibitors of HCV RNA-dependent RNA polymerase, respectively^[2]. Each of these DAAs can be used as a component of combination regimens (with or without PEG-IFN and Ribavirin) and result in SVR rates as high as 60%-100%^[2]. The SVR rate depends on the DAA used, the HCV genotype, pre-existing amino acid substitutions (that might confer resistance to some DAAs) and the severity of liver disease^[2]. These new regimens could change both the epidemiology and the natural progression of hepatitis C.

Here we discuss the potential use, in pregnant and breastfeeding women, of the antiviral therapies (including DAAs) licensed for the treatment of chronic C hepatitis. We also examine the adverse effects of anti-HCV drugs on fertility, pregnancy and lactation (in particular, embryo toxic and teratogenic effects). In this context, no antiviral therapy has yet been approved for use in childbearing women, and therefore little is known about the effects of anti-HCV drugs on pregnancy and lactation in this population. Consequently, our discussion and conclusions are based principally on data derived from animal studies^[9-15].

ANTIVIRAL THERAPY OF HEPATITIS C IN CHILDBEARING WOMEN

Hepatitis C infection in pregnant and breastfeeding women is not a negligible problem. About 1%-8% of pregnant women have markers of HCV infection, and the prevalence is lower in western/northern countries than in Eastern/Southern countries^[11,15]. Since, HCV infection is usually asymptomatic, most infected women are unaware of their status and may be diagnosed with chronic C hepatitis incidentally when undergoing serological tests during pregnancy or before delivery. For example, in Italy, free-of-charge screening for HCV, HBV and the human immunodeficiency virus, is routinely offered to all pregnant women from the 33rd to the 37th week of gestation^[16] and reveals many cases of previously undiagnosed chronic C hepatitis. Notably, the number of HCV-infected childbearing women is expected to increase with the increase in the migratory flow from developing countries to Western/Northern countries. As mentioned above, the vertical transmission

of HCV infection is now one of most frequent routes of transmission^[1]. Consequently, eradication of the virus in pregnant women and women of childbearing age is the main target in the prevention and control of HCV infection^[4]. Problems related to the treatment of HCV infection in pregnant and breastfeeding women are not rare. In a developed country such as the United States, pregnancy is the third most common contraindication to treatment and delayed treatment onset in about 2% of more than 45000 HCV-infected patients^[17]. In addition, in the same study about 1.3% of women undergoing antiviral therapy for HCV became pregnant during therapy^[17]. Thus, the problem is not only whether or not to start treatment in a pregnant and/or breastfeeding woman, but also how to manage a woman who becomes pregnant during antiviral therapy.

The past of antiviral therapy: PEG-IFN/Ribavirin and PEG-IFN/Ribavirin plus first-generation DAAs

For many years, the two cornerstones of the standard-of-care treatment of HCV infection were IFN and Ribavirin, both of which have side-effects and contraindications that limited their use in the setting of pregnant/breastfeeding patients^[18,19]. IFN- α is a protein released in response to viral infections. It binds to specific receptors on the cell surface thereby promoting a complex cascade of protein-protein interactions that rapidly activate gene transcription. IFN-stimulated genes regulate many biologic effects (*i.e.*, inhibition of viral replication in infected cells, inhibition of cell proliferation and immunomodulation). The United States Food and Drug Administration (FDA) classified the first pharmacological formulation of IFN- α in Pregnancy Category C since the molecule had an abortifacient effect in animals (rhesus monkeys) during the early/middle fetal period of organogenesis and late fetal development^[20,21]. The drug may also impair fertility; in fact, menstrual cycle irregularities, namely, prolonged or shortened menstrual periods and erratic bleeding, have been observed in nonhuman primates, and menstrual rhythm normalized upon treatment discontinuation^[22]. Decreased serum estradiol and progesterone concentrations have been reported in women treated with human leukocyte IFN although no mutagenic effect or toxicity has been reported^[22]. Given the species-specificity of IFN, effects in animals are unlikely to be predictive of those in humans^[20,21]. Nevertheless, in clinical practice, IFN- α is widely used in most pregnant women affected by essential thrombocythemia to prevent or reduce the risk of thrombocythemia-related fetal loss^[22]. The risk of major malformation, miscarriage, stillbirth or preterm delivery does not seem to be significantly higher in this setting than in the general population^[22].

Also the pegylated formulation of IFN- α (PEG-IFN- α) should be assumed to have abortifacient potential despite the lack of well-controlled studies in pregnant women^[23,24]. Apart from the potential risks for the fetus, a major concern is the risk of serious IFN-related adverse effects on the patient's psychological status,

namely exacerbation of postpartum depression^[18]. Therefore, pregnant candidates for PEG-IFN treatment should undergo psychiatric evaluation. The degree of IFN excretion in human milk is unknown. However, given the potential risk of serious adverse reactions to the drug in nursing infants, IFN is contraindicated in children below the age of 2 years^[20,21,23,24]. The decision whether to discontinue nursing and to initiate antiviral therapy depends solely on whether or not the progression of maternal liver disease must be immediately blocked. Given its low SVR rate (< 30%), PEG-IFN mono-therapy has been widely used in recent years in association with Ribavirin^[25]. The combination of PEG-IFN and Ribavirin increased the SVR24 to 40% in North America and to 50% in western Europe in patients infected with HCV genotype 1^[25]. Even better results were obtained in patients with genotypes 2, 3, 5 and 6: The best SVR was achieved in patients with genotype 2 (up to 80% SVR)^[25]. The results of combined treatment in genotype 4 patients are the same as those obtained in genotype 1 patients or slightly better^[25].

Ribavirin is a guanosine analog nucleotide inhibitor that acts by interrupting viral RNA synthesis and viral mRNA capping. It is a prodrug that, when metabolized (into purine RNA nucleotides), interferes with RNA metabolism required for viral replication^[26,27]. The mechanism underlying this effect is unknown. The FDA classified Ribavirin in Pregnancy Category X^[26,27] because of its embryocidal and teratogenic effects in animals^[26-28]. The fetal malformations reported in animal studies include abnormalities of the skull, palate, eye, jaw, limbs, skeleton and gastrointestinal tract^[26-28]. Therefore, Ribavirin is absolutely contraindicated for both HCV-infected childbearing women and HCV-infected male partners of pregnant women unless they take effective contraceptive measures. In addition, since Ribavirin-induced spermatogenic abnormalities (cell toxicity, mutagenicity and a decreased epididymal sperm count) reverted only 4-8 mo after treatment withdrawal in all animal species studied^[29,30], women are advised to avoid pregnancy for at least 6 mo after partners of men taking Ribavirin treatment^[26,27].

Between 2003 and 2009, the United States Ribavirin Registry collected the data of 118 babies born to mothers exposed to the drug (49 direct and 69 indirect exposures) during pregnancy: Only six cases of birth defects were reported (torticollis, hypospadias, polydactyly, neonatal teeth, glucose-6-phosphate dehydrogenase deficiency, ventricular septal defect, and cyst of the fourth ventricle of the brain)^[28]. Despite the low rate of birth defects, it seems reasonable not to encourage or support the use of Ribavirin in pregnant women, and, moreover, to recommend that women avoid pregnancy during Ribavirin treatment.

