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REVIEW

Neurologic complications after liver transplantation

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Abstract

Neurologic complications are relatively common after solid organ transplantation and affect 15%-30% of liver transplant recipients. Etiology is often related to immunosuppressant neurotoxicity and opportunistic infections. Most common complications include seizures and encephalopathy, and occurrence of central pontine myelinolysis is relatively specific for liver transplant recipients. Delayed allograft function may precipitate hepatic encephalopathy and neurotoxicity of calcineurin inhibitors typically manifests with tremor, headaches and encephalopathy. Reduction of neurotoxic immunosuppressants or conversion to an alternative medication usually result in clinical improvement. Standard preventive and diagnostic protocols have helped to reduce the prevalence of opportunistic central nervous system (CNS) infections, but viral and fungal CNS infections still affect 1% of liver transplant recipients, and the morbidity and mortality in the affected patients remain fairly high. Critical illness myopathy may also affect up to 7% of liver transplant recipients. Liver insufficiency is also associated with various neurologic disorders which may improve or resolve after successful liver transplantation. Accurate diagnosis and timely intervention are essential to improve outcomes, while advances in clinical management and extended post-transplant survival are increasingly shifting the focus to chronic post-transplant complications which are often encountered in a community hospital and an outpatient setting.

Key words: Liver transplantation; Neurologic complications; Meningoencephalitis; Seizures; Stroke; Critical illness myopathy

Core tip: Neurologic complications after liver transplantation are still a major source of morbidity and mortality and careful approach to possible immunosuppressant neurotoxicity and opportunistic infections is needed. Most common neurologic complications include encephalopathy, seizures and cerebrovascular complications, but opportunistic central nervous system infections and central pontine myelinolysis may be associated with significant morbidity as well. Accurate diagnosis and timely intervention are essential to improve outcomes, while advances in clinical management of neurologic post-transplant complications and extended post-transplant survival are increasingly shifting the focus to chronic post-transplant complications which are often encountered in a community hospital and an outpatient setting.

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INTRODUCTION

There have been more than 6000 liver transplantations performed in United States in 2011, including living donor and cadaveric allografts. Since the first successful liver transplantation by Dr Starzl in 1963, there has been tremendous and dynamic progress of surgical and postoperative protocols leading to 1-year and 5-year post-

transplant survival rates at 88% and 74%, respectively[1]. Successful liver transplantation is also associated with improved quality of life for allograft recipients[2]. However, a variety of post-transplant complications, which may not be directly related to allograft function, complicate recovery of transplant recipients. Neurologic complications are still fairly common after solid organ transplantation and affect 30%-60% of allograft recipients[3], including 15%-30% of liver allograft recipients (Table 1)[4-8]. More recent studies demonstrate decreasing frequency of neurologic complications reflecting improved transplant outcomes and advanced management regimens. Improved survival of transplant recipients has also somewhat shifted the spectrum of neurologic complications from acute post-transplant inpatient emergencies towards chronic outpatient complications, although severe post-transplant complications may occur at any given time. Decreased frequency of neurologic complications was also reported for patients receiving living donor allografts when compared to cadaveric allografts (20% *vs* 27%), but there is a donor-related morbidity and living-liver donors may rarely suffer neurologic complications as well[7]. Major neurologic complications include alterations of consciousness, seizures, cerebrovascular complications and central nervous system (CNS) infections, similarly as with other solid organ transplants, and also central pontine myelinolysis (CPM) which is characteristic for liver transplantation. Pre-transplant liver failure may be associated with various neurologic complications directly related to liver dysfunction (*e.g.*, hepatic encephalopathy), or related to systemic disorders associated with major neurologic manifestations (*e.g.*, Wilson's disease) (Table 2). Stabilization of liver function following successful transplantation may subsequently lead to improvement of neurologic symptoms as well. During the post-transplant clinical course, the surgical procedure of transplantation, chronic immunosuppression and various toxic-metabolic disorders may precipitate a wide spectrum of neurologic complications[4-7,9]. Premorbid and new onset psychiatric and behavioral disorders may also negatively affect compliance with immunosuppression regimens and complicate post-transplant management[10]. Neurologic post-transplant complications can develop at any given time after transplantation and often multiple risk factors are present. The outcome of transplantation is usually not affected by neurologic complications but additional morbidity may delay post-transplant recovery[11]. Additionally, opportunistic infections and immunosuppressant neurotoxicity may complicate management of immunosuppressive medications and effective prevention of allograft rejection.

Overall, post-transplant neurologic morbidity in liver allograft recipients is often related to opportunistic infections and immunosuppressant neurotoxicity, but most patients will have multiple risk factors for development of neurologic complications (Table 3).

NEUROTOXICITY OF IMMUNOSUPPRESSIVE MEDICATIONS

Long-term immunosuppression increases the risk of opportunistic infections, but immunosuppressive medications can also exhibit direct neurotoxicity[12-15]. Maintenance immunosuppressive regimens typically include a combination of inhibitors of calcineurin or mTOR, antimetabolites and corticosteroids, while induction and rejection treatments may also include polyclonal and monoclonal antibodies[12,16].

Calcineurin inhibitors (CNI), cyclosporine and tacrolimus, are one of mainstays of immunosuppression after organ transplantation. However their use may be associated with various neurologic complications. In the early posttransplant period, there is a greater risk of CNI neurotoxicity due to higher doses, intravenous use and impaired blood-brain barrier. Clinical manifestations of CNI toxicity are usually accompanied by elevated levels of tacrolimus or cyclosporine, but symptoms may present even with normal serum levels. Hypomagnesemia and hypocholesterolemia may also increase the risk of CNI toxicity[14]. Neurotoxicity of CNI typically manifests with tremor, headache, and encephalopathy[12-15]. Presence of altered consciousness and cortical blindness may indicate posterior reversible encephalopathy syndrome (PRES) with characteristic imaging findings on MRI of the brain with an increased T2-signal posteriorly in white matter[17-19]. PRES is associated with reversible vasogenic subcortical edema and may accompany various medical conditions and drug neurotoxicity[17]. In transplant recipients, PRES is most commonly reported with cyclosporine or tacrolimus neurotoxicity, but there are also rare reports of PRES with sirolimus[20]. There is no specific therapy for CNI neurotoxicity other than symptomatic treatment (*e.g.*, antiepileptics for seizure management), and with dose reduction or switching to alternative immunosuppressives[3,14,21].

Recently introduced mTor inhibitors, sirolimus and everolimus, are usually better tolerated than CNI, but rare case of sirolimus-induced PRES was reported as well[20,22].

Toxicity of other immunosuppressive medications may also precipitate various neurologic symptoms. Corticosteroids may increase the risk of critical illness myopathy, or precipitate steroid myopathy, mood disorders or psychosis. Other common side-effects of corticosteroids include weight gain, osteoporosis and hyperglycemia. Rarely, corticosteroid use may lead to epidural lipomatosis which may manifest with radiculopathies or compressive myelopathy[23]. Mycophenolate is usually well tolerated, but some patients may complain of headaches.

OPPORTUNISTIC INFECTIONS

Chronic immunosuppression increases the risk of opportunistic infection as a result of imbalance between immune status and exposure to infectious agents[24,25].

Table 1 Neurologic complications of liver transplantation

Ref.	*n*	Total	Seizure	Stroke	Brain hemorrhage	Encephalopathy	CNS infection
Bronster *et al*[4]	463	20%	8.20%	0.60%	1.50%	11.8	1.2
Lewis *et al*[6]	657	27%	6	4%[1]	-[1]	11.0	1.1
Saner *et al*[7]	174	25%	2.8	0.60%	1.20%	17.8	0.0
Kim *et al*[5]	319	15%	1.60%	0.30%	0.60%	2.0	0.3
Vizzini *et al*[8]	395	16%	1%	1.30%	2.5	5.3	0.0

[1]Combined ischemic strokes and brain hemorrhage. CNS: Central nervous system.

Table 2 Liver failure and associated neurologic complications

Underlying condition	Neurologic complication
Hepatic failure	Encephalopathy, acquired hepatocerebral degeneration, parkinsonism, neuropathy, myelopathy, asterixis
Wilson's disease	Psychiatric complications, dystonia, parkinsonism
Primary biliary cirrhosis	Neuropathy, dysautonomia
Familial amyloidosis (transthyretin)	Neuropathy, dysautonomia

Table 3 Risk factors for neurologic complications after liver transplantation

Underlying condition	Neurologic complication
Chronic hyponatremia	Central pontine and extrapontine myelinolysis
High levels of immunosuppression	Immunosuppressant neurotoxicity, opportunistic infections
Endemic and nosocomial exposures	Opportunistic infections
Sepsis	CIM/CIP, septic encephalopathy
Multiple organ failure	CIM/CIP
Hepatic dysfunction	Encephalopathy
History of alcohol abuse	Encephalopathy

Modified from Linden *et al*[11]. CIM/CIP: Critical illness myopathy/polyneuropathy.

Early signs of infection in immunosuppressed patients may be masked by medications or other medical problems, including allograft rejection. Exposure to infectious agents may stem from donor-related infections, recipient-related infections, nosocomial infections and community infections. Rate of opportunistic infections has declined due to improved surveillance measures and prophylaxis. Most common causes of opportunistic CNS infections in immunosuppressed transplant recipients are fungi and viruses, while bacterial and protozoic infections are less common[24,25]. During initial post-transplant period (first 30 d), acquired infections are often related to pre-transplant colonization or surgical procedures, and there are also rare donor-to-recipient transmissions *via* allograft[26,27]. The risk of acquired opportunistic infections increases at 1 mo following the transplantation[25]. At 6 mo after transplantation, the risk of infections gradually diminishes as immunosuppressants are often tapered down. However, the risk does persist long-term and this may further rise with rejection episodes requiring more aggressive immunotherapy. Endemic exposure and travel may result in unusual causative agents including Naegleria, Leishmania, Coccidioides or Histoplasma[28]. Prevalence of CNS infections in transplant patients has been previously estimated at 5%, but most recent series demonstrate much less frequent infections with better immunosuppressive and preventive strategies[3,8]. However, potential signs of CNS infections are always to be taken seriously in transplant recipients, and due to immunosuppression and complex metabolic disturbances, the initial onset of symptoms may be obscured in some patients.

Clinically, opportunistic CNS infections may present with signs of meningitis or meningoencephalitis, or with more focal findings suggestive of an abscess (fungi, bacteria). Viral infections may also present with focal signs due to preferential involvement of some brain regions [*e.g.*, limbic encephalitis with Human herpesvirus (HHV)-6 infection][29]. New onset of severe back pain may suggest spinal epidural abscess, requiring prompt intervention[30].

Viral infections are often caused by Cytomegalovirus (CMV), herpes simplex virus, varicella zoster virus (VZV), Epstein-Barr virus (EBV) or HHV-6. CMV infections are fairly common in transplant recipients, but CNS involvement is rare. Dermatomal zoster may precede CNS infection caused by VZV. Infrequently, EBV infection may precipitate posttransplant lymphoproliferative disorder with CNS involvement[31]. Treatment of post-transplant lymphoproliferative disease may include irradiation, chemotherapy or biological (rituximab), and immunosuppression is often reduced as well. Reactivation of HHV-6 infection after liver transplantation is typically asymptomatic, but rarely it may lead to limbic encephalitis and post-encephalitic epilepsy[32]. Progressive multifocal leukoencephalopathy (PML) is an uncommon fatal brain disorder caused by JC virus, and its imaging features may resemble PRES[19]. However, PML does not improve with reduction of CNI dosing and typically follows a progressive course. It does not usually respond to antiviral treatment or reduction of immunosuppression, and is typically associated with gradual progression over several months[33].

Most common fungal CNS infections are caused by Cryptococcus neoformans and Aspergillus species[24,34], while opportunistic bacterial CNS infections are less common after liver transplantation. Fungal CNS infections are usually associated with systemic fungal infections, and may also extend locally following fungal sinusitis[34]. Increased risk of CNS aspergillosis has been reported after liver retransplantation[35]. Treatment of

fungal CNS infections in immunocompromised patients is often limited by delayed diagnosis and drug toxicity (nephrotoxicity with Amphotericin), so morbidity and mortality still remain high. Clinical presentations include meningitis, brain hemorrhage or abscesses. Vasoinvasive CNS fungal infections (*e.g.*, Aspergillus species) are often associated with hemorrhagic strokes[36]. Fungal brain abscesses may also manifest with seizures or focal neurologic signs. Candida is the most common fungal infection after liver transplantation, but CNS infections caused by Candida species are rare.

Bacterial CNS infections are relatively rare after liver transplantation, but the risk may be increased with environmental exposure. Exposure to contaminated dairy may lead to CNS listeriosis which may present with rhombencephalitis and usually responds to treatment, especially with early diagnosis[37]. Nocardia species are ubiquitous saprophytes and are unlikely to infect non-immunocompromised individuals. Clinical manifestations range from pulmonary infection to abscesses and CNS infection[38]. Timely diagnosis usually leads to an effective treatment, although surgical drainage may be needed for cerebral abscesses.

Toxoplasmosis is the most common protozoal infection in transplant recipients and other immunocompromised individuals. Toxoplasmosis has been reported in up to 0.18% of solid organ transplant recipients and may be associated with exposure to cats. Seronegative transplant recipients are at 15-fold greater risk of toxoplasmosis and should be treated prophylactically with trimethoprim-sulfametoxazole or pyrimethamine[39]. Freshwater swimming may lead to amebic encephalitis which carries almost 100% mortality[40].

Despite improved prevention and therapeutic advances, opportunistic CNS infections still carry severe morbidity and high mortality. Therefore, prompt and accurate diagnosis and early institution of therapy of CNS infections are essential for improvement of outcomes.

HEPATIC ENCEPHALOPATHY

Chronic pretransplant hepatic encephalopathy increases the risk of posttransplant neurologic complications[41], but the improving graft function may gradually lead to significant cognitive improvement. Delayed allograft function can precipitate hepatic encephalopathy and affect pharmacokinetics of different hepatically-metabolized medications. Clinical manifestations of hepatic encephalopathy range from subtle cognitive slowing and memory difficulties, to somnolence, stupor and coma[42]. Patients often exhibit asterixis and parkinsonism. Pathophysiology of brain dysfunction associated with hepatic encephalopathy is not completely understood, but the role has been proposed for ammonia and manganese[43].