In conclusion, because of its low SVR rate unless combined with Ribavirin, PEG-IFN should not be administered in childbearing women even though it has not been reported to have abortifacient and/or teratogenic effects.

Table 1 Main pharmacokinetic properties of the new direct-acting antivirals

Drug	Molecular weight	Effect of food on absorption	Cytochrome P450 enzymes interaction			Binding to plasma protein	Half-life
			Enzyme	Effect of the drug on the enzyme	Effect of the enzyme on the drug		
Sofosbuvir	529.45 Da	Increased absorption, slower rate	NO	None	None	85%	0.5 h/26 h ¹
Simeprevir	749.93 Da	Increased absorption, slower rate	CYP3A4	Inhibitor	Alter AUC	> 99.9%	10-41 h
Daclatasvir	738.98 Da	Decreased absorption ²	CYP3A4	Weak inducer	Alter AUC	> 99%	12-15 h
Ledipasvir	888.9 Da	No effect	CYP3A4	Weak inducer	None	> 99.8%	47 h
Viekirax	Ombitasvir 894.1	Increased absorption	CYP3A4	Inhibitor	Alter AUC	99.9%	21-25 h
	Paritaprevir 765.8 Da					98.6%	5.5 h
	Ritonavir 720.9 Da					99%	4 h
Dasabuvir	493.5 Da	Increased absorption	CYP3A4	Inducer	None	99.5% (94.5% ³)	6 h
			CYP2C8	None	Alter AUC		
			CYP3A4	None	Alter AUC ³		
			CYP3A4	Inducer	None		

¹GS-331007 metabolite; ²Following a high-fat meal; ³Dasabuvir M1 metabolite. AUC: Area under the curve; NO: Not metabolized by P450 enzymes; CYP: Cytochrome.

The year 2011 saw the advent of DAAs that target essential components of the HCV life cycle. The first-generation DAAs were the protease inhibitors boceprevir and telaprevir, which were indicated mainly for the treatment of chronic hepatitis C patients infected by genotype 1 virus. Boceprevir is an inhibitor of HCV NS3/4A protease, an enzyme required for the proteolytic cleavage of HCV-encoded polyprotein into mature forms of the non-structural proteins NS4A, NS4B, NS5A and NS5B. Telaprevir is an NS3-4A protease inhibitor that competes with NS5A/5B for its substrate-binding site. The FDA classified both these first-generation DAAs in Pregnancy Category B^[31,32]. In fact, neither boceprevir nor telaprevir negatively affected fetal development in animals (mice, rats and rabbits). Consequently, in the absence of well-controlled human studies, "no evidence of risk in humans" has been supposed. Nevertheless, the major limitation to the use of these drugs is that they must be administered in association with PEG-IFN and Ribavirin as part of a triple-therapy regimen. Consequently, both boceprevir and telaprevir are contraindicated during pregnancy and adequate contraceptive measures are strongly recommended for both childbearing women and their male sexual partners throughout treatment duration and up to 6 mo after withdrawal^[31,32]. Lastly, the excretion of protease inhibitors into human breast milk remains to be clarified; the levels of these drugs in the milk of lactating rats can be higher than those observed in maternal blood^[31,32].

Second-generation DAAs

The second-generation DAAs, which became available in 2015, and their principal pharmacokinetic properties are listed in Table 1. Pharmacokinetic data are not complete for all second-generation DAAs. In the absence of data on their properties and effects on pregnant and lactating human females, clinicians can only try to predict the effect that pregnancy-associated physiological changes may have on the peak plasma dose, drug metabolism, and the ability of the drug to cross the placental barrier and/or enter into the mother's milk. Generally, drugs

that are more likely to cross the placenta are lipids or weak acids with a molecular weight below 500 Da, are poorly bound to plasma proteins and have a long half-life. The concentration of the drug in breast milk, and therefore its potential effect on the newborn, depends on dosage, rate of absorption in the maternal circulation, maternal drug metabolism and the time from drug administration to breastfeeding^[33,34]. In the following section we will briefly review the data on the pharmacokinetics and teratogenicity in animals of the DAAs currently available to try to identify the ones that could potentially be used in childbearing women.

Sofosbuvir (Sovaldi®) is a pangenotypic nucleotide prodrug converted by hepatocytes into its active form that acts by competitively inhibiting the HCV NS5B polymerase active site and thus blocking viral RNA synthesis. It is indicated for the treatment of chronic hepatitis C as a component of a combination antiviral regimen. Neither the area under the curve (AUC) nor the product's absorption changes when the drug is taken with food, which suggests that the prolonged gastric emptying observed in pregnancy would not affect absorption of the drug or the time-to-peak plasma dose. Sofosbuvir is readily available after oral administration, and undergoes extensive first pass metabolism. Gender does not appear to significantly affect its pharmacokinetics^[35]. Since P450 enzymes do not seem to be involved in metabolizing Sofosbuvir, increased activity of these enzymes in pregnancy is unlikely to affect its plasma concentration. On the other hand, Sofosbuvir has strong affinity for the P-glycoprotein efflux protein (Table 1). The drug is eliminated as GS-331007 in urine. The glomerular filtration rate usually increases during pregnancy and consequently renal drug elimination is generally greater than elimination in the non-pregnant state; however, it is unclear whether this process could alter the plasma concentration of Sofosbuvir to the point of requiring dose adjustment to attain a clinical response. Similarly, it is unclear whether the drug could cross the placental barrier. In studies conducted on animals (rats and rabbits), Sofosbuvir

Table 2 Effect of new direct-acting antivirals in pregnancy and Food and Drug Administration Pregnancy Categories

Drug	Embryotoxicity and/or teratogenicity ¹	Dose-escalation ²	Transfer across placenta	Transfer into milk	FDA Pregnancy Category ³
Sofosbuvir	No	28-fold	Yes	Yes	B
Simeprevir	Yes	4-fold	Yes	Yes	C
Daclatasvir	Yes	4-fold	Yes	Yes	NA ⁴
Ledipasvir	No	Maternal toxic doses	Yes	Yes	B
Viekirax	Yes	4-fold	Minimal	Yes	B
	Ombitasvir	32-fold			
	Paritaprevir	8-fold			
	Ritonavir	48-fold			
Dasabuvir	No	48-fold	Minimal	Yes	B

¹Based on animal studies; ²Dose escalation above therapeutic dose; ³FDA Pregnancy Category without association with ribavirin. NA: Not available; FDA: The Food and Drug Administration.

metabolites crossed the placenta and entered the milk of lactating animals. However, this process did not appear to significantly affect the viability or the development of embryos or fetuses^[34,35]. Little is known regarding the use of Sofosbuvir in pregnant women. The outcomes of less than 300 pregnancies are mentioned in the product characteristics reports of the European Medical Agency, but no data about those outcomes are available on the Pubmed database. The FDA classified Sofosbuvir in Pregnancy Category B when used alone or with Ledipasvir, and in Pregnancy Category X when used in combination with Ribavirin. The latter combination is strongly contraindicated during pregnancy and adequate contraceptive measures are highly recommended for both childbearing women and their male sexual partners throughout treatment duration and up to 6 mo after treatment withdrawal (Table 2)^[36].

Simeprevir (Olysio®) is a specific NS3/4A HCV serine protease inhibitor that interrupts the processing of the HCV-encoded polypeptide thereby blocking the HCV viral life cycle. Simeprevir is considered a second-generation HCV protease inhibitor because its binding affinity and specificity for NS3/4A is higher than that of first-generation protease inhibitors that have a linear structure. It has been approved as part of combination regimens with PEG-IFN and Ribavirin or with Sofosbuvir for the treatment of chronic hepatitis C genotype 1 infection in adults. When Simeprevir is taken with food, its absorption is delayed so that its bioavailability reaches 62% (Table 1). It is therefore possible that the prolongation of gastric emptying observed in pregnancy may also affect absorption and the time-to-peak plasma dose of Simeprevir. After its absorption, Simeprevir undergoes first-pass metabolism by the P450 cytochrome enzymes, mainly the CYP3A4 system (Table 1). It is also a substrate of the P-glycoprotein drug transporters. Plasma levels of Simeprevir change significantly when administered with inducers or inhibitors of CYP3A4. Plasma exposure of simeprevir is greatly affected also by the state of the liver, and there may be an increase of up to 5-fold in the AUC depending on the degree of hepatic impairment. Therefore, the increased activity of the P450 enzymes in pregnancy, and the possible physiopathological changes that may

affect the liver of pregnant women may affect the plasma concentration of Simeprevir. Metabolites of Simeprevir are mainly eliminated *via* biliary excretion. Gender did not appear to have a clinically relevant role on the pharmacokinetics of Simeprevir.

As yet, there are no data concerning the passage of Simeprevir across the human placenta (Table 2), however, animal studies established that the drug is transferred across the placenta, and that it exerts teratogenic effects on the foetal skeletal system, namely supernumerary ribs and delayed ossification at exposures 4-fold higher than those observed at the recommended dose (Table 2). Moreover, Simeprevir can be excreted in the milk of lactating animals. The drug is classified in FDA Pregnancy Category C when administered alone, and Pregnancy Category X when used in combination with Ribavirin^[37-39].

Daclatasvir (Daklinza®) inhibits the NS5A protein (Table 1), and appears to act on viral replication, and on the assembly and secretion stages of the viral life cycle, thereby causing a rapid decline in both intra- and extracellular levels of HCV RNA. It is the first NS5A complex inhibitor approved for use in the European Union as part of combined regimens with Sofosbuvir, Ribavirin and PEG-IFN for the treatment of chronic HCV infection in adults. Oral clearance (CL/F) of Daclatasvir is significantly lower in women than in men^[40]. However, this gender difference does not appear to be clinically relevant. It remains unclear whether the documented non-significant gender difference in oral clearance, and the expected changes in drug bioavailability and clearance in the pregnant state may, together, significantly affect Daclatasvir exposure in pregnant women.

Daclatasvir is a substrate of P-glycoprotein and is metabolized by the CYP3A4 enzyme (Table 1). Dose adjustments are recommended when it is administered with strong inducers of this class of cytochrome enzymes. It is therefore likely that the increased activity of the P450 enzymes in pregnancy would affect the plasma concentration of Daclatasvir.

Daclatasvir is primarily excreted unchanged through the biliary route. Overall, based on its chemical characteristics, it is unlikely that Daclatasvir could cross the materno-fetal circulation at therapeutic doses. However,

Daclatasvir was found to cross the placenta in a study conducted in rats and rabbits^[40] (Table 2). In the latter study, there was a decrease in the gestational weight of mothers exposed to the drug. Daclatasvir exerted an embryotoxic and teratogenic effect at exposures 4-fold to 16-fold higher than the clinical AUC exposure, and the potential toxic exposure was exponentially greater with the increase in the animals' body surface area^[40]. In other studies, Daclatasvir was excreted in the milk of lactating animals at concentrations 1.7- to 2-fold higher than maternal plasma concentrations^[40,41-43]. Daclatasvir has recently received FDA approval for marketing in the United States. At the time of writing this article, it has not been included in a Pregnancy Category.

Ledipasvir is available in a combined formulation with Sofosbuvir called Harvoni. Harvoni is administered alone or in combination with Ribavirin in patients with chronic hepatitis C infection^[2]. Ledipasvir acts on the replication, assembly and secretion phases of HCV by inhibiting HCV NS5A phosphoprotein^[44]. Based on the limited data available, Ledipasvir acts only on genotypes 1, 3 and 4. It is gradually absorbed after oral administration; the AUC does not appear to be affected when the drug is administered with meals. Moreover, Ledipasvir does not appear to undergo significant first pass and/or pre-excretory metabolism and it is mainly excreted unchanged through the biliary route, in faeces. Like Sofosbuvir, Ledipasvir is not metabolized by the P450 enzymes. It is therefore unlikely that increased activity of these enzymes in pregnancy affects its plasma concentration. Slow oxidative metabolism of Ledipasvir into M19 has been demonstrated *in vivo*, although the mechanism underlying this process is unknown. However, it is not possible to make any assumption regarding changes in this particular metabolic route in pregnant women. Both the AUC and C-max of Ledipasvir appear to be greater in females than in males, but this difference has not been considered clinically significant by the regulating authorities^[44] (Table 1).

Studies conducted with animals showed that Ledipasvir crosses the placenta and is excreted in the milk of lactating animals. In non pregnant animals, the number of corpora lutea and implantation sites were decreased with a 6-fold increase in exposure, while in pregnant animals the effects on offspring, *i.e.*, mainly alterations in body weight, were observed at a concentration 4-fold higher than the recommended clinical dosage^[44]. The FDA categorized Ledipasvir in the Pregnancy Category B when used with Sofosbuvir without Ribavirin^[42-44] (Table 2).

Viekirax[®] is a combination formulation composed of three pharmacologically active substances, namely Ombitasvir, Paritaprevir and Ritonavir. The combination acts on different steps of the HCV lifecycle: Ombitasvir inhibits HCV NS5A and Paritaprevir inhibits HCV NS3/4A, while Ritonavir, which does not directly affect HCV, acts as a booster of Paritaprevir through its inhibitory effect on CYP3A. Viekirax is indicated only in combination with Ribavirin and/or Dasabuvir (see below) for the treatment

of chronic hepatitis C in adults. The combination reaches T-max 4-5 h after oral administration and requires up to 12 d of dosing to reach steady state^[45,46]. Exposures of the individual components are affected by drug-to-drug interactions, even with the other components of Viekirax and with Dasabuvir. Food also significantly affects Viekirax absorption. In fact, absorption of the drug is much lower when administered in the fasting state. All three components are highly-bound to plasma proteins and undergo extensive hepatic metabolism. Notably, Paritaprevir is predominantly metabolized by CYP3A4, and therefore requires boosting with Ritonavir, which is also metabolized by the same enzyme. The components of the combination have different half-lives: Ombitasvir has the longest half-life, around 21-25 h and is mainly excreted by the biliary route. Paritaprevir and Ritonavir have a mean half-life of 5.5 and 4 h, respectively and are excreted mainly in faeces with only a small proportion being eliminated renally (8.8% for Paritaprevir and 11.3% for Ritonavir). Since exposures of the three individual components of Viekirax do not seem to vary significantly irrespectively of the degree of renal impairment, therefore renal elimination does not appear to be significant. Exposure of all the three active components of Viekirax is related to gender. In fact, concentrations of Ombitasvir and Paritaprevir were found to be 0.5- and 1-fold higher, respectively in women^[47]. Moreover, exposure of Ombitasvir was found to be related to body weights. Body weight also affects Ombitasvir exposure but not Paritaprevir exposure (Table 1).