High ammonia level with improving graft function may also be related to acquired urea cycle enzyme abnormalities, and these may be difficult to treat[44].

POST-TRANSPLANT ENCEPHALOPATHY

Hepatic encephalopathy is a frequent complication of advanced liver diseases and it usually improves after successful liver transplantation. Failure to awaken after liver transplantation may be one of the first signs of abnormal graft function[45], and is always taken seriously. Primary graft failure may manifest with unresponsiveness, hepatorenal syndrome and severe coagulopathy, and carries high mortality.

Overall, common causes of post-transplant encephalopathy in liver allograft recipients include hepatic dysfunction, medication toxicity, infectious causes (CNS infections or septic encephalopathy), complex metabolic disturbances (uremia, CPM), cerebrovascular events or seizures[45]. Higher risk of encephalopathy was reported in patients with history of severe hepatic encephalopathy, ethanol-related hepatic failure, metabolic liver disease, greater severity of pre-transplant liver injury (defined by Child-Pugh or MELD scores) and non-elective liver transplantation[41,46,47]. Quite often, multiple co-existing risk factors will be identified in individual patients and careful clinical evaluation will be needed to determine the most appropriate course of action. In early post-transplant course, a delayed arousal can be related to persisting hepatic dysfunction, CNI neurotoxicity or intracranial hemorrhage. Higher doses and intravenous delivery increase the risk of CNI neurotoxicity. Neuroimaging studies may show evidence of PRES or intracerebral hemorrhage, while EEG might reveal triphasic waves suggestive of hepatic encephalopathy or nonconvulsive status epilepticus[19,48]. Chronic immunosuppression later increases the risk of opportunistic infections with direct CNS involvement, and lumbar puncture should be considered in patients with possible CNS infection Opportunistic CNS infection often present in the setting of systemic infection, but systemic infections may also precipitate septic encephalopathy in the absence of direct CNS involvement.

CPM

Relatively high prevalence of CPM or extrapontine myelinolysis (EPM) in the early period after liver transplantation is probably attributable to large fluid shifts, similarly as in rapid correction of hyponatremia (Table 2). Due to massive fluid shifts in early posttransplant period, the risk of CPM/EPM is also higher in first 48 h after transplantation. It has been estimated that up to 1%-2% of liver transplant recipients may develop CPM/EPM[5,6,49]. True prevalence of CPM remains uncertain as symptoms may be overshadowed by other complications and imaging changes may resolve over time[50]. Greater risk of CPM/EPM has been reported in patients with preoperative hyponatremia and worse liver dysfunction[49]. Clinical manifestations of CPM include stupor and spastic tetraparesis[51]. Neuroimaging studies typically show area of T2 hyperintensity on MRI imaging in central pons[19]. Supportive treatment of CPM is the standard of care,

and at this time there is no sufficient evidence to support other types of treatment for CPM, including plasma exchange or IVIG.

SEIZURES

Seizures after liver transplantation are often precipitated by CNI neurotoxicity, followed by CNS infections and cerebrovascular complications[52,53]. Complex metabolic and toxic disturbances may also precipitate nonconvulsive status epilepticus which may go unnoticed if EEG is not done. In patients with failure to awaken, EEG will also provide critical distinction between toxic metabolic encephalopathy and NCSE, although interpretation may present a unique challenge with multiple medical and technical factors to be considered[48]. Focal brain lesions after cerebrovascular complications, CNS infections, or even PRES, may precipitate symptomatic epilepsy requiring long-term maintenance therapy with antiepileptic medications. While acute treatment of status epilepticus after transplantation is usually the same as in non-transplant patients, long-term maintenance treatment of seizures has to take into account complex metabolic disturbances, altered pharmacokinetics and drug-drug interactions[54]. The presence of liver and kidney insufficiency will affect levels of antiepileptics, and we usually try to avoid potentially hepatotoxic medications after liver transplantation. Additionally, for long-term maintenance treatment of seizures after liver transplantation it is preferable to avoid medications which can affect CNI pharmacokinetics. Preferential choices include levetiracetam, gabapentin and lacosamide, but phenytoin is still often used due to availability and price[55].

CEREBROVASCULAR COMPLICATIONS

Cerebrovascular complications, including ischemic strokes and intracranial hemorrhage, are rare after liver transplantation and most studies report prevalence of 2%-4% in transplant recipients[4-7]. Higher risk of cerebrovascular complications has been reported in older recipients and with pretransplant diabetes, similarly as in general population[56].

Ischemic strokes are overall less common than intracranial hemorrhages, and are often associated with similar risk factors as in general population, including hypertension and hyperlipidemia. Sudden clinical deterioration may be related to intracranial bleeding, and vasoinvasive fungal CNS infections may manifest with hemorrhagic strokes. Increased risk of brain hemorrhage has been demonstrated in patients with thrombocytopenia and overwhelming infections[57]. That risk is further compounded by coagulopathy associated with hepatic failure. Hepatic encephalopathy is also associated with dysregulation of cerebral blood flow autoregulation[58].

Rarely, thrombotic microangiopathy resembling thrombotic thrombocytopenic purpura may develop leading to kidney failure and even brain ischemia. This may improve with a switch or reduction of CNI dosage and plasma exchange[59,60].

NEUROMUSCULAR COMPLICATIONS

Neuromuscular complications after liver transplantation are relatively uncommon, but post-transplant recovery may be complicated by critical illness myopathy in 7% of liver transplant recipients[61]. Perioperative neuropathies are also relatively rare and few patients may develop post-transplant demyelinating inflammatory polyneuropathy[62,63]. Uncommonly, an injury of the phrenic nerve during liver transplantation may result in hemidiaphragm paralysis, and trauma associated with venovenous bypass may lead to brachial plexus injury[64].

Alcohol-induced toxic neuropathy is relatively common in patients with alcoholic liver cirrhosis. Neuropathy related to alcohol toxicity may improve or even resolve after successful liver transplantation[65]. Neuropathy related to familial amyloidosis may improve after liver transplantation, although some symptoms usually persist (Table 2)[66].

Herpes zoster has been reported in 5.7% of liver transplant recipients with median onset of 9 mo after transplantation, and frequent occurrence of postherpetic neuralgia[67].

NEUROLOGIC COMPLICATIONS IN LIVE LIVER DONORS

Live-donor liver transplantation is a life-saving procedure, especially in the absence of appropriate cadaveric liver allografts. However, this is not an entirely benign procedure and it is associated with postoperative complications in about 16% of live donors and a donor mortality of 0.2%[68]. Rarely, live-donor liver transplantation may be also associated with donor neurologic complications, including brachial plexopathy[69].

NEUROLOGIC DISORDERS ASSOCIATED WITH LIVER FAILURE

Various liver diseases are often associated with neurologic complications including Wilson's disease, hepatitis C (with or without cryoglobulinemia), primary biliary cirrhosis, and alcoholic cirrhosis. Liver failure resulting from different causes may also manifest with various neurologic symptoms including hepatic encephalopathy, parkinsonism associated with hepatocerebral degeneration, asterixis, tremor and hepatic neuropathy[42,70-72]. Additionally, multisystemic disorders associated with liver failure (*e.g.*, familial amyloidosis) may also precipitate various neurologic complications which may improve or resolve after successful liver transplantation (Table 2)[65,72-74].

REFERENCES

1 2012 Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of

Transplant Recipients: 2002-2011. Rockville (MD): Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation; Richmond (VA): United Network for Organ Sharing; Ann Arbor (MI): University Renal Research and Education Association; 2012: 91-94
2 **Moore KA**, McL Jones R, Burrows GD. Quality of life and cognitive function of liver transplant patients: a prospective study. *Liver Transpl* 2000; **6**: 633-642 [PMID: 10980064 DOI: 10.1053/jlts.2000.9743]
3 **Zivković SA**, Abdel-Hamid H. Neurologic manifestations of transplant complications. *Neurol Clin* 2010; **28**: 235-251 [PMID: 19932384 DOI: 10.1016/j.ncl.2009.09.011]
4 **Bronster DJ**, Emre S, Boccagni P, Sheiner PA, Schwartz ME, Miller CM. Central nervous system complications in liver transplant recipients--incidence, timing, and long-term follow-up. *Clin Transplant* 2000; **14**: 1-7 [PMID: 10693627 DOI: 10.1034/j.1399-0012.2000.140101.x]
5 **Kim BS**, Lee SG, Hwang S, Park KM, Kim KH, Ahn CS, Moon DB, Ha TY, Song GW, Kim DS, Moon KM, Jung DH. Neurologic complications in adult living donor liver transplant recipients. *Clin Transplant* 2007; **21**: 544-547 [PMID: 17645717 DOI: 10.1111/j.1399-0012.2007.00687.x]
6 **Lewis MB**, Howdle PD. Neurologic complications of liver transplantation in adults. *Neurology* 2003; **61**: 1174-1178 [PMID: 14610116 DOI: 10.1212/01.WNL.0000089487.42870.C6]
7 **Saner F**, Gu Y, Minouchehr S, Ilker K, Fruhauf NR, Paul A, Radtke A, Dammann M, Katsarava Z, Koeppen S, Malagó M, Broelsch CE. Neurological complications after cadaveric and living donor liver transplantation. *J Neurol* 2006; **253**: 612-617 [PMID: 16511638 DOI: 10.1007/s00415-006-0069-3]
8 **Vizzini G**, Asaro M, Miraglia R, Gruttadauria S, Filì D, D'Antoni A, Petridis I, Marrone G, Pagano D, Gridelli B. Changing picture of central nervous system complications in liver transplant recipients. *Liver Transpl* 2011; **17**: 1279-1285 [PMID: 21770016 DOI: 10.1002/lt.22383]
9 **Pujol A**, Graus F, Rimola A, Beltrán J, Garcia-Valdecasas JC, Navasa M, Grande L, Galofré J, Visa J, Rodés J. Predictive factors of in-hospital CNS complications following liver transplantation. *Neurology* 1994; **44**: 1226-1230 [PMID: 8035920 DOI: 10.1212/WNL.44.7.1226]
10 **Huffman JC**, Popkin MK, Stern TA. Psychiatric considerations in the patient receiving organ transplantation: a clinical case conference. *Gen Hosp Psychiatry* 2003; **25**: 484-491 [PMID: 14706415 DOI: 10.1016/S0163-8343(03)00090-2]
11 **Linden P**, Zivkovic SA. Liver transplantation. In: Biller J, editor. The Interface of Neurology and Internal Medicine. Philadelphia: Lippincott Williams and Wilkins; 2008: 290-294
12 **Wijdicks EF**, Wiesner RH, Krom RA. Neurotoxicity in liver transplant recipients with cyclosporine immunosuppression. *Neurology* 1995; **45**: 1962-1964 [PMID: 7501141 DOI: 10.1212/WNL.45.11.1962]
13 **Eidelman BH**, Abu-Elmagd K, Wilson J, Fung JJ, Alessiani M, Jain A, Takaya S, Todo SN, Tzakis A, Van Thiel D. Neurologic complications of FK 506. *Transplant Proc* 1991; **23**: 3175-3178 [PMID: 1721398]
14 **Wijdicks EF**. Neurotoxicity of immunosuppressive drugs. *Liver Transpl* 2001; **7**: 937-942 [PMID: 11699028 DOI: 10.1053/jlts.2001.27475]
15 **Wijdicks EF**, Wiesner RH, Dahlke LJ, Krom RA. FK506-induced neurotoxicity in liver transplantation. *Ann Neurol* 1994; **35**: 498-501 [PMID: 7512320 DOI: 10.1002/ana.410350422]
16 **Pillai AA**, Levitsky J. Overview of immunosuppression in liver transplantation. *World J Gastroenterol* 2009; **15**: 4225-4233 [PMID: 19750565 DOI: 10.3748/wjg.15.4225]
17 **Lee VH**, Wijdicks EF, Manno EM, Rabinstein AA. Clinical spectrum of reversible posterior leukoencephalopathy syndrome. *Arch Neurol* 2008; **65**: 205-210 [PMID: 18268188 DOI: 10.1001/archneurol.2007.46]
18 **Small SL**, Fukui MB, Bramblett GT, Eidelman BH. Immunosuppression-induced leukoencephalopathy from tacrolimus (FK506). *Ann Neurol* 1996; **40**: 575-580 [PMID: 8871576 DOI: 10.1002/ana.410400406]
19 **Zivković S**. Neuroimaging and neurologic complications after organ transplantation. *J Neuroimaging* 2007; **17**: 110-123 [PMID: 17441832 DOI: 10.1111/j.1552-6569.2007.00097.x]
20 **Bodkin CL**, Eidelman BH. Sirolimus-induced posterior reversible encephalopathy. *Neurology* 2007; **68**: 2039-2040 [PMID: 17548556 DOI: 10.1212/01.wnl.0000264428.76387.87]
21 **Guarino M**, Benito-Leon J, Decruyenaere J, Schmutzhard E, Weissenborn K, Stracciari A. EFNS guidelines on management of neurological problems in liver transplantation. *Eur J Neurol* 2006; **13**: 2-9 [PMID: 16420387 DOI: 10.1111/j.1468-1331.2006.01353.x]
22 **van de Beek D**, Kremers WK, Kushwaha SS, McGregor CG, Wijdicks EF. No major neurologic complications with sirolimus use in heart transplant recipients. *Mayo Clin Proc* 2009; **84**: 330-332 [PMID: 19339650]
23 **Zampella EJ**, Duvall ER, Sekar BC, Langford KH, Epstein AE, Kirklin JK, Morawetz RB. Symptomatic spinal epidural lipomatosis as a complication of steroid immunosuppression in cardiac transplant patients. Report of two cases. *J Neurosurg* 1987; **67**: 760-764 [PMID: 3312515 DOI: 10.3171/jns.1987.67.5.0760]
24 **Fishman JA**. Infection in solid-organ transplant recipients. *N Engl J Med* 2007; **357**: 2601-2614 [PMID: 18094380 DOI: 10.1056/NEJMra064928]
25 **Linden PK**. Approach to the immunocompromised host with infection in the intensive care unit. *Infect Dis Clin North Am* 2009; **23**: 535-556 [PMID: 19665082 DOI: 10.1016/j.idc.2009.04.014]
26 **Fischer L**, Sterneck M, Claus M, Costard-Jäckle A, Fleischer B, Herbst H, Rogiers X, Broelsch CE. Transmission of malaria tertiana by multi-organ donation. *Clin Transplant* 1999; **13**: 491-495 [PMID: 10617239 DOI: 10.1034/j.1399-0012.1999.130609.x]
27 **Rodriguez-Hernandez MJ**, Ruiz-Perez-Pipaon M, Cañas E, Bernal C, Gavilan F. Strongyloides stercoralis hyperinfection transmitted by liver allograft in a transplant recipient. *Am J Transplant* 2009; **9**: 2637-2640 [PMID: 19843038 DOI: 10.1111/j.1600-6143.2009.02828.x]
28 **Kotton CN**. Travel and transplantation: travel-related diseases in transplant recipients. *Curr Opin Organ Transplant* 2012; **17**: 594-600 [PMID: 23147910]
29 **Baldwin K**. Ganciclovir-resistant human herpesvirus-6 encephalitis in a liver transplant patient: a case report. *J Neurovirol* 2011; **17**: 193-195 [PMID: 21287317 DOI: 10.1007/s13365-011-0019-4]
30 **Wszolek ZK**, McCashland TM, Witte RJ, Brandenberg GA, Steg RE. Spinal epidural abscess in a liver transplant recipient. *Transplant Proc* 1996; **28**: 2978-2979 [PMID: 8908144]
31 **Cavaliere R**, Petroni G, Lopes MB, Schiff D. Primary central nervous system post-transplantation lymphoproliferative disorder: an International Primary Central Nervous System Lymphoma Collaborative Group Report. *Cancer* 2010; **116**: 863-870 [PMID: 20052713 DOI: 10.1002/cncr.24834]
32 **Abdel Massih RC**, Razonable RR. Human herpesvirus 6 infections after liver transplantation. *World J Gastroenterol* 2009; **15**: 2561-2569 [PMID: 19496184 DOI: 10.3748/wjg.15.2561]
33 **Verhelst X**, Vanhooren G, Vanopdenbosch L, Casselman J, Laleman W, Pirenne J, Nevens F, Orlent H. Progressive multifocal leukoencephalopathy in liver transplant recipients: a case report and review of the literature. *Transpl Int* 2011; **24**: e30-e34 [PMID: 21134241 DOI: 10.1111/j.1432-2277.2010.01190.x]
34 **Wu G**, Vilchez RA, Eidelman B, Fung J, Kormos R, Kusne S. Cryptococcal meningitis: an analysis among 5,521 consecu-