Both Ombitasvir and Paritaprevir/Ritonavir caused malformations in the eyes and teeth of animals at exposures 4-fold higher than the AUC. In the case of Paritaprevir/Ritonavir, an exposure 32/8-fold higher than those observed at the recommended dose resulted in malformation in the offspring of animals, again involving the eyes. Passage of Ombitasvir and Paritaprevir metabolites in the milk of lactating animals, and to a lesser extent through the placenta, has been demonstrated, but no effect was observed in lactating pups. The FDA categorized Viekirax in Pregnancy Category B^[45-47] (Table 2).

Dasabuvir (Exviera[®]) is a non-nucleoside inhibitor of the HCV RNA-dependent RNA polymerase. It is indicated for the treatment of chronic hepatitis C infection in adults only in combination with Viekirax, thereby forming the "Viekira pak". Dasabuvir reaches T-max 4-5 h after oral administration. Viekira pak reaches steady state after 12 d of dosing. Like Viekirax, Dasabuvir must be administered with food. In fact, taken with food, its exposure is 30% higher than in the fasting state. It is metabolised by the P450 enzymes, namely CYP2C8 and to a lesser extent by CYP3A. Its metabolites are mainly eliminated through the biliary route. Exposure is 30% higher in women than in men. Also Dasabuvir exposure is affected by body weight and by impairment of renal and hepatic functions, albeit not in a clinically significant way (Table 1).

At doses of Dasabuvir 48-fold higher than the maximum recommended dose, Dasabuvir did not cause any embryocidal and/or teratogenic effects in animals^[47]. The drug was excreted in the milk of lactating animals probably by the breast cancer resistance protein efflux transporter of which Dasabuvir is a substrate. However, the drug did not affect nursing pups. The FDA categorized Dasabuvir in Pregnancy Category B^[45,46,48] (Table 2).

CONCLUSION

Given the lack of human studies, no DAA has yet been approved for use in pregnancy or during breast feeding. Consequently, we have reviewed the features of the DAAs approved for treatment of chronic HCV infection in adults in the attempt to identify the most promising candidates, in terms of pharmacokinetic profile and adverse effects, for use in pregnancy or during breast feeding. Sofosbuvir appears to have a favourable pharmacokinetic profile and animal studies indicate that it may be safe during pregnancy. Thus, Sofosbuvir, used in Ribavirin-free regimens, may become the drug of choice for women of childbearing age affected by HCV infection. On the contrary, Simeprevir is not suitable for use in pregnant or breast-feeding women, because its AUC and half-life are greatly affected by liver performance and by drug-drug interactions. Moreover, Simeprevir was associated with teratogenic effects in animals at doses only 4-fold higher than recommended doses. Ledipasvir has a highly favourable pharmacokinetic profile, and too moreover was safe in animal embryos and fetuses. Consequently, its combined formulation with Sofosbuvir (Harvoni), appears to be a good choice in women of child-bearing potential. Daclatasvir, based on its pharmacokinetic profile, appears to have a wide safety margin when used at therapeutic levels. It also appears that dosages may have to be increased in pregnant women. However, in contrast to its expected safety, it was found to cross the placenta and exert a teratogenic effect in animals. It is still awaiting FDA pregnancy categorization.

Although the Ombitasvir/Paritaprevir/Ritonavir combination is in FDA Pregnancy Category B, the pharmacokinetic profile of the individual components, namely absorption and affinity for P450 enzymes, suggest a potential for variability in AUC exposures with the physiological changes of pregnancy to the point that dose adjustment may be required. Furthermore, the components of the combination exerted a teratogenic effect on animals. Lastly, given its indication for use in combination with Ribavirin, it would not be suitable for women of childbearing potential. Dasabuvir is supposed to be relatively safe in pregnancy based on its pharmacokinetic profile and animal studies.

Until very recently infection with HCV genotype 2 would have posed a further treatment challenge in infected pregnant women, because all the recommended regimens for its treatment included Ribavirin. Even

though Italian authorities have very recently approved the use of Daclatasvir for the treatment of adult patients chronically infected by HCV genotype 2 in Ribavirin-free regimen associated with Sofosbuvir^[49], a safety data on pregnant women are lacking.

In conclusion, second-generation anti-HCV DAAs have revolutionized the standard of care and prognosis of patients suffering from chronic hepatitis C infection, however, childbearing women cannot benefit from this advance. As concluded by other authors^[4], despite promising safety profiles, there are no approved therapies to prevent vertical HCV transmission. Therefore the only achievable goal seems to be universal screening of fertile women to identify and treat those with HCV infection before they become pregnant.

Lastly, it would be useful to create a registry similar to the Ribavirin Pregnancy Registry in order to monitor the effect of the second-generation DAAs on women who become pregnant during therapy, in terms of outcome on both the mother and the product of conception.

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Retrospective Study

Predictors of fifty days in-hospital mortality in decompensated cirrhosis patients with spontaneous bacterial peritonitis

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Abstract

AIM: To determine the predictors of 50 d in-hospital mortality in decompensated cirrhosis patients with spontaneous bacterial peritonitis (SBP).

METHODS: Two hundred and eighteen patients admitted to an intensive care unit in a tertiary care hospital between June 2013 and June 2014 with the diagnosis of SBP (during hospitalization) and cirrhosis were retrospectively analysed. SBP was diagnosed by abdominal paracentesis in the presence of polymorphonuclear cell count ≥ 250 cells/mm³ in the peritoneal fluid. Student's *t* test, multivariate logistic regression, cox proportional hazard ratio (HR), receiver operating characteristics (ROC) curves and Kaplan-Meier survival analysis were utilized for statistical analysis. Predictive abilities of several variables identified by multivariate analysis were compared using the area under ROC curve. $P < 0.05$ were considered statistical significant.

RESULTS: The 50 d in-hospital mortality rate attributable to SBP is 43.11% ($n = 94$). Median survival duration for those who died was 9 d. In univariate analysis acute kidney injury (AKI), hepatic encephalopathy, septic shock, serum bilirubin, international normalized ratio, aspartate transaminase, and model for end-stage liver disease - sodium (MELD-Na) were significantly associated with in - hospital mortality in patients with SBP ($P \leq 0.001$). Multivariate cox

proportional regression analysis showed AKI (HR = 2.16, 95%CI: 1.36-3.42, $P = 0.001$) septic shock (HR = 1.73, 95%CI: 1.05-2.83, $P = 0.029$) MELD-Na (HR = 1.06, 95%CI: 1.02-1.09, $P \leq 0.001$) was significantly associated with 50 d in-hospital mortality. The prognostic accuracy for AKI, MELD-Na and septic shock was 77%, 74% and 71% respectively associated with 50 d in-hospital mortality in SBP patients.