tive organ transplant recipients. *Transpl Infect Dis* 2002; **4**: 183-188 [PMID: 12535260 DOI: 10.1034/j.1399-3062.2002.t01-1-02005.x]

35 **Singh N**, Pruett TL, Houston S, Muñoz P, Cacciarelli TV, Wagener MM, Husain S. Invasive aspergillosis in the recipients of liver retransplantation. *Liver Transpl* 2006; **12**: 1205-1209 [PMID: 16598780 DOI: 10.1002/lt.20756]

36 **Torre-Cisneros J**, Lopez OL, Kusne S, Martinez AJ, Starzl TE, Simmons RL, Martin M. CNS aspergillosis in organ transplantation: a clinicopathological study. *J Neurol Neurosurg Psychiatry* 1993; **56**: 188-193 [PMID: 8437008 DOI: 10.1136/jnnp.56.2.188]

37 **Mizuno S**, Zendejas IR, Reed AI, Kim RD, Howard RJ, Hemming AW, Schain DC, Soldevila-Pico C, Firpi RJ, Fujita S. Listeria monocytogenes following orthotopic liver transplantation: central nervous system involvement and review of the literature. *World J Gastroenterol* 2007; **13**: 4391-4393 [PMID: 17708617]

38 **Clark NM**. Nocardia in solid organ transplant recipients. *Am J Transplant* 2009; **9** Suppl 4: S70-S77 [PMID: 20070699 DOI: 10.1111/j.1600-6143.2009.02896.x]

39 **Fernàndez-Sabé N**, Cervera C, Fariñas MC, Bodro M, Muñoz P, Gurguí M, Torre-Cisneros J, Martín-Dávila P, Noblejas A, Len O, García-Reyne A, Del Pozo JL, Carratalà J. Risk factors, clinical features, and outcomes of toxoplasmosis in solid-organ transplant recipients: a matched case-control study. *Clin Infect Dis* 2012; **54**: 355-361 [PMID: 22075795 DOI: 10.1093/cid/cir806]

40 **Yoder JS**, Eddy BA, Visvesvara GS, Capewell L, Beach MJ. The epidemiology of primary amoebic meningoencephalitis in the USA, 1962-2008. *Epidemiol Infect* 2010; **138**: 968-975 [PMID: 19845995 DOI: 10.1017/S0950268809991014]

41 **Dhar R**, Young GB, Marotta P. Perioperative neurological complications after liver transplantation are best predicted by pre-transplant hepatic encephalopathy. *Neurocrit Care* 2008; **8**: 253-258 [PMID: 17928960 DOI: 10.1007/s12028-007-9020-4]

42 **Ferenci P**, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy--definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002; **35**: 716-721 [PMID: 11870389 DOI: 10.1053/jhep.2002.31250]

43 **Lockwood AH**. Blood ammonia levels and hepatic encephalopathy. *Metab Brain Dis* 2004; **19**: 345-349 [PMID: 15554426]

44 **Ghabril M**, Nguyen J, Kramer D, Genco T, Mai M, Rosser BG. Presentation of an acquired urea cycle disorder post liver transplantation. *Liver Transpl* 2007; **13**: 1714-1716 [PMID: 18044746 DOI: 10.1002/lt.21291]

45 **Wijdicks EF**. Impaired consciousness after liver transplantation. *Liver Transpl Surg* 1995; **1**: 329-334 [PMID: 9346591 DOI: 10.1002/lt.500010511]

46 **Buis CI**, Wiesner RH, Krom RA, Kremers WK, Wijdicks EF. Acute confusional state following liver transplantation for alcoholic liver disease. *Neurology* 2002; **59**: 601-605 [PMID: 12196657 DOI: 10.1212/WNL.59.4.601]

47 **Kanwal F**, Chen D, Ting L, Gornbein J, Saab S, Durazo F, Yersiz H, Farmer D, Ghobrial RM, Busuttil RW, Han SH. A model to predict the development of mental status changes of unclear cause after liver transplantation. *Liver Transpl* 2003; **9**: 1312-1319 [PMID: 14625832 DOI: 10.1016/j.lts.2003.09.023]

48 **Brenner RP**. The interpretation of the EEG in stupor and coma. *Neurologist* 2005; **11**: 271-284 [PMID: 16148734 DOI: 10.1097/01.nrl.0000178756.44055.f6]

49 **Lee EM**, Kang JK, Yun SC, Kim KH, Kim SJ, Hwang KS, Lee SG. Risk factors for central pontine and extrapontine myelinolysis following orthotopic liver transplantation. *Eur Neurol* 2009; **62**: 362-368 [PMID: 19797900 DOI: 10.1159/000242426]

50 **Buis CI**, Wijdicks EF. Serial magnetic resonance imaging of central pontine myelinolysis. *Liver Transpl* 2002; **8**: 643-645 [PMID: 12089721 DOI: 10.1053/jlts.2002.34023]

51 **Wijdicks EF**, Blue PR, Steers JL, Wiesner RH. Central pontine myelinolysis with stupor alone after orthotopic liver transplantation. *Liver Transpl Surg* 1996; **2**: 14-16 [PMID: 9346623 DOI: 10.1002/lt.500020104]

52 **Estol CJ**, Lopez O, Brenner RP, Martinez AJ. Seizures after liver transplantation: a clinicopathologic study. *Neurology* 1989; **39**: 1297-1301 [PMID: 2797452 DOI: 10.1212/WNL.39.10.1297]

53 **Wijdicks EF**, Plevak DJ, Wiesner RH, Steers JL. Causes and outcome of seizures in liver transplant recipients. *Neurology* 1996; **47**: 1523-1525 [PMID: 8960738 DOI: 10.1212/WNL.47.6.1523]

54 **Chabolla DR**, Harnois DM, Meschia JF. Levetiracetam monotherapy for liver transplant patients with seizures. *Transplant Proc* 2003; **35**: 1480-1481 [PMID: 12826199 DOI: 10.1016/S0041-1345(03)00361-0]

55 **Shepard PW**, St Louis EK. Seizure treatment in transplant patients. *Curr Treat Options Neurol* 2012; **14**: 332-347 [PMID: 22660960 DOI: 10.1007/s11940-012-0180-y]

56 **Gaynor JJ**, Moon JI, Kato T, Nishida S, Selvaggi G, Levi DM, Island ER, Pyrsopoulos N, Weppler D, Ganz S, Ruiz P, Tzakis AG. A cause-specific hazard rate analysis of prognostic factors among 877 adults who received primary orthotopic liver transplantation. *Transplantation* 2007; **84**: 155-165 [PMID: 17667806 DOI: 10.1097/01.tp.0000269090.90068.0f]

57 **Wijdicks EF**, de Groen PC, Wiesner RH, Krom RA. Intracerebral hemorrhage in liver transplant recipients. *Mayo Clin Proc* 1995; **70**: 443-446 [PMID: 7731253 DOI: 10.4065/70.5.443]

58 **Zheng Y**, Villamayor AJ, Merritt W, Pustavoitau A, Latif A, Bhambhani R, Frank S, Gurakar A, Singer A, Cameron A, Stevens RD, Hogue CW. Continuous cerebral blood flow autoregulation monitoring in patients undergoing liver transplantation. *Neurocrit Care* 2012; **17**: 77-84 [PMID: 22644887 DOI: 10.1007/s12028-012-9721-1]

59 **Nwaba A**, MacQuillan G, Adams LA, Garas G, Delriviere L, Augustson B, DeBoer B, Moody H, Jeffrey GP. Tacrolimus-induced thrombotic microangiopathy in orthotopic liver transplant patients: case series of four patients. *Intern Med J* 2013; **43**: 328-333 [PMID: 23441660 DOI: 10.1111/imj.12048]

60 **Trimarchi HM**, Truong LD, Brennan S, Gonzalez JM, Suki WN. FK506-associated thrombotic microangiopathy: report of two cases and review of the literature. *Transplantation* 1999; **67**: 539-544 [PMID: 10071024 DOI: 10.1097/00007890-199902270-00009]

61 **Campellone JV**, Lacomis D, Kramer DJ, Van Cott AC, Giuliani MJ. Acute myopathy after liver transplantation. *Neurology* 1998; **50**: 46-53 [PMID: 9443456 DOI: 10.1212/WNL.50.1.46]

62 **Wijdicks EF**, Litchy WJ, Wiesner RH, Krom RA. Neuromuscular complications associated with liver transplantation. *Muscle Nerve* 1996; **19**: 696-700 [PMID: 8609918]

63 **Wilson JR**, Conwit RA, Eidelman BH, Starzl T, Abu-Elmagd K. Sensorimotor neuropathy resembling CIDP in patients receiving FK506. *Muscle Nerve* 1994; **17**: 528-532 [PMID: 7512691 DOI: 10.1002/mus.880170510]

64 **Katirji MB**. Brachial plexus injury following liver transplantation. *Neurology* 1989; **39**: 736-738 [PMID: 2651971 DOI: 10.1212/WNL.39.5.736]

65 **Gane E**, Bergman R, Hutchinson D. Resolution of alcoholic neuropathy following liver transplantation. *Liver Transpl* 2004; **10**: 1545-1548 [PMID: 15558589 DOI: 10.1002/lt.20282]

66 **Shimojima Y**, Morita H, Kobayashi S, Takei Y, Ikeda S. Ten-year follow-up of peripheral nerve function in patients with familial amyloid polyneuropathy after liver transplantation. *J Neurol* 2008; **255**: 1220-1225 [PMID: 18484233 DOI: 10.1007/s00415-008-0884-9]

67 **Gourishankar S**, McDermid JC, Jhangri GS, Preiksaitis JK.

Herpes zoster infection following solid organ transplantation: incidence, risk factors and outcomes in the current immunosuppressive era. *Am J Transplant* 2004; **4**: 108-115 [PMID: 14678041 DOI: 10.1046/j.1600-6143.2003.00287.x]

68 **Middleton PF**, Duffield M, Lynch SV, Padbury RT, House T, Stanton P, Verran D, Maddern G. Living donor liver transplantation--adult donor outcomes: a systematic review. *Liver Transpl* 2006; **12**: 24-30 [PMID: 16498709 DOI: 10.1002/lt.20663]

69 **Shackleton CR**, Vierling JM, Nissen N, Martin P, Poordad F, Tran T, Colquhoun SD. Morbidity in live liver donors: standards-based adverse event reporting further refined. *Arch Surg* 2005; **140**: 888-895; discussion 895-896 [PMID: 16172298]

70 **Chaudhry V**, Corse AM, O'Brian R, Cornblath DR, Klein AS, Thuluvath PJ. Autonomic and peripheral (sensorimotor) neuropathy in chronic liver disease: a clinical and electrophysiologic study. *Hepatology* 1999; **29**: 1698-1703 [PMID: 10347110 DOI: 10.1002/hep.510290630]

71 **Ferrara J**, Jankovic J. Acquired hepatocerebral degeneration. *J Neurol* 2009; **256**: 320-332 [PMID: 19224314 DOI: 10.1007/s00415-009-0144-7]

72 **Stracciari A**, Baldin E, Cretella L, Delaj L, D'Alessandro R, Guarino M. Chronic acquired hepatocerebral degeneration: effects of liver transplantation on neurological manifestations. *Neurol Sci* 2011; **32**: 411-415 [PMID: 21328073 DOI: 10.1007/s10072-011-0481-z]

73 **Qu B**, Liu C, Guo L, Yang Y, Li JH, Yu L, Lv Y. The role of liver transplantation in the treatment of hepatic myelopathy: case report with review of the literature. *Transplant Proc* 2009; **41**: 1987-1989 [PMID: 19545775 DOI: 10.1016/j.transproceed.2009.01.105]

74 **Stracciari A**, Tempestini A, Borghi A, Guarino M. Effect of liver transplantation on neurological manifestations in Wilson disease. *Arch Neurol* 2000; **57**: 384-386 [PMID: 10714666 DOI: 10.1001/archneur.57.3.384]

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MINIREVIEWS

Imaging appearance of treated hepatocellular carcinoma

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Abstract

Surgical resection and imaging guided treatments play a crucial role in the management of hepatocellular carcinoma (HCC). Although the primary end point of treatment of HCC is survival, radiological response could be a surrogate end point of survival, and has a key role in HCC decision-making process. However, radiological assessment of HCC treatment efficacy is often controversial. There are few doubts on the evaluation of surgical resection; in fact, all known tumor sites should be removed. However, an unenhancing partial linear peripheral halo, in most cases, surrounding a fluid collection reducing in size during follow-up is demonstrated in successfully resected tumor with bipolar radiofrequency electrosurgical device. Efficacy assessment of locoregional therapies is more controversial and differs between percutaneous ablation (*e.g.*, radiofrequency ablation and percutaneous ethanol injection) and transarterial treatments (*e.g.*, conventional transarterial chemoembolization, transarterial chemoembolization with drug eluting beads and radioembolization). Finally, a different approach should be used for new systemic agent that, though not reducing tumor mass, could have a benefit on survival by delaying tumor progression and death. The purpose of this brief article is to review HCC imaging appearance after treatment.