CONCLUSION: AKI, MELD-Na and septic shock were predictors of 50 d in-hospital mortality in decompensated cirrhosis patients with SBP.

Key words: Decompensated cirrhosis; Acute kidney injury; Model for end-stage liver disease sodium; Septic shock; Spontaneous bacterial peritonitis

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Core tip: Spontaneous bacterial peritonitis (SBP) is associated with poor prognosis especially with in-hospital patients. The mortality rate ranges from 20%-40%. The model for end-stage liver disease (MELD) has been suggested as a predictor of the in-hospital mortality in patients with SBP. However, the role of other predictors has not been established. The goal of this study is to identify other prognostic factors for mortality in decompensated cirrhotic patients with SBP and to evaluate the predictive power of acute kidney injury, MELD-sodium and septic shock.

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INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is defined as acute infection of ascitic fluid without any identifiable surgically treatable intra-abdominal source^[1]. SBP is a major complication of decompensated cirrhosis with ascites^[2]. The SBP is diagnosed by abdominal paracentesis with an elevated ascitic fluid neutrophil count (≥ 250 cells/mm³) and/or a positive ascitic fluid culture. In up to 60% cases, gram-negative bacteria (*Escherichia coli* or *Klebsiella pneumoniae*) are most prevalent organism involved^[3]. In about 25% of the cases, gram-positive bacteria (mainly *Streptococcus species* and *Enterococci*) are involved^[3]. The prevalence of SBP is up to 30% in hospitalized cirrhotic patients with ascites^[4]. Despite intensive management, the in-hospital mortality remains between 20%-40%^[5]. Model for end-stage liver disease (MELD) scores have been investigated with their predictive accuracy; however it is vulnerable to variations in laboratory measurements, making their utili-

zation in prediction of SBP related in-hospital mortality affected^[6,7]. In addition, decompensated cirrhotics with major complication like SBP may have low MELD scores with high mortality^[7]. Acute kidney injury (AKI) is common in patients with decompensated cirrhosis with ascites. AKI in cirrhosis was diagnosed by AKI network (AKIN) based on serum creatinine/urine output. AKI can be used to predict mortality in decompensated cirrhotic with ascites^[8]. SBP-associated septic shock carries significant mortality in cirrhosis^[9]. Thus this study aimed to have a comprehensive approach to determine possible prognostic factors predicting SBP related in-hospital mortality, compare the predictive power of AKI, MELD-sodium (MELD-Na), and septic shock and to identify the best cut-off point of MELD-Na scores to predict 50 d in-hospital mortality.

MATERIALS AND METHODS

Patients

Medical records of 218 adult patients admitted to hepatology intensive care unit (ICU) of the Institute of Liver and Biliary Sciences, New Delhi between June 2013 and June 2014 with the diagnosis of SBP (during hospitalization) and cirrhosis were reviewed. The study was approved by the Institutional Ethics Committee and the guidelines of Helsinki declaration were followed^[10]. The Ethics Committee waived the requirement for the consent for data analysis.

The diagnosis of cirrhosis was based on clinical, laboratory and imaging findings. SBP was diagnosed by abdominal paracentesis in the presence of neutrophil count ≥ 250 cells/mm³ in the ascitic fluid and the absence of the secondary features suggestive of secondary bacterial peritonitis^[11]. We also required a positive culture.

Patient charts were retrospectively reviewed. Data include patient demographics, etiology, severity of liver disease, laboratory values, co-existing medical diagnoses (diabetes mellitus, hepatocellular carcinoma), medication use, organ failure, ascitic fluid analysis results, duration of ICU stay, and patient outcome. In the case of culture-positive infections, all microorganisms and their antibiotic susceptibility patterns were recorded. Most patients admitted to the ICU were referred after a variable antibiotic exposure; prior systemic or non-absorbable antibiotic data could not be collected.

The laboratory parameters [bilirubin, creatinine levels and international normalized ratio (INR)] at admission to intensive care unit were used to calculate MELD-Na score using the UNOS Internet site^[12]. As a protocol, all patients admitted/transferred to the ICU with ascites, underwent an ascitic fluid analysis within 24 h of admission, in the absence of severe coagulopathy. Ascitic fluid was sent for albumin and cell count with differential and cultured by inoculation of 10 mL of ascitic fluid in blood culture bottles. Paired blood culture samples were also collected at admission in all patients. Antibiotic choice varied from patient to patient, and no standard first-line

drugs were used. The choice of antibiotic was decided based on previous antibiotic exposure of the patient before development of SBP, whether the patient was on quinolone prophylaxis, and physician discretion based on the perceived severity of patient illness. The antibiotic use was narrowed and modified as per the gram-stain and antibiotic sensitivity results. No patient underwent fluid restriction or hypertonic saline for management of dilution hyponatremia.

Renal dysfunction was defined by AKIN criteria^[8] and managed by albumin infusions and intravenous terlipressin, with dose titrated as per response and tolerance. Intravenous albumin was used in all patients, with a minimal daily dose of 20 g and increased to up to 60 g/d^[13], titrated by clinical monitoring and hourly urine output. We did not stratify renal dysfunction into hepatorenal syndrome (HRS), and non-HRS. However, any cause of secondary renal dysfunction was actively investigated, with urine sediment, 24-h urine protein excretion, and bedside-renal ultrasound. All patients were evaluated daily by a nephrologist. Hepatic encephalopathy was treated with oral and rectal lactulose and rifaximin. No patient received neomycin. We suspected secondary peritonitis in patients with inadequate response to therapy, severe abdominal tenderness or when multiple organisms were identified in the ascitic fluid. These patients underwent a non-contrast computed tomography (CT) scan of abdomen.

American College of Chest Physicians/Society of Critical Care Medicine consensus conference criteria were used to diagnose septic shock^[14].

Exclusion criteria

Patients with cirrhosis and ascites fluid polymorphonuclear cell (PMN) < 250 cells/mm³. Patients admitted from the community with SBP. Patients presented with ascites unrelated to cirrhosis. Patients with variceal haemorrhage advanced malignancy and human immunodeficiency virus.

Statistical analysis

Stata version 14 for Windows was used for analysis. All the variables were normally distributed with equal variance. The continuous variables were described as mean \pm SD. The means of continuous variables were compared using student's *t* test. Categorical variables were described as proportions. The means of categorical variables were compared with logistics regression. Multivariate logistics regression was employed to analyse statistically significant variables. Cox proportional hazard model was used to analyse the hazard rates of the predictors adjusted by age and gender. The predictive accuracy of the prognostic variables like MELD-Na, AKI and septic shock was measured using receiver operating characteristics (ROC) curves. The best cut-off point for MELD-Na was created using acceptable sensitivity and specificity in the ROC analysis to determine 50 d in-hospital mortality risk. For each predictor variable, sensitivity, specificity, positive predictive value (PPV),

negative predictive values (NPV), positive likelihood ratio (+LR) and negative LR (-LR) were calculated to fit into the prognostic model. Two tailed *P* value < 0.05 was considered statistically significant. The power of the study was set at 80%. STROBE checklist for retrospective analysis was performed.