Key words: Hepatocellular carcinoma; Imaging; Treatment

Core tip: Surgical resection and imaging guided treatments play a crucial role in the management of hepatocellular carcinoma (HCC). Moreover, recent studies have underlined the potential of antiangiogenetic treatment in patients with untreatable, unresectable HCCs. The purpose of this article is to review HCC imaging appearance after treatment.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary malignant liver tumor. Surgical resection and liver transplantation are the only potentially curative options, but they are contraindicated in most patients[1,2]. Other available imaging guided tools for the treatment of HCC are radiofrequency ablation, ethanol injection, transarterial chemoembolization and radioembolization. These options are not curative, they do increase survival and they

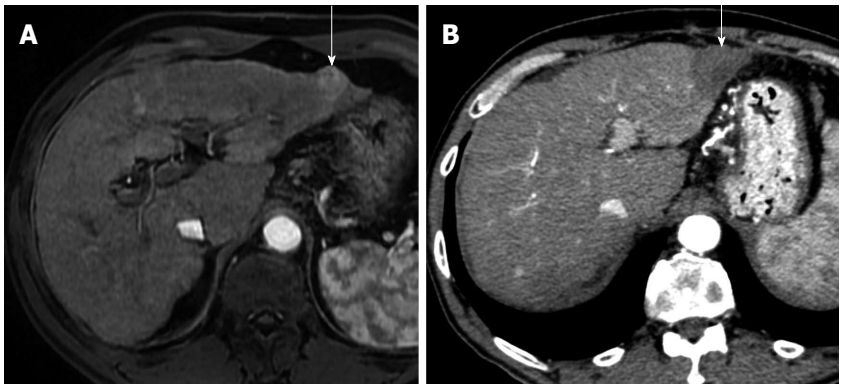

Figure 1 Successful hepatocellular carcinoma resection with radiofrequency tissue coagulation device. A: Pretreatment arterial phase T1-weighted gradient-echo magnetic resonance shows hypervascular hepatocellular carcinoma (HCC) (arrow); B: On arterial phase computed tomography obtained 4 mo after treatment no hypervascular focus is evident (arrow). Note that resected area is larger to preexisting HCC.

are used to downstage patients in order to be suitable to liver transplantation. Moreover, recent studies have underlined the potential of antiangiogenetic treatment in patients with untreatable, unresectable HCCs[3,4]. Imaging follow-up plays a crucial role in evaluating treatment effectiveness and therefore in taking important decisions in the management of these patients. In this article, we will review the imaging appearance of HCC after treatment.

EVALUATION OF TUMOR RESPONSE WITH IMAGING

Currently there are no recommendations regarding the timing for radiological follow-up to assess treatment response and schedules shaped by randomized controlled trials (RCTs) are very heterogeneous[5]. At our institution, treatment efficacy is evaluated using dynamic computed tomography (CT) or magnetic resonance (MR) examinations at one, 3 and 6 mo after treatment, and every 6 mo afterwards. The first evaluation performed at one month is crucial to assess response to treatment and the presence of postprocedural complications. Subsequent follow-up is essential to detect tumor recurrence or the occurrence of new foci of HCC.

Liver resection with radiofrequency tissue coagulation device

Radiofrequency (RF)-assisted liver resection technique uses RF energy to obtain parenchymal dissection by creating a zone of coagulative necrosis along the transection plane[6]. This reduces the risk of intraoperative blood loss when compared with conventional liver resection[6]. Liver resection is indicated in patients with preserved liver function and single HCC, ideally in a subcapsular location[1]. Resected area must be larger than the original tumor and with a margin greater than 5 mm[7]. On CT, resection margin typically appears as an hypoattenuating, non enhancing halo[8,9] (Figure 1). On MR, resection margin is commonly hyperintense on T2-weighted images and hypointense on T1-weighted images and does not enhance after gadolinium injection[8,9]. Residual viable tissue, when present, is usually located along the resection site and shows arterial enhancement and venous wash-out[8,9]. A fluid collection within the resected area is commonly found at initial follow-up and disappears with time[9]. Uncommon complications include biloma, hepatic abscess, pleural effusion and adjacent organ injury (*i.e.*, small bowel perforation).

Percutaneous ablation

Percutaneous ablation induces coagulative necrosis by modifying tumor temperature using RF, microwave, laser, cryotherapy or by direct intratumoral injection of chemical substances (ethanol or acetic acid). This procedure is recommended for patients with preserved liver function and a maximum of 3 small (≤ 3 cm) HCCs[1].

RF ablation: RF ablation (RFA) is the most widely used ablation therapy. RFA ablation consists of placing a needle electrode directly into the tumor, by US or CT guidance, and heating tissue to temperatures exceeding 60 ℃[10] to obtain the coagulative necrosis of the tumor. Similarly to resected area, ablation zone should be 5-10 mm larger in comparison to the preexisting tumor[11]. Due to a coagulative necrosis and hemorrhagic products, treated HCC is typically hypoattenuating or heterogeneously hyperattenuating on unenhanced CT. Contrast enhanced images help differentiate viable from necrotic tumor. In general, successfully treated HCC shows absence of arterial enhancement (Figure 2), whereas any nodular arterially enhancing area within or along the margin of the ablated zone is suspicious of viable tumor[10]. Moreover, patients treated with RFA are considered at higher risk in comparison to the general cirrhotic population for the occurrence of new foci of HCC (Figure 3). However, absence of arterial enhancement at CT does not imperatively correspond to absence of viable tissue[11]. Lu *et al*[11] reported 100% specificity and 36% sensitivity of CT for the depiction of residual or recurrent tumor. In their study, only 4/11 (37%) HCCs with positive histological findings were detected at CT[11]. These observations are in agreement with a study by Dromain *et al*[12] who also found that MRI allowed earlier detection of residual tumor than does CT. At MR, successfully treated HCC shows T2-hypointensity and strong T1-hyperintensity[12]. The inclusion of subtraction images in the MR protocol increases detection of residual arterial enhancement in those cases of HCC with spontaneuous T1 hyperintensity[13]. At initial follow-up studies, treated HCC may show a thin and peripheral

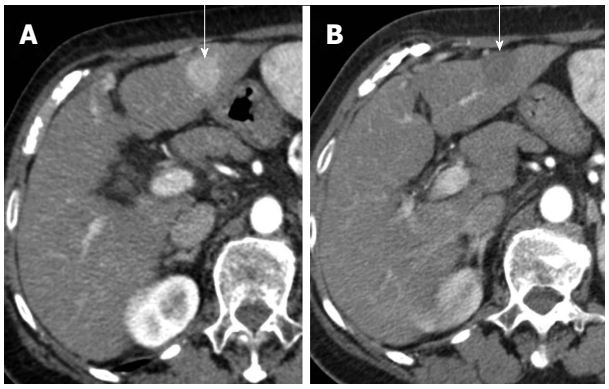

Figure 2 Complete necrosis after radiofrequency ablation for hepatocellular carcinoma. A: Pretreatment arterial phase computed tomography (CT) shows hypervascular hepatocellular carcinoma (arrow); B: Arterial phase CT obtained 1 mo after treatment shows ablated area (arrow). Absence of arterial enhancement suggests complete tumor necrosis.

arterially enhancing rim, due to inflammatory reaction to thermal necrosis[14,15]. This rim sometimes shows hypoattenuation on unenhanced CT and mild hypertintensity on T2-weighted images. More rarely, needle electrode placing can cause formation of arterio-venous shunts[14,15]. These shunts can be easily diagnosed on the basis of wedge-shaped morphology, peripheral location and lack of wash out on portal venous phase (Figure 4). However, if the arterially enhancing zone is small and nodular, a correct characterization may be difficult and a 3-6 mo follow-up is recommended[16]. Size increase of arterially enhancing area, or the appearance of wash-out may suggest presence of viable tumor. Other complications include biloma, hepatic abscess, portal vein thrombosis (Figure 5), arterial pseudoaneurysm (Figure 6), tumor seeding (Figure 7) and adjacent organ injury[17].

Percutaneous ethanol injection: Percutaneous ethanol injection (PEI) consists of percutaneous ethanol instillation into the HCC by sonographic or CT guidance. It is a well-tolerated, inexpensive procedure with few adverse effects. This alternative procedure may be performed in those patients with small HCCs, in whom RFA is not suitable to be performed due to tumor location[18]. In fact, some tumors are located at risky sites (defined as less than 5 mm from a large vessel or an extrahepatic organ, near gallbladder, or in subphrenic locations) and RFA treatment can cause severe complications. In addition, in tumors larger than 2 cm in size, initial RF may leave a tiny nest of viable tissue that will easily be ablated by ethanol with a relevant saving of resources. However, several studies demonstrated that PEI provides a lower rate of complete necrosis in comparison to RFA[19-21]. CT and MR post-treatment imaging features are similar to those obtained after RFA[22] (Figure 8).

Transarterial chemoembolization

Transarterial chemoembolization (TACE) consists of transarterial administration of a mixture of chemotherapy

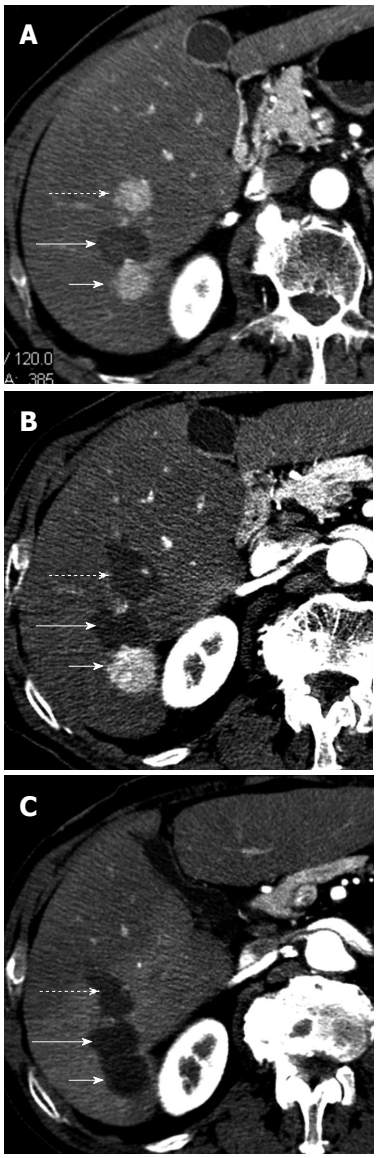

Figure 3 Hepatocellular carcinoma after multiple radiofrequency ablations. A: Arterial phase computed tomography (CT) obtained 8 mo after radiofrequency ablation shows two new enhancing hepatocellular carcinoma (HCC) nodules located anteriorly (dotted arrow) and posteriorly (short arrow) to ablated HCC (long arrow). These findings suggest occurrence of new HCC nodules; B: Arterial phase CT obtained 2 mo after additional radiofrequency (RF) ablation shows that HCC nodule located anteriorly (dotted arrow) has been replaced by a hypoattenuating, non enhancing area (arrow) that is larger than preexisting tumor. These findings suggest complete necrosis. Posteriorly located HCC (short arrow) increased in size; C: Arterial phase CT obtained 2 mo after posterior. HCC had been replaced by a hypoattenuating, nonenhancing ablation area as a result of additional RF ablation. This example shows that RF ablation is a repeatable procedure.

(Doxorubicin or Cisplatin in most cases) and iodized oil (Lipiodol, Guerbert, France), followed by embolizing particles[23]. Since HCC receives blood supply almost completely from the hepatic artery (as opposed to normal liver), these agents accumulate preferentially into HCC lesions. Iodized oil acts as chemotherapy carrier, while particles occlude tumor feeding arteries. Therefore, TACE combines delivery of high dose chemotherapy to the tumor with embolization of its feeding arteries. The

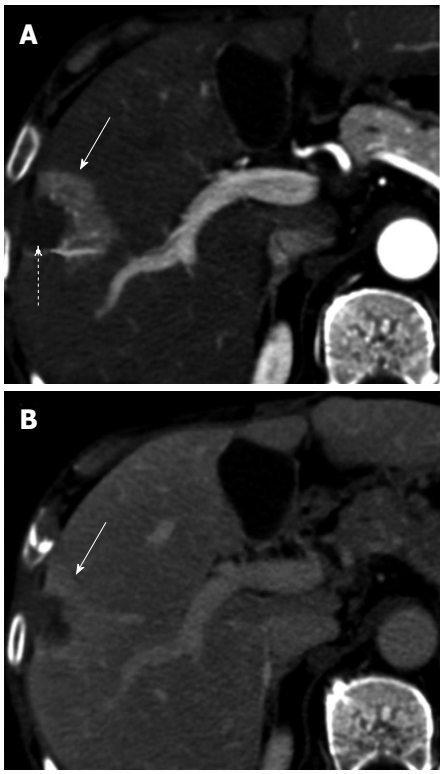

Figure 4 Perfusion alteration after radiofrequency ablation for hepatocellular carcinoma. A: Arterial phase computed tomography (CT) obtained 1 mo after treatment shows ablated zone (dotted arrow) and a semilunar enhancing area (solid arrow) medial and anterior to ablated zone; B: Delayed phase CT shows persistent enhancement of the semilunar area (solid arrow), suggesting that the arterial enhancement is due to perfusion alteration rather than residual tumor.

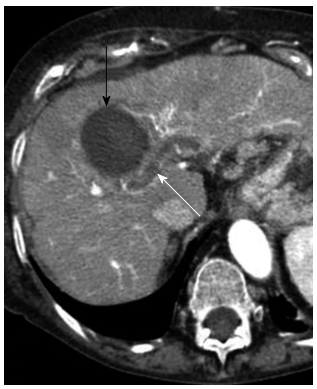

Figure 5 Portal vein thrombosis after radiofrequency ablation for hepatocellular carcinoma. Arterial phase computed tomography obtained 1 mo after radiofrequency ablation shows a non enhancing thrombus in intrahepatic portal vein (white arrow), in proximity of ablated area (black arrow).

major indications for TACE are multiple HCCs without vascular invasion or extrahepatic spread and HCCs for which percutaneous ablation is precluded by position (*e.g.*, pericholecistic, subphrenic, *etc.*) or size[1]. TACE is also indicated in those patients in whom previous procedures have failed[1,24]. At initial post-treatment examinations, treated HCC usually presents the same size of the preexisting tumor. The evaluation of CT images is based on the

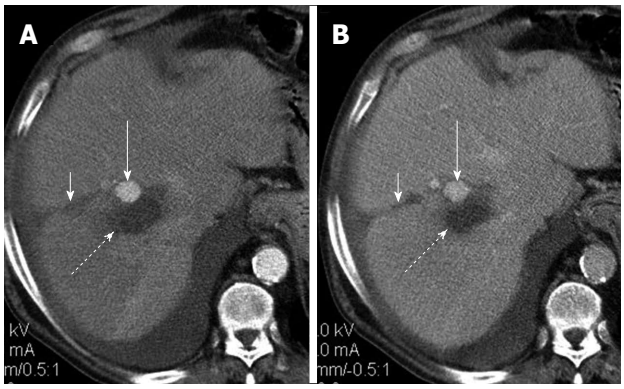

Figure 6 Arterial pseudoaneurysm after radiofrequency ablation for hepatocellular carcinoma. Post-treatment arterial phase (A) computed tomography (CT) shows a round enhancing area (long arrow) anterior to ablated zone (dotted arrow) with persistent enhancement on portal venous phase (B) CT. Round shape, isoattenuation to aorta and absence of wash-out suggest diagnosis of iatrogenic pseudoaneurysm. Note probe track (short arrow on A and B).

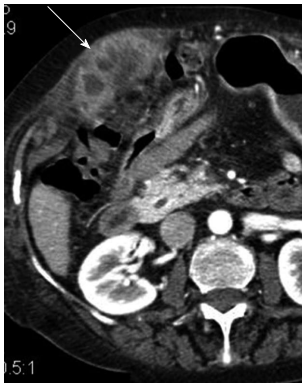

Figure 7 Tumor seeding after radiofrequency ablation for hepatocellular carcinoma. Arterial phase computed tomography shows heterogeneously enhancing hepatocellular carcinoma tissue (arrow) invading muscles of the anterior abdominal wall along needle tract.

assumption that tumor portion that retains iodized oil is necrotic, while enhancing foci represent viable tissue[10]. However, it is sometimes difficult to detect viable tissue, because beam hardening artefacts produced by iodized oil can impair evaluation of arterial enhancement[10] (Figure 9A and B). Kim *et al*[25] reported that use of unenhanced phase in conjunction with biphasic CT could improve the detection of additional foci of viable tumor. According to this study, an HCC treated with TACE could be considered viable if it showed hyperattenuation or isoattenuating on hepatic arterial phase and hypoattenuation on unenhanced and portal venous phases. MRI is known to be superior to CT for the evaluation of HCC after performing TACE[26] (Figure 9C and D). Completely necrotic HCC usually shows variable signal intensity on unenhanced T1-weighted images and T2-weighted images and lack of enhancement after gadolinium injection[27]. MR accuracy is however relatively low. Hunt *et al*[28] reported an overall accuracy rate of 55%, with 43% sensitivity and 75% specificity. Treated HCC sometimes shows a thin and peripheral pseudocapsule that enhances on hepatic arte-

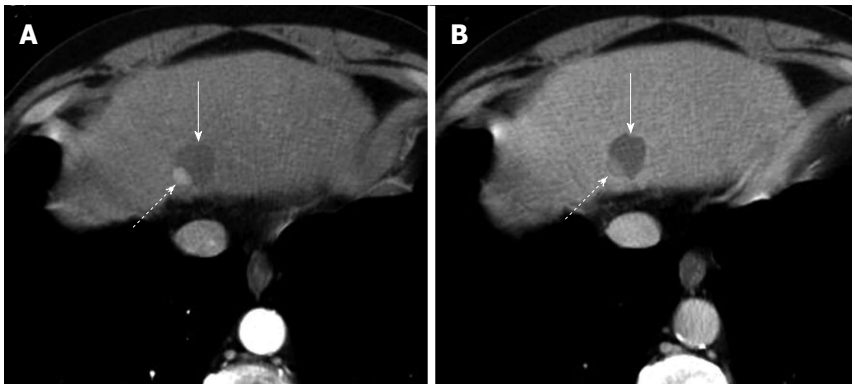

Figure 8 Incomplete hepatocellular carcinoma necrosis after percutaneous ethanol injection. Arterial (A) and portal venous (B) computed tomography obtained 1 mo after treatment shows that approximately 10%-20% of the tumor, located in the dorsal and lateral portion of the treated, hypoattenuating area, is still viable as demonstrated by the presence of enhancement in arterial phase and hypoattenuation ("washout") on portal venous phase (dotted arrow on A and B). The majority of tumor (solid arrow) does not show enhancement as a result of the treatment.

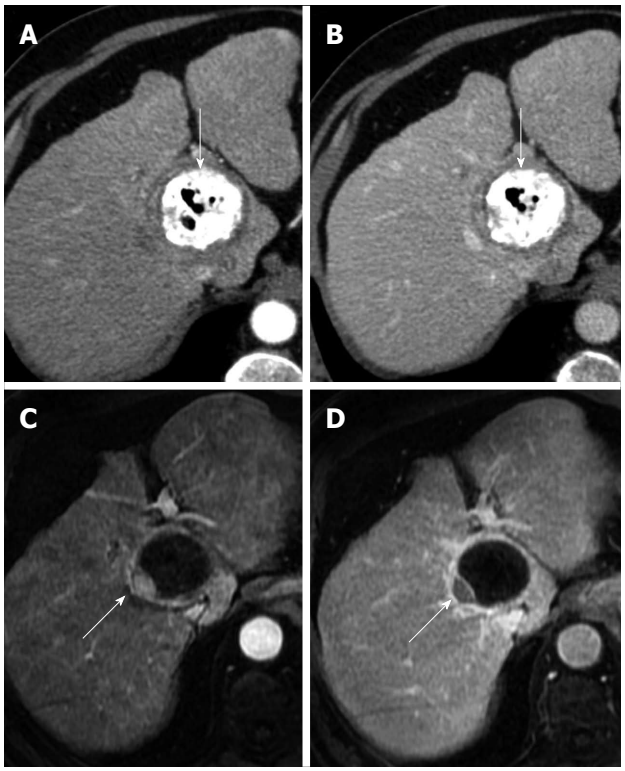

Figure 9 Incomplete hepatocellular carcinoma necrosis after transarterial chemoembolization. Arterial (A) and portal venous (B) phase computed tomography (CT) obtained 1 mo after transarterial chemoembolization (TACE) shows that hepatocellular carcinoma (HCC) is entirely replaced by Lipiodol accumulation (arrow). No evidence of residual tumor was found. Arterial (C) and portal venous (D) phase T1-weighted gradient-echo magnetic resonance (MR) obtained 1 wk after CT shows residual viable tumor (arrow) in the posterolateral portion of the tumor. This case shows higher accuracy of MR in comparison to CT in assessing HCC response after TACE.

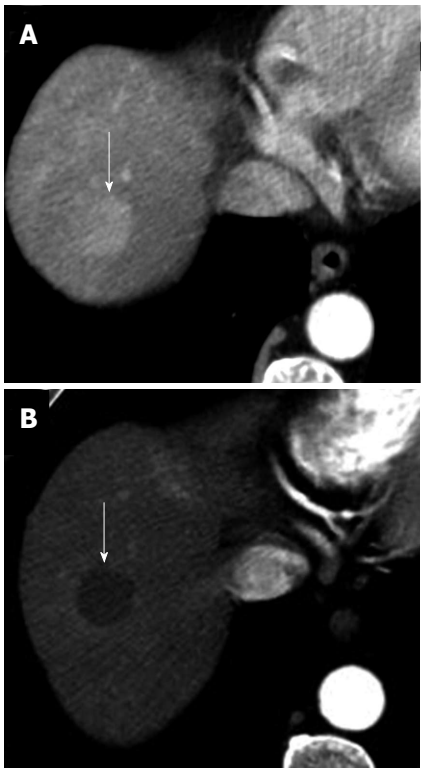

Figure 10 Complete hepatocellular carcinoma necrosis after transarterial chemoembolization with drug-eluting beads. A: Pretreatment arterial phase computed tomography (CT) shows a hypervascular hepatocellular carcinoma (HCC) (arrow); B: Arterial phase CT obtained 3 mo after transarterial chemoembolization shows a hypoattenuating, non enhancing nodule (arrow). Absence of arterial enhancement suggests complete HCC necrosis.

rial and delayed phases. Moreover, iodized oil can sometimes injury small hepatic arteries and cause formation of arterio-portal shunts. Absence of wash-out is crucial to differentiate these pseudolesions from residual viable tumor. Other complications include hepatic artery injuries (dissection or thrombosis), biloma, hepatic abscess and embolization of non target vessels[29]. Non target vessels include arteries that arise from the hepatic circulation (gastroduodenal, right gastric, accessory left gastric, cystic arteries)[30]. Embolization of these vessels results in gastrointestinal ulcers, skin ulcerations, and, rarely, ischemic cholecystitis[29,30]. Furthermore, although the purpose of TACE is to obtain a selective intratumoral delivery of chemioterapy, several studies demonstrated that many patients may have higher plasma levels of chemotherapeutic agents with systemic toxic effects[31]. Therefore, alternative techniques that increase the precision of drug delivery are required. TACE with drug eluting beads (DEB) is recently emerging as an alternative option to conventional TACE. TACE with DEB consists of transarterial injection of microspheres that sequester chemotherapy immediately before administration and release it into the tumor in a sustained and controlled manner[18]. In addition, absence of iodized oil does not mask arterial enhancement[32]

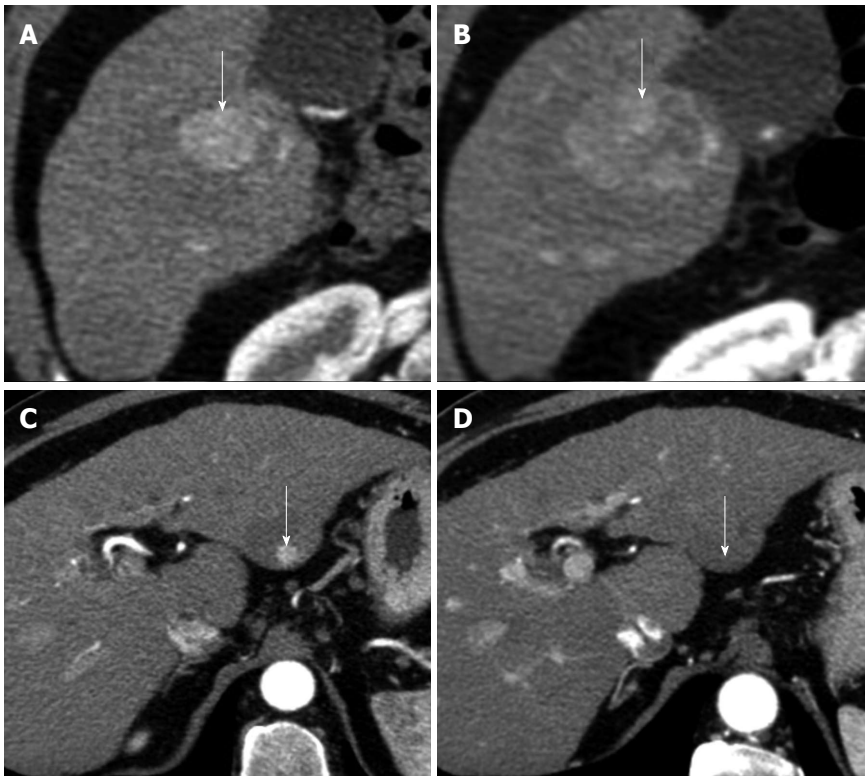

Figure 11 Hepatocellular carcinomas after sorafenib. A: Pretreatment arterial phase computed tomography (CT) shows a large hypervascular hepatocellular carcinoma (HCC) (arrow) in right hepatic lobe; B: Arterial phase CT obtained at the same level of A, 9 mo after the start of sorafenib, shows increase in size of HCC (arrow); C: Pretreatment arterial phase CT image shows a second, small hypervascular HCC (arrow) arising within a larger hypoattenuating dysplastic nodule in left hepatic lobe; D: Arterial phase CT obtained at the same level of C, 9 mo after the start of sorafenib, shows disappearance of HCC enhancement (arrow). This case shows that different HCC response to sorafenib may occur in the same patient.

(Figure 10), allowing easier assessment of residual, viable tumor in comparison to traditional TACE performed with iodized oil.

Radioembolization

An alternative technique to treat advanced HCC is radioembolization. It consists of transarterial administration of yttrium-90 microspheres that are preferentially deposited within hypervascular tumors and that emit lethal beta radiation[18]. This induces tumor coagulative necrosis and avascularity. Post-treatment HCC appearance is similar to that obtained following RFA and PEI[33].

Targeted therapy

Targeted therapies are a new generation of anticancer drugs designed to interfere with tumor growth and progression. Sorafenib, a multikinase inhibitor, is the only drug that has been demonstrated to significantly improve survival in patients with untreatable, unresectable HCCs in a large randomized controlled trial[3]. Sorafenib reduces tumor vascularization and subsequently induces tumor necrosis and hemorrhage[3] (Figure 11). For these reasons, the traditional WHO and RECIST criteria based on evaluation of tumor size may not be ideal to determine tumor response. Therefore, different criteria based on assessment of viable and enhancing tumor are needed. Modified RECIST (mRECIST) differ from RECIST criteria, since they consider only the diameter of viable tumor, defined as the portion of the tumor that shows arterial enhancement and venous wash-out[5,34]. Recent studies have reported the potential of perfusion CT and MR[35,36]. However, high CT radiation dose, MR breathing, cardiac motion and high costs limit the use of these techniques[35,37,38].