RESULTS

Total of 218 patients with decompensated cirrhosis with ascites and SBP were included in the study. Two hundred and eleven (97%) patients were diagnosed with SBP for the first time and only 7 patients (0.03%) had previous episodes (more than once). The 50 d in-hospital mortality rate was 43.11% (*n* = 94). Median survival duration for those who died was 9 d. In univariate analysis AKI, hepatic encephalopathy, septic shock, total leucocyte count, serum bilirubin, INR, aspartate transaminase (SGOT), and MELD-Na were significantly associated with in-hospital mortality in patients with SBP (Table 1).

The baseline characteristics of the demographics, etiology, clinical and laboratory data is shown in Table 1. Mean age was 49.90 \pm 12.52 years and the male was predominant (83%). Most common etiology of liver cirrhosis was ethanol-induced (45.87%) followed by crypto/non-alcoholic fatty liver disease-NAFLD (28.9%). Hepatitis C virus related cirrhosis constitute only 11% in this study. A total of 109 subjects (50.0%) had hepatic encephalopathy with 59 deaths (62.77%), *P* = 0.001. Overall, 99 patients (45.11%) had AKI in hospitalized patients out of which 64 died (68.09%), *P* < 0.001. Compared with survivors the deceased had a higher proportion of septic shock (25.53% vs 3.23%), *P* < 0.001. Total leukocyte counts, bilirubin, INR, SGOT were significantly higher in the patients who died compared to the survivors. Mean MELD-Na score was higher among the deaths comparing to the survivors (30.59 \pm 6.62 vs 25.21 \pm 7.44) with statistical significance (*P* < 0.001). Child-Turcotte-Pugh (CTP) (B/C) score was not different among the groups. The mean CTP scores were high with mean 10.72 (SD: 1.82).

On multivariate regression analysis, AKI (*P* = 0.001), septic shock (*P* = 0.029), MELD-Na (*P* < 0.001) were found to be independent predictors of 50 d in-hospital mortality in patients with SBP (Table 2). Cox proportional hazard model showed the hazard ratio (HR) of AKI was 2.16 (95%CI: 1.36-3.42), septic shock (HR = 1.73, 95%CI: 1.05-2.83) and MELD-Na (HR = 1.1, 95%CI: 1.02-1.21). ROC curve for AKI, septic shock and MELD-Na had better prognostic accuracy for 50 d in-hospital mortality in patients with SBP (Figure 1). AKI had highest area under the curve (AUC) 0.77 (95%CI: 0.71-0.83), followed by MELD-Na (AUC: 0.74, 95%CI: 0.69-0.79), septic shock (AUC: 0.71, 95%CI: 0.65-0.77). Table 3 reports the sensitivity, specificity, PPV, NPV, +LR and -LR for these predictors. The cut off for MELD-Na derived from the ROC with the best ability to predict 50 d in-hospital mortality in decompensated cirrhotic patient with SBP was 28, with sensitivity 92.9%, specificity 60.3%, and NPV of 97.9%. The Kaplan-Meier

Table 1 Baseline characteristics of the hospitalized patients with spontaneous bacterial peritonitis in decompensated cirrhosis

Variables	Overall (n = 218)	Survivors (n = 124)	Deaths (n = 94)	P value
Demographic data				
Age (yr) mean ± SD	49.90 ± 12.52	49.86 ± 13.37	49.96 ± 11.37	0.950
Male (%)	177 (81.19)	99 (79.84)	78 (82.98)	0.557
Etiology of cirrhosis (%)				
Ethanol	100 (45.87)	48 (38.71)	52 (55.32)	0.689
Crypto/NAFLD	63 (28.90)	38 (30.65)	25 (26.60)	0.104
HCV	23 (10.55)	16 (12.905)	7 (7.45)	0.068
Clinical data (%)				
Hepatocellular carcinoma	17 (7.80)	9 (7.26)	8 (8.51)	0.733
Diabetes	47 (21.56)	27 (21.77)	20 (21.28)	0.929
Acute kidney injury	99 (45.41)	35 (28.23)	64 (68.09)	< 0.001
Respiratory failure	10 (4.59)	6 (4.84)	4 (4.26)	0.978
Hepatic encephalopathy	109 (50.0)	50 (40.32)	59 (62.77)	0.001
Septic shock	28 (12.84)	4 (3.23)	24 (25.53)	< 0.001
Positive culture	48 (22.02)	21 (16.94)	27 (28.72)	0.038
Laboratory data ¹ (mean ± SD)				
Ascitic neutrophil count (cells/mm ³)	3346.07 ± 4700.60	3899.28 ± 5003.75	2616.30 ± 4182.81	0.040
Hemoglobin (g/dL)	9.42 ± 1.88	9.58 ± 1.77	9.21 ± 2.01	0.154
Platelet count (mmol/L)	128.24 ± 102.11	138.43 ± 111.25	115.03 ± 87.69	0.095
Leucocyte count (10 ³ /μL)	13.30 ± 9.35	11.86 ± 8.65	15.17 ± 9.92	0.009
Sodium (mEq/L)	132.14 ± 7.69	132.50 ± 6.54	131.67 ± 9.01	0.454
Bilirubin (mg/dL)	8.17 ± 8.81	5.85 ± 6.27	11.24 ± 10.61	< 0.001
Albumin (g/dL)	2.32 ± 0.50	2.35 ± 0.48	2.28 ± 0.52	0.250
INR	2.31 ± 1.11	2.09 ± 1.08	2.59 ± 1.08	0.001
AST (U/L)	59.66 ± 109.81	79.23 ± 98.71	171.3 ± 321.94	0.003
ALT (U/L)	59.66 ± 109.81	46.49 ± 72.93	77.04 ± 143.41	0.041
Urea (mg/dL)	70.31 ± 52.42	62.24 ± 48.23	80.94 ± 55.98	0.008
Creatinine (mg/dL)	1.67 ± 1.29	1.58 ± 1.39	1.80 ± 1.15	0.217
Scores (mean ± SD)				
CTP (B/C)	10.72 ± 1.82	10.50 ± 1.95	11.02 ± 1.60	0.034
MELD	24.79 ± 8.28	22.20 ± 7.59	28.20 ± 7.94	< 0.001
MELD-Na	27.53 ± 7.57	25.21 ± 7.44	30.59 ± 6.62	< 0.001

¹Results obtained on the day of diagnosis of spontaneous bacterial peritonitis. MELD-Na: Model for end-stage liver disease-sodium; MELD: Model for end-stage liver disease; HCV: Hepatitis C virus; NAFLD: Non-alcoholic fatty liver disease; INR: International normalized ratio; AST: Aspartate aminotransferase; ALT: Alanineaminotransferase; CTP: Child-Turcotte-Pugh.

Table 2 Cox proportional regression analysis of risk factors for spontaneous bacterial peritonitis related in-hospital related mortality

Variables	¹ HR (95%CI)	P value
AKI	2.16 (1.36-3.42)	0.001
Septic shock	1.73 (1.05-2.83)	0.029
MELD-Na	1.06 (1.02-1.09)	< 0.001

¹Hazard ratio (HR) adjusted for age and gender. AKI: Acute kidney injury; MELD-Na: Model for end-stage liver disease-sodium.