CONCLUSION

Advances in HCC treatment have led to an increased use of therapeutic tools such as RF and percutaneous ablation, TACE and antiangiogenetic therapy. In this scenario, CT and MR play an important role in the assessment of response to treatment. Therefore, every radiologist and hepatologist should be familiar with the HCC appearance after treatment and should be able to distinguish normal post-treatment changes from residual or recurrent tumor.

REFERENCES

1 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
2 **Genco C**, Cabibbo G, Maida M, Brancatelli G, Galia M, Alessi N, Butera G, Genova C, Romano P, Raineri M, Giarratano A, Midiri M, Cammà C. Treatment of hepatocellular carcinoma: present and future. *Expert Rev Anticancer Ther* 2013; **13**: 469-479 [PMID: 23560841 DOI: 10.1586/era.13.21]
3 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz

M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

4 **Iavarone M**, Cabibbo G, Piscaglia F, Zavaglia C, Grieco A, Villa E, Cammà C, Colombo M. Field-practice study of sorafenib therapy for hepatocellular carcinoma: a prospective multicenter study in Italy. *Hepatology* 2011; **54**: 2055-2063 [PMID: 21898496 DOI: 10.1002/hep.24644]

5 **Maida M**, Cabibbo G, Brancatelli G, Genco C, Alessi N, Genova C, Romano P, Raineri M, Giarratano A, Midiri M, Cammà C. Assessment of treatment response in hepatocellular carcinoma: a review of the literature. *Future Oncol* 2013; **9**: 845-854 [PMID: 23718305 DOI: 10.2217/fon.13.33]

6 **Curro G**, Jiao L, Scisca C, Baccarani U, Mucciardi M, Habib N, Navarra G. Radiofrequency-assisted liver resection in cirrhotic patients with hepatocellular carcinoma. *J Surg Oncol* 2008; **98**: 407-410 [PMID: 18683211 DOI: 10.1002/jso.21129]

7 **Kleinert R**, Wahba R, Bangard C, Prenzel K, Hölscher AH, Stippel D. Radiomorphology of the Habib sealer-induced resection plane during long-time followup: a longitudinal single center experience after 64 radiofrequency-assisted liver resections. *HPB Surg* 2010; **2010**: 403097 [PMID: 20862384 DOI: 10.1155/2010/403097]

8 **McGahan JP**, Khatri VP. Imaging findings after liver resection by using radiofrequency parenchymal coagulation devices: initial experiences. *Radiology* 2008; **247**: 896-902 [PMID: 18487541 DOI: 10.1148/radiol.2473070949]

9 **Taibbi A**, Furlan A, Sandonato L, Bova V, Galia M, Marin D, Cabibbo G, Soresi M, Bartolotta TV, Midiri M, Lagalla R, Brancatelli G. Imaging findings of liver resection using a bipolar radiofrequency electrosurgical device--initial observations. *Eur J Radiol* 2012; **81**: 663-670 [PMID: 21306849 DOI: 10.1016/j.ejrad.2011.01.015]

10 **Yaghmai V**, Besa C, Kim E, Gatlin JL, Siddiqui NA, Taouli B. Imaging assessment of hepatocellular carcinoma response to locoregional and systemic therapy. *AJR Am J Roentgenol* 2013; **201**: 80-96 [PMID: 23789661 DOI: 10.2214/AJR.13.10706]

11 **Lu DS**, Yu NC, Raman SS, Limanond P, Lassman C, Murray K, Tong MJ, Amado RG, Busuttil RW. Radiofrequency ablation of hepatocellular carcinoma: treatment success as defined by histologic examination of the explanted liver. *Radiology* 2005; **234**: 954-960 [PMID: 15681691 DOI: 10.1148/radiol.2343040153]

12 **Dromain C**, de Baere T, Elias D, Kuoch V, Ducreux M, Boige V, Petrow P, Roche A, Sigal R. Hepatic tumors treated with percutaneous radio-frequency ablation: CT and MR imaging follow-up. *Radiology* 2002; **223**: 255-262 [PMID: 11930075 DOI: 10.1148/radiol.2231010780]

13 **Winters SD**, Jackson S, Armstrong GA, Birchall IW, Lee KH, Low G. Value of subtraction MRI in assessing treatment response following image-guided loco-regional therapies for hepatocellular carcinoma. *Clin Radiol* 2012; **67**: 649-655 [PMID: 22300821 DOI: 10.1016/j.crad.2011.11.013]

14 **Jiang T**, Zhu AX, Sahani DV. Established and novel imaging biomarkers for assessing response to therapy in hepatocellular carcinoma. *J Hepatol* 2013; **58**: 169-177 [PMID: 22944253 DOI: 10.1016/j.jhep.2012.08.022]

15 **Sainani NI**, Gervais DA, Mueller PR, Arellano RS. Imaging after percutaneous radiofrequency ablation of hepatic tumors: Part 1, Normal findings. *AJR Am J Roentgenol* 2013; **200**: 184-193 [PMID: 23255761 DOI: 10.2214/AJR.12.8478]

16 **Park Y**, Choi D, Lim HK, Rhim H, Kim YS, Kim SH, Lee WJ. Growth rate of new hepatocellular carcinoma after percutaneous radiofrequency ablation: evaluation with multiphase CT. *AJR Am J Roentgenol* 2008; **191**: 215-220 [PMID: 18562748 DOI: 10.2214/AJR.07.3297]

17 **Sainani NI**, Gervais DA, Mueller PR, Arellano RS. Imaging after percutaneous radiofrequency ablation of hepatic tumors: Part 2, Abnormal findings. *AJR Am J Roentgenol* 2013; **200**: 194-204 [PMID: 23255762 DOI: 10.2214/AJR.12.8479]

18 **Lencioni R**, Crocetti L. Local-regional treatment of hepatocellular carcinoma. *Radiology* 2012; **262**: 43-58 [PMID: 22190656 DOI: 10.1148/radiol.11110144]

19 **Orlando A**, Leandro G, Olivo M, Andriulli A, Cottone M. Radiofrequency thermal ablation vs. percutaneous ethanol injection for small hepatocellular carcinoma in cirrhosis: meta-analysis of randomized controlled trials. *Am J Gastroenterol* 2009; **104**: 514-524 [PMID: 19174803 DOI: 10.1038/ajg.2008.80]

20 **Oeda S**, Mizuta T, Isoda H, Kuwashiro T, Iwane S, Takahashi H, Kawaguchi Y, Eguchi Y, Ozaki I, Tanaka K, Fujimoto K. Survival advantage of radiofrequency ablation for hepatocellular carcinoma: Comparison with ethanol injection. *Hepatogastroenterology* 2013 May 1; Epub ahead of print [PMID: 23635508 DOI: 10.5754/hge121270]

21 **Cho YK**, Kim JK, Kim MY, Rhim H, Han JK. Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. *Hepatology* 2009; **49**: 453-459 [PMID: 19065676 DOI: 10.1002/hep.22648]

22 **Bartolozzi C**, Lencioni R, Caramella D, Mazzeo S, Ciancia EM. Treatment of hepatocellular carcinoma with percutaneous ethanol injection: evaluation with contrast-enhanced MR imaging. *AJR Am J Roentgenol* 1994; **162**: 827-831 [PMID: 8141000]

23 **Brown DB**, Gould JE, Gervais DA, Goldberg SN, Murthy R, Millward SF, Rilling WS, Geschwind JF, Salem R, Vedantham S, Cardella JF, Soulen MC. Transcatheter therapy for hepatic malignancy: standardization of terminology and reporting criteria. *J Vasc Interv Radiol* 2009; **20**: S425-S434 [PMID: 19560030 DOI: 10.1016/j.jvir.2009.04.021]

24 **Cabibbo G**, Genco C, Di Marco V, Barbara M, Enea M, Parisi P, Brancatelli G, Romano P, Craxì A, Cammà C. Predicting survival in patients with hepatocellular carcinoma treated by transarterial chemoembolisation. *Aliment Pharmacol Ther* 2011; **34**: 196-204 [PMID: 21564144 DOI: 10.1111/j.1365-2036.2011.04694.x]

25 **Kim HC**, Kim AY, Han JK, Chung JW, Lee JY, Park JH, Choi BI. Hepatic arterial and portal venous phase helical CT in patients treated with transcatheter arterial chemoembolization for hepatocellular carcinoma: added value of unenhanced images. *Radiology* 2002; **225**: 773-780 [PMID: 12461260]

26 **Kloeckner R**, Otto G, Biesterfeld S, Oberholzer K, Dueber C, Pitton MB. MDCT versus MRI assessment of tumor response after transarterial chemoembolization for the treatment of hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 2010; **33**: 532-540 [PMID: 19847482 DOI: 10.1007/s00270-009-9728-y]

27 **Mannelli L**, Kim S, Hajdu CH, Babb JS, Clark TW, Taouli B. Assessment of tumor necrosis of hepatocellular carcinoma after chemoembolization: diffusion-weighted and contrast-enhanced MRI with histopathologic correlation of the explanted liver. *AJR Am J Roentgenol* 2009; **193**: 1044-1052 [PMID: 19770328 DOI: 10.2214/AJR.08.1461]

28 **Hunt SJ**, Yu W, Weintraub J, Prince MR, Kothary N. Radiologic monitoring of hepatocellular carcinoma tumor viability after transhepatic arterial chemoembolization: estimating the accuracy of contrast-enhanced cross-sectional imaging with histopathologic correlation. *J Vasc Interv Radiol* 2009; **20**: 30-38 [PMID: 19028117 DOI: 10.1016/j.jvir.2008.09.034]

29 **Clark TW**. Complications of hepatic chemoembolization. *Semin Intervent Radiol* 2006; **23**: 119-125 [PMID: 21326755 DOI: 10.1055/s-2006-941442]

30 **Lee AJ**, Gomes AS, Liu DM, Kee ST, Loh CT, McWilliams JP. The road less traveled: importance of the lesser branches of the celiac axis in liver embolotherapy. *Radiographics* 2012; **32**: 1121-1132 [PMID: 22786998 DOI: 10.1148/rg.324115114]

31 **Lammer J**, Malagari K, Vogl T, Pilleul F, Denys A, Watkinson A, Pitton M, Sergent G, Pfammatter T, Terraz S,

Benhamou Y, Avajon Y, Gruenberger T, Pomoni M, Langenberger H, Schuchmann M, Dumortier J, Mueller C, Chevallier P, Lencioni R. Prospective randomized study of doxorubicin-eluting-bead embolization in the treatment of hepatocellular carcinoma: results of the PRECISION V study. *Cardiovasc Intervent Radiol* 2010; **33**: 41-52 [PMID: 19908093 DOI: 10.1007/s00270-009-9711-7]

32 **Chung WS**, Lee KH, Park MS, Lee YJ, Kwon J, Baek SE, Kim MJ. Enhancement patterns of hepatocellular carcinoma after transarterial chemoembolization using drug-eluting beads on arterial phase CT images: a pilot retrospective study. *AJR Am J Roentgenol* 2012; **199**: 349-359 [PMID: 22826396 DOI: 10.2214/AJR.11.7563]

33 **Atassi B**, Bangash AK, Bahrani A, Pizzi G, Lewandowski RJ, Ryu RK, Sato KT, Gates VL, Mulcahy MF, Kulik L, Miller F, Yaghmai V, Murthy R, Larson A, Omary RA, Salem R. Multimodality imaging following 90Y radioembolization: a comprehensive review and pictorial essay. *Radiographics* 2008; **28**: 81-99 [PMID: 18203932 DOI: 10.1148/rg.281065721]

34 **Lencioni R**, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: 20175033 DOI: 10.1055/s-0030-1247132]

35 **Zhu AX**, Chabner BA, Tanabe KK. New trends and novel treatment for hepatocellular carcinoma: a global perspective. *Oncologist* 2010; **15** Suppl 4: 1-4 [PMID: 21115575 DOI: 10.1634/theoncologist.2010-S4-01]

36 **Sacco R**, Faggioni L, Bargellini I, Ginanni B, Battaglia V, Romano A, Bertini M, Bresci G, Bartolozzi C. Assessment of response to sorafenib in advanced hepatocellular carcinoma using perfusion computed tomography: Results of a pilot study. *Dig Liver Dis* 2013 Apr 8; Epub ahead of print [PMID: 23578581 DOI: 10.1016/j.dld.2013.03.004]

37 **Sabir A**, Schor-Bardach R, Wilcox CJ, Rahmanuddin S, Atkins MB, Kruskal JB, Signoretti S, Raptopoulos VD, Goldberg SN. Perfusion MDCT enables early detection of therapeutic response to antiangiogenic therapy. *AJR Am J Roentgenol* 2008; **191**: 133-139 [PMID: 18562736 DOI: 10.2214/AJR.07.2848]

38 **Goh V**, Halligan S, Hugill JA, Bassett P, Bartram CI. Quantitative assessment of colorectal cancer perfusion using MDCT: inter- and intraobserver agreement. *AJR Am J Roentgenol* 2005; **185**: 225-231 [PMID: 15972428]

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ORIGINAL ARTICLE

Participation of peribiliary glands in biliary tract pathophysiologies

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Author contributions: Igarashi S, Sato Y and Ren XS performed the majority of experiments; Harada K and Sasaki M provided vital reagents and advice; Nakanuma Y supervised this study and wrote the manuscript.

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Abstract

AIM: To investigate the roles of peribiliary glands around the bile ducts in the pathophysiology of the biliary tract.

METHODS: The expression of fetal pancreatic markers, pancreatic duodenal homeobox factor 1 (PDX1) and hairy and enhancer of split 1 (HES1) and endodermal stem/progenitor (S/P) cell markers [CD44s, chemokine receptor type 4 (CXCR4), SOX9 and epithelial cell adhesion molecule (EpCAM)] were examined immunohistochemically in 32 normal adult livers (autopsy livers) and 22 hepatolithiatic livers (surgically resected livers). The latter was characterized by the proliferation of the peribiliary glands. Immunohistochemistry was performed using formalin-fixed, paraffin-embedded tissue sections after deparaffinization. Although PDX1 and HES1 were expressed in both the nucleus and cytoplasm of epithelial cells, only nuclear staining was evaluated. SOX9 was expressed in the nucleus, while CD44s, CXCR4 and EpCAM were expressed in the cell membranes. The frequency and extent of the expression of these molecules in the lining epithelia and peribiliary glands were evaluated semi-quantitatively based on the percentage of positive cells: 0, 1+ (focal), 2+ (moderate) and 3+ (extensive).

RESULTS: In normal livers, PDX1 was infrequently expressed in the lining epithelia, but was frequently expressed in the peribiliary glands. In contrast, HES1 was frequently expressed in the lining epithelia, but its expression in the peribiliary glands was focal, suggesting that the peribiliary glands retain the potential of differentiation toward the pancreas and the lining epithelia exhibit properties to inhibit such differentiation. This unique combination was also seen in hepatolithiatic livers. The expression of endodermal S/P cell markers varied in the peribiliary glands in normal livers: SOX9 and EpCAM were frequently expressed, CD44s infrequently, and CXCR4 almost not at all. The expression of these markers, particularly CD44s and CXCR4, increased in the peribiliary glands and lining epithelia in hepatolithiatic livers. This increased expression of endodermal S/P cell markers may be related to the increased production of intestinal and gastric mucin and also to the biliary neoplasia associated with the gastric and intestinal phenotypes reported in hepatolithiasis.

CONCLUSION: The unique expression pattern of PDX1 and HES1 and increased expression of endodermal S/P cell markers in the peribiliary glands may be involved in biliary pathophysiologies.

Key words: Biliary tree; Peribiliary glands; Pancreatic duodenal homeobox factor 1; Stem cells; Differentiation; Pancreas

Core tip: Immunohistochemical analysis showed that pancreatic duodenal homeobox factor 1 was more frequently expressed in the peribiliary glands than epithelia lining the bile duct and was accompanied by the reciprocal expression pattern of hairy and enhancer of

split 1. These results may reflect maintenance of the biliary tract and the increased expression of endodermal stem/progenitor cell markers may be involved in the unique pathophysiologies of the peribiliary glands.

Igarashi S, Sato Y, Ren XS, Harada K, Sasaki M, Nakanuma Y. Participation of peribiliary glands in biliary tract pathophysiologies. *World J Hepatol* 2013; 5(8): 425-432 Available from: URL: http://www.wjgnet.com/1948-5182/full/v5/i8/425.htm DOI: http://dx.doi.org/10.4254/wjh.v5.i8.425

INTRODUCTION

The biliary tract and pancreas are embryologically derived from the foregut and several factors, such as pancreatic duodenal homeobox factor 1 (PDX1) and hairy and enhancer of split 1 (HES1), are reportedly involved in their development and differentiation[1-3]. For example, PDX1, a transcription factor crucial for the development of the pancreas and a marker of pancreatic progenitor cells, was shown to be expressed in the fetal biliary tract[4,5]. In contrast, HES1, which is also a fetal transcription factor that represses pancreatic exocrine and endocrine differentiation, is also important for the development and differentiation of the biliary tract[4,5]. In $HES1^{-}/HES1^{-}$ mice, the biliary tract was shown to continuously express PDX1 and the pancreatic acini appeared and replaced biliary epithelial cells in the biliary tract[5]. Our previous study showed that PDX1 was expressed extensively in epithelia lining the fetal bile ducts, but not at all in adult bile ducts, whereas HES1 was expressed in the adult bile ducts[4]. These findings supported reciprocal roles for PDX1 and HES1 in the development of bile ducts. However, the expression and significance of these proteins in the peribiliary glands remain to be clarified.

Peribiliary glands composed of branched tubuloalveolar seromucinous glands are found around the extrahepatic and intrahepatic large bile ducts of humans at all ages[1,6-10]. These glands communicate with bile duct lumens through their own conduits[6-8] and are relatively dense in the hilar bile ducts, cystic duct and periampullary region[7-10]. They secrete several substances such as lactoferrin and lysozyme[7]. Recently, the peribiliary glands were reported to be stem cell niches of the biliary tree[11] and these stem cells were shown to be capable of differentiating into hepatobiliary and pancreatic cells[11-13]. According to Carpino *et al*[12], these peribiliary glands harbor stem/progenitor (S/P) cells of the liver, bile duct and pancreas, which express endodermal S/P cell markers, such as C-X-C chemokine receptor type 4 (CXCR4), PDX1, HES1, SOX9/17, epithelial cell adhesion molecule (EpCAM) and CD44s, and these cells also weakly express adult liver, bile duct and pancreatic markers, such as albumin and cystic fibrosis transmembrane conductance regulator (CFTR)[11-13].

These S/P cells in the peribiliary glands are likely to be central to normal tissue turnover and injury repair, and may play key roles in the pathophysiology of several biliary tract diseases[12]. In hepatolithiasis, the peribiliary glands proliferate markedly, secrete large amounts of mucin into the bile duct lumen[14,15] and may be involved in stone formation and even cholangiocarcinogenesis[16]. However, the roles of S/P cells in the peribiliary glands in hepatolithiasis have not been examined.

In this study, we examined the expression of fetal pancreatic markers (PDX1 and HES1) and endodermal S/P cell markers (CD44s, CXCR4, SOX9 and EpCAM) in the lining epithelia and peribiliary glands immunohistochemically, using 32 normal livers and 22 hepatolithiatic livers, and then tried to evaluate the roles and significance of these markers in the biliary tract pathophysiologies in hepatolithiasis.

MATERIALS AND METHODS

Case selection and tissue preparation

Case selection: Thirty-two histologically normal livers from 32 patients (range of age: 45-81 years old with an average age of 63 years; 20 males and 12 females) were obtained from our recent autopsy series with minimal autolytic changes and at least one tissue section was obtained from the hepatic hilus containing hilar bile duct(s) with peribiliary glands in each case. In addition, 22 hepatolithiatic livers were obtained from our surgical cases and the age and sex of these cases were similar to those of normal livers. All stone-containing bile ducts exhibited the marked proliferation of peribiliary glands and failed to show neoplastic biliary epithelial lesions[6,7,14] and at least two tissue sections were obtained from these stone-containing bile ducts in each case.

Tissue preparation: All tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. More than 20 consecutive 4-μm-thick sections were cut from each paraffin block and one section was stained with hematoxylin and eosin (HE) for histological observations. The remaining sections were used for immunohistochemistry.

Immunohistochemistry and its evaluation

Immunostaining was performed using formalin-fixed, paraffin-embedded tissue sections of normal and hepatolithiatic livers. The antibodies and their sources, optimal dilution and antigen retrieval are shown in Table 1. After deparaffinization and the blocking of endogenous peroxidase, the sections were incubated first in a protein block solution (DakoCytomation), then overnight at 4 ℃ with the primary antibodies against PDX1 and HES1 and the markers for endodermal S/P cells (CD44s, CXCR4, SOX9 and EpCAM). Sections were then treated with secondary antibodies conjugated to a peroxidase-labeled polymer (EnVision system, Dako Cytomation). Color development was performed using DAB and the sections were counterstained with hematoxylin or methyl green.

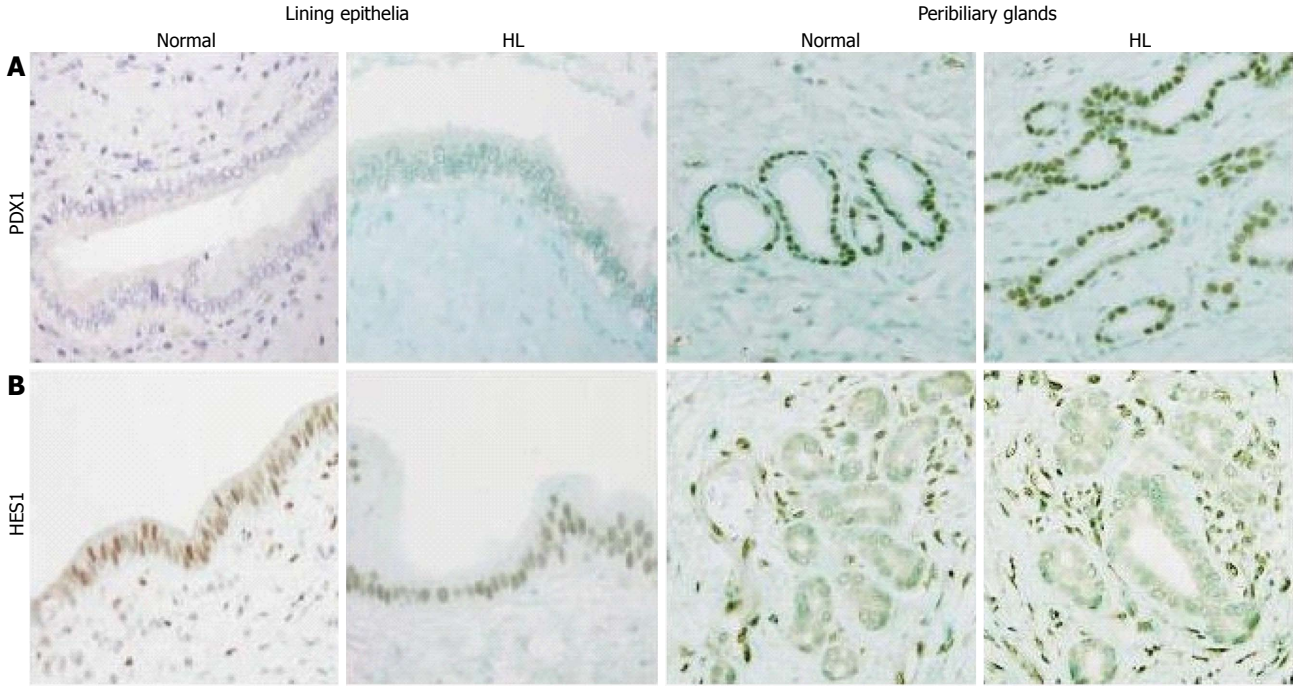

Figure 1 Expression of pancreatic duodenal homeobox factor 1 and hairy and enhancer of split 1. A: Pancreatic duodenal homeobox factor 1 (PDX1) was not detected in the lining epithelia of hilar bile ducts in normal or hepatolithiatic livers (HL), while it was strongly expressed in peribiliary glands in normal and hepatolithiatic livers. Immunostaining of PDX1 × 200; B: Hairy and enhancer of split 1 (HES1) was frequently expressed in the lining epithelia of hilar bile ducts in normal and hepatolithiatic livers , while its expression in peribiliary glands was infrequent in normal and hepatolithiatic livers. Mesenchymal cells were positive for HES1. Immunostaining of HES1 × 200.

As positive controls, islet cells in normal pancreatic tissue (one case) for PDX1, fibroblasts in cirrhotic liver tissue (one case) for HES1, neural cells in a normal human brain (one case) for CXCR4, fibroblasts in cirrhotic liver tissue (one case) for CD44s, normal human embryonic tissue (one case) for SOX9, and bile ducts in a normal liver (one case) for EpCAM were used as shown in Table 1. Negative controls were carried out with non-immunized serum substituted for the primary antibodies, resulting in no signal detection.

Although PDX1 and HES1 were expressed in both the nucleus and cytoplasm of epithelial cells, only nuclear staining was evaluated. Sox9 was expressed in the nucleus, while CD44s, CXCR4 and EpCAM were expressed in the cell membranes. The immunoreactivity of epithelial cells in the lining epithelia and peribiliary glands was semi-quantitatively graded based on the percentage of positive cells, as follows: 0, no expression of each marker in the lining epithelia or peribiliary glands; 1+ (focal), the expression of each marker in less than one third of the lining epithelia and peribiliary glands, respectively; 3+ (extensive), the expression of each marker in more than two thirds of the lining epithelia and peribiliary glands, respectively; and 2+ (moderate), the expression of each marker in the lining epithelia and peribiliary glands between 1+ and 3+, respectively. The staining intensity was rather stronger in the cases of extensive expression and was rather weaker in the cases of focal expression.

Statistical analysis

Intergroup comparisons were made using Mann-Whitney's *U* test. The results were considered significant if the *P* value < 0.05.

RESULTS

Expression of the fetal pancreatic markers (PDX1 and HES1)

In normal livers, PDX1 was infrequently and focally expressed in the lining epithelia but was extensively expressed in the peribiliary glands, with 28 of 32 cases showing moderate to extensive expression (Figures 1A and 2A). In contrast, HES1 was extensively expressed in the lining epithelia, with 28 of 32 cases showing moderate to extensive expression (Figures 1B and 2B), while its expression was infrequent and focal in the peribiliary glands. The patterns for the expression of PDX1 and HES1 in the peribiliary glands and lining epithelia in hepatolithiatic livers were similar to those in normal livers.

Expression of the endoderm S/P cell markers (SOX9, EpCAM, CD44s and CXCR4)

In normal livers, SOX9 was frequently expressed in the lining epithelia and also in the peribiliary glands, with 17 and 18 cases showing moderate to extensive expression, respectively (Figure 3A). EpCAM was frequently expressed in the lining epithelia and peribiliary glands (Figure 3B), with the level of moderate to extensive expression. In hepatolithiatic livers, the degree of SOX9 expression was relatively low in the lining epithelia and relatively high in the peribiliary glands in comparison

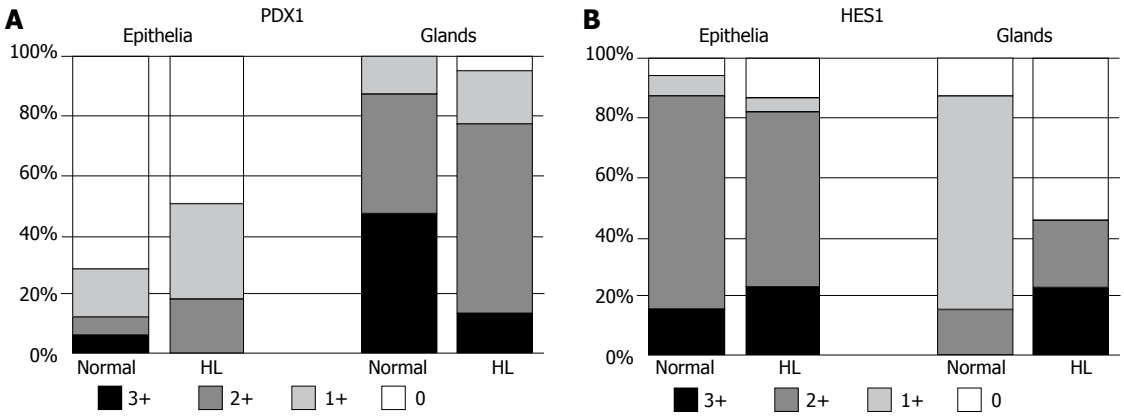

Figure 2 Distribution of pancreatic duodenal homeobox factor 1 and hairy and enhancer of split 1 expression in normal and hepatolithiatic livers. A: Pancreatic duodenal homeobox factor 1 (PDX1) was more frequently expressed in the peribiliary glands in normal livers (0, 0 case; 1+, 4 cases; 2+, 13 cases; 3+, 15 cases) than in the lining epithelia (0, 23 cases; 1+, 5 cases; 2+, 2 cases; 3+, 2 cases). This distribution pattern was similar in hepatolithiatic livers (HL), too; B: In normal livers, hairy and enhancer of split 1 (HES1) was more frequently expressed in the lining epithelia (0, 2 cases; 1+, 2 cases; 2+, 23 cases; 3+, 5 cases) than in the peribiliary glands (0, 7 cases; 1+, 20 cases; 2+, 5 cases; 3+, 0 case). Although such a distribution pattern was similar in hepatolithiatic livers, HES1 expression in the peribiliary glands was decreased.