Table 3 Diagnostic accuracy of prognostic variables to predict spontaneous bacterial peritonitis related in-hospital mortality

Predictors	Sensitivity	Specificity	PPV	NPV	+LR	-LR
AKI	64.6	74.8	68.1	71.8	2.56	0.47
Septic shock	85.7	63.2	25.5	96.8	2.33	0.23
MELD-Na(28) ¹	92.9	60.3	24.5	97.9	2.34	0.12

¹Cut off score for MELD-Na. PPV: Positive predictive value; NPV: Negative predictive value; +LR: Positive likelihood ratio; -LR: Negative likelihood ratio; AKI: Acute kidney injury; MELD-Na: Model for end stage liver disease-sodium.

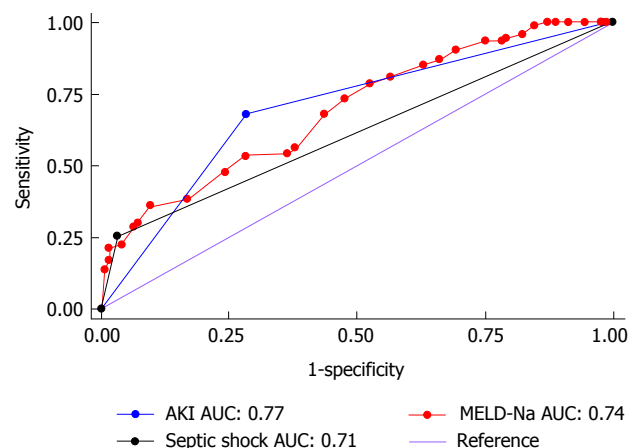


Figure 1 Receiver operator characteristic curve for acute kidney injury, septic shock and model for end-stage liver disease - sodium had better prognostic accuracy for 50 d in-hospital mortality in patients with spontaneous bacterial peritonitis. AKI: Acute kidney injury; AUC: Area under the curve; MELD-Na: Model for end stage liver disease-sodium.

survival analysis was plotted for the 50-d survival in SBP patients along with individual prognostic variables like AKI, MELD-Na, and septic shock (Figure 2).

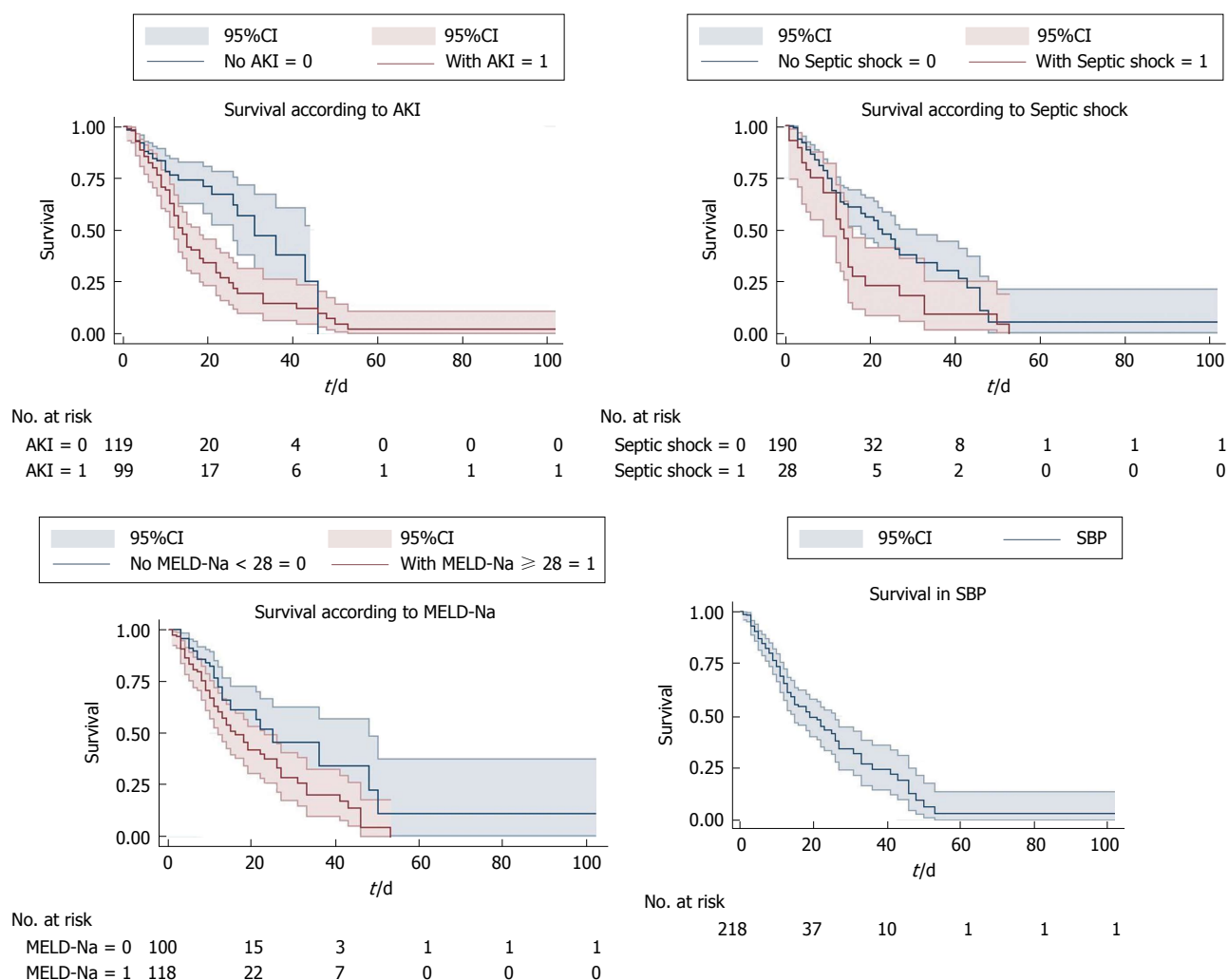


Figure 2 Kaplan-Meier survival analysis was plotted for the 50-d survival in spontaneous bacterial peritonitis patients along with individual prognostic variables like acute kidney injury, model for end-stage liver disease - sodium, and septic shock. AKI: Acute kidney injury; MELD-Na: Model for end stage liver disease-sodium; SBP: Spontaneous bacterial peritonitis.

DISCUSSION

The prevalence of SBP in outpatients has been reported to be 1.5%-3.5%^[15]. Among in-patients the prevalence is around 10%^[15]. Half of the episodes of SBP are acquired during hospitalization. In the present observational study SBP related 50 d in-hospital mortality in decompensated cirrhosis was 43%. Of total 94 cases, 93 patients with SBP (99%) died on or before 50th d of hospitalisation.

This study assessed different prognostic factors which can be used to predict mortality in hospitalized patient with SBP and corroborates that hepatic encephalopathy, total leukocyte count, serum bilirubin, SGOT, INR and child pugh score significantly associated with mortality^[5,7,16]. The MELD score shows promise as a means for risk - stratifying patients with SBP including those waiting for liver transplantation^[7]. Certain limitations of MELD model^[17] prompted us to include MELD-Na as hyponatremia is a well-known predictor of death in cirrhotic patients. Isolated creatinine is inaccurate measurement of renal failure in decompensated

liver cirrhosis due to significant reduction in creatinine production in liver and muscle wasting^[18]. We found AKI, MELD-Na and septic shock to be the important predictors of mortality. We did not incorporate other independent variables like total leukocyte count, serum bilirubin, INR since these were the components in the present predictive model like MELD-Na and septic shock.

In this study AKI has the single best predictive ability (AUC: 0.77) followed by MELD-Na (AUC: 0.74) and septic shock (AUC: 0.71). In addition, we identified MELD-Na cut-off 28 with sensitivity 92.9% and NPV of 97.9%. The hazard ratio of mortality for patients with AKI was significantly higher 2.16 (95%CI: 1.36-3.42) compared to septic shock (HR = 1.73, 95%CI: 1.05-2.83) and MELD-Na (HR = 1.06, 95%CI: 1.02-1.09). Kaplan-Meier survival analysis showed AKI, MELD-Na, and septic shock as predictors for the 50 d in-hospital mortality in decompensated patients with SBP. It can help in the further improvement of the quality of care of hospitalized SBP patients with reduction of their short-term mortality. The cut-off for MELD-Na can be applied to prioritize high-risk patients

upon hospital admission who would benefit by expectant management.

Diagnosis of SBP is based on the demonstration of an absolute number of PMNs in ascitic fluid equal to or greater than 250/mm³. However, the best specificity for diagnosis has been reported with a cut-off of 500 PMN/mm³. It is unclear whether a positive culture in the absence of elevated ascitic fluid PMN count (bacteriascites), requires antibiotic therapy. In these cases, some guidelines recommend antibiotic treatment only if the patient shows signs of infection^[18]. Ascitic fluid culture is positive in 40% of all cases. The most common isolates include GNB, usually *Escherichia coli* and Gram-positive cocci (mainly *Streptococcus* species and *Enterococci*)^[3]. Gram negative organism infections predominate in community acquired and gram-positive organisms in nosocomial infections^[3]. Recommended first-line antibiotics for treatment of SBP include third generation cephalosporins (mainly Cefotaxime), Amoxicillin-Clavulanic acid, ciprofloxacin, and ofloxacin^[19], with an expected resolution rate of over 90%. These guidelines from the western medicine acknowledge the increasing problem of antibiotic resistance^[20] and recommend coverage for resistant organisms if there is no evidence of infection resolution at repeat ascitic fluid analysis at 48 h. Resistant infections are usually caused by *Enterococcus faecium* and extended-spectrum β -lactamase-producing *Enterobacteriaceae*, which are resistant to the current recommended empirical antibiotic therapy^[21]. These findings led to the suggestion that nosocomial SBP should be treated with carbapenems or with tigecycline^[22]. We included only patients with hospital-acquired SBP because most of the present ICU admissions include transferred patients already hospitalised, with a variable but consistent antibiotic exposure. Only a minority of our patients are admitted directly from the community, and usually to the wards and not to the ICU. These patients would be expected to have a higher prevalence of resistant infections. A hospital-acquired infection was an independent predictor of death, likely due to a higher rate of multidrug resistance (resistance to third-generation cephalosporin)^[23].

This study has certain strengths and limitations. The results clearly show AKI has greater predictive ability than septic shock and MELD-Na as far as 50 d in-hospital mortality in SBP patient is concerned. This study did not account for the stages of ascites. We didn't stratify our patients according to different stages of AKI as per AKIN criteria. We didn't consider HRS into account in this study. We didn't evaluate the antibiotic resistance in SBP patients who are culture positive at the baseline. We included only nosocomial acquired SBP. Most of our patients presented with advanced decompensated liver cirrhosis at the time of SBP diagnosis. The advanced liver cirrhosis was assessed by lower serum albumin, high serum bilirubin and INR values. This study is a single centre study, these findings needed to be supplemented by multicentre prospective studies.

COMMENTS

Background

Spontaneous bacterial peritonitis (SBP) is associated with poor prognosis especially in-hospital patients. The mortality rate ranges from 20%-40%. The model for end-stage liver disease (MELD) has been suggested as a predictor of the in-hospital mortality in patients with SBP. The authors' goal is to identify other prognostic factors for mortality in decompensated cirrhotic patients with SBP and to evaluate the predictive power of acute kidney injury (AKI), MELD-sodium (MELD-Na) and septic shock to predict mortality.

Research frontiers

The prognostic factors for mortality with SBP patients in liver cirrhosis are important in determining the management. MELD has been considered as an important predictive factor. But it's not clear about role of other prognostic factors.

Innovations and breakthroughs

In this study, 50 d in-hospital mortality rate attributable to SBP is 43.11%. receiver operating characteristic (ROC) curve, Kaplan Meier survival analysis was useful tool in predicting 50 d in-hospital mortality in SBP with liver cirrhosis. Multivariate cox proportional regression analysis showed AKI [hazrd ratio (HR) = 2.16, 95%CI: 1.36-3.42, $P = 0.001$] septic shock (HR = 1.73, 95%CI: 1.05-2.83, $P = 0.029$) MELD-Na (HR = 1.06, 95%CI: 1.02-1.09, $P \leq 0.001$) were significantly associated with 50 d in-hospital mortality. The prognostic accuracy for AKI, MELD-Na and septic shock was 77%, 74% and 71% respectively.

Applications

Liver transplant is potentially only curative therapeutic option with long term result in patients with decompensated cirrhosis and SBP. The cost of liver transplant and the shortages of the liver donor is a point of concern. The findings of this study can be used as a strategic approach in advanced liver cirrhosis patients on hospital admission that would benefit from intensive management where liver transplant is not a plausible option. It can help in the further improvement of the quality of care of hospitalized SBP patients with reduction of their short-term mortality. The cut-off for MELD-Na can be used to stratify high-risk patients on hospital admission who would benefit by intensive management.

Terminology

The ROC analysis is a graphical plot in statistical methods to create a cut off value for the predictors. The graph is plotted with true positive value against false positive value. The accuracy of cut off value is interpenetrated by the area under curve (AUC) in ROC curve. AUC = 1 is gold standard, 0.9-1 = excellent, 0.8-0.9 = good, 0.7-0.8 = fair, 0.6-0.7 = poor, ≤ 0.5 = fail. Kaplan-Meier survival curve is a time to event analysis of series of events over a period of time recorded in horizontal and declining horizontal steps. When a person withdraws from the study (censored), lost follow up, or died there will be a sudden drop in the curve. HR: It's a ratio of hazard rates. It is the probability of an event in the study group or population at a particular time. HRs are used in time to event analysis.

Peer-review

This is a retrospective study to analyze predictors of 50 d in-hospital mortality in decompensated cirrhosis patients with spontaneous bacterial peritonitis. The authors review the medical records of 218 adults admitted with SBP in period of one year, to identify factors related to mortality. The article is very well described; it was properly planned and conducted.

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