Table 1 Primary antibodies and positive control tissue

Primary antibody	Clone (product code)	Company	Optional dilution	Antigen retrieval method	Positive control (N/C/M)
PDX1	goat poly. (sc-14664)	Santa Cruz	1:100	Citrate buf. AC	Islet cells in the human pancreas
HES1	rabbit poly. (H2034-35)	US Biological	1:500	Citrate buf. MW	Human fibroblasts (N)
CXCR4	mouse mono. (35-8800)	ZYMED	1:100	PK	Neural cells in the human brain (M)
CD44s	mouse mono. (M 7082)	Dako	1:100	Dako Target Retrieval Solution No.S1700	Human fibroblasts (M/C)
SOX9	rabbit poly. (AB5535)	MILLIPORE	1:1000	Boro buf. PC	Embryonic tissue (N)
EpCAM	mouse mono. (ab46714)	Abcam	1:5	Dako Target Retrieval Solution No.S1700	Bile ducts in the human liver (M)

Poly: Polyclonal; Mono: Monoclonal; AC: Autoclave; MW: Microwave; PK: Proteinase K; PC: Pressure Cooker; N: Nuclear; C: Cytoplasm; M: Membrane; PDX1: Pancreatic duodenal homeobox factor 1; HES1: Hairy and enhancer of split 1; CXCR4: Chemokine receptor type 4; EpCAM: Epithelial cell adhesion molecule.

with normal livers, whereas the frequency and degree of EpCAM expression in both the lining epithelia and peribiliary glands were similar to those in normal livers (Figure 4A and B).

CD44s was moderately to extensively expressed in the lining epithelia and peribiliary glands in 7 and 8 of 32 normal livers, respectively (Figure 3C), and its expression in the lining epithelia and peribiliary glands was increased in the frequency and degree in the hepatolithiatic livers, with this increase being significant in the lining epithelia ($P < 0.01$) (Figure 4C). CXCR4 was expressed in the lining epithelia and peribiliary glands of two and one normal livers, respectively. Its expression in the lining epithelia and peribiliary glands was increased in the frequency and degree in the hepatolithiatic livers (Figure 3D), with this increase being significant in the lining epithelia ($P < 0.01$) (Figure 4D).

The number of peribiliary glands in hepatolithiatic livers was markedly higher than that in normal livers[6,7,14]. Therefore, the actual number of glandular cells expressing each marker of these fetal pancreatic markers and endodermal S/P cell markers in the peribiliary glands could be regarded as more in hepatolithiatic livers than expected by the above-mentioned semi-quantitative approach.

DISCUSSION

The results of this study can be summarized as follows: (1) In normal livers, PDX1 was extensively expressed in the peribiliary glands and was infrequently expressed in the lining epithelia of the bile ducts, and while the opposite was true for HES1, this unique expression pattern was retained in hepatolithiatic livers; (2) Endodermal S/P cell markers were variably expressed in the peribiliary glands and EpCAM and SOX9 were frequently detected in the peribiliary glands of normal livers, whereas CXCR4 was rarely detected; and (3) In hepatolithiatic livers, the expression of the S/P markers, particularly CD44s and CXCR4, in the lining epithelia and peribiliary glands was increased. Taken together, the reciprocal expression of PDX1 and HES1 in the lining epithelia and peribiliary glands may be important for physiological maintenance of the biliary tract. The expression of endodermal S/P cell markers was variable in the peribiliary glands and tended to be higher in hepatolithiatic livers than in normal livers, implying the pathogenetic roles of S/P cells in the biliary pathophysiologies of hepatolithiasis.

HES1 is known to inhibit or repress the differentiation of pancreas, whereas PDX1 is involved in its promotion[2-5]. Our recent study supported their roles in the

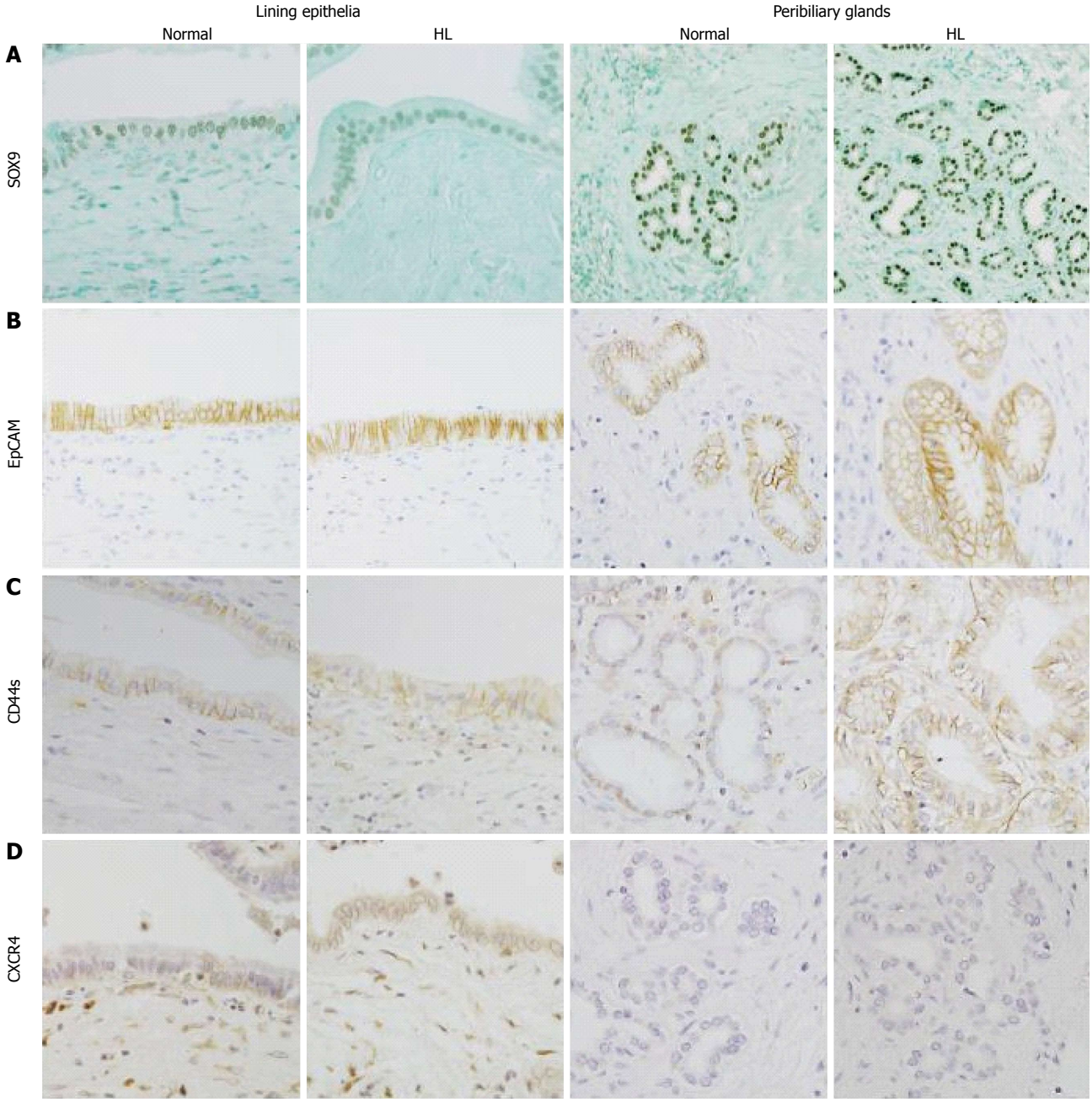

Figure 3 Expression of SOX9, epithelial cell adhesion molecule, CD44s and chemokine receptor type 4. A: SOX9 was expressed in the nuclei of the lining epithelia of hilar bile ducts in normal livers and of the peribiliary glands in hepatolithiatic livers (HL) (Immunostaining of SOX9, × 200); B: Many of the lining epithelial cells in the hilar bile ducts of normal livers were positive for epithelial cell adhesion molecule (EpCAM) and many of the acini in the peribiliary glands of hepatolithiatic livers were positive for EpCAM (Immunostaining of EpCAM, × 200); C: CD44s was focally expressed in the lining epithelia and peribiliary glands of hepatolithiatic livers (Immunostaining of CD44s, × 200); D: Chemokine receptor type 4 (CXCR4) was focally expressed in the lining epithelia and peribiliary glands of hepatolithiatic livers (Immunostaining of CXCR4, × 200).

biliary tract differentiation and maturation in humans[1,4]. The present study showed that PDX1 was infrequently and focally expressed in the lining epithelia of normal livers, but was extensively expressed in the peribiliary glands. In contrast, HES1 was extensively expressed in the lining epithelia, although infrequently and usually focally expressed in the peribiliary glands. Taken together, it seems possible that the peribiliary glands expressing PDX1 in adults retain potential properties for the promotion of pancreatic development as seen in the biliary epithelia of the fetal bile duct, while the lining epithelia of the bile duct expressing HES1 keep properties for the inhibition of pancreatic development.

Our previous studies showed that the pancreatic exocrine acini were occasionally present in the peribiliary glands of adults and expressed enzymes such as amylase, lipase and chymotrypsin[17,18]. Carpino *et al*[12] also reported that pancreatic genes such as CFTR were weakly expressed in the peribiliary glands[11,13]. Thus, it seems possible that the peribiliary glands may have the

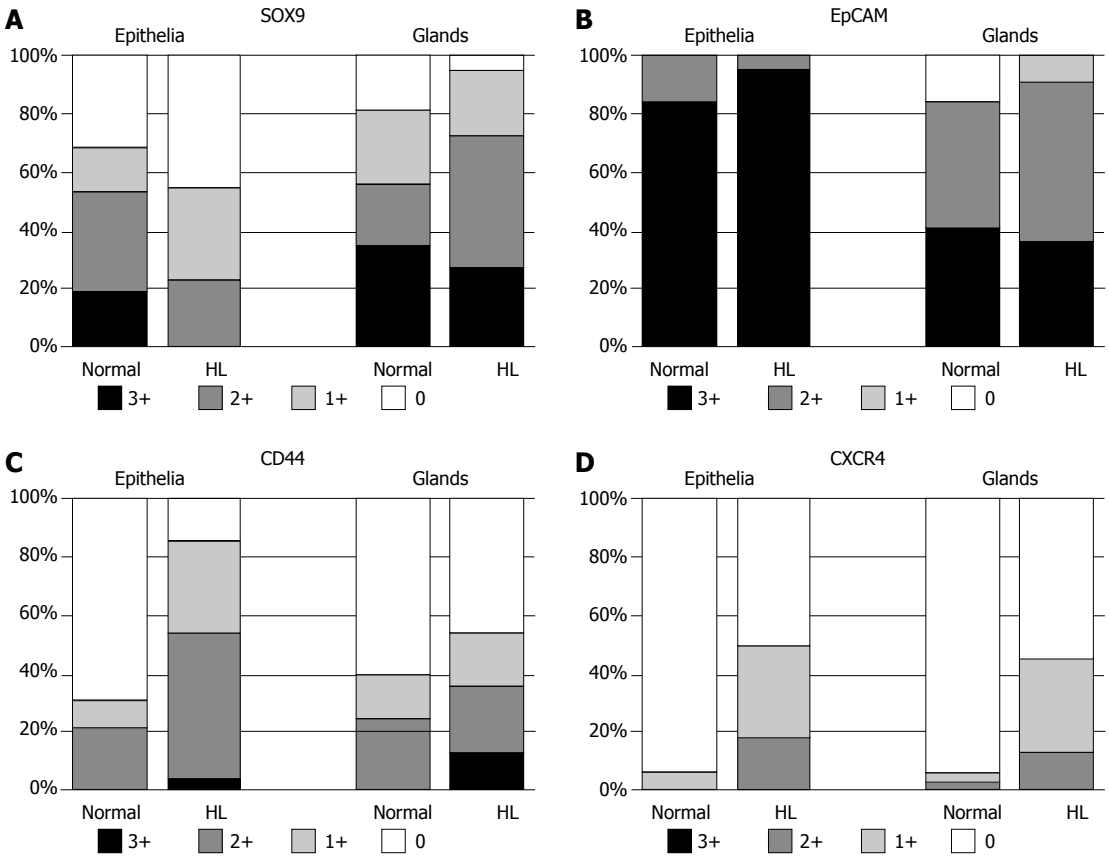

Figure 4 Distribution of SOX9, epithelial cell adhesion molecule, CD44s and chemokine receptor type 4 expression in normal and hepatolithiatic livers. A: SOX9 was frequently expressed in the lining epithelia and peribiliary glands of normal livers (0, 10 and 6 cases; 1+, 5 and 8 cases; 2+, 11 and 7 cases; 3+, 6 and 11 cases, respectively) and hepatolithiatic livers (0, 10 cases and 1 case; 1+, 7 and 5 cases; 2+, 5 and 10 cases; 3+, 0 and 6 cases, respectively). Its expression was rather higher in the peribiliary glands than in the lining epithelia of hepatolithiatic livers; B: Epithelial cell adhesion molecule (EpCAM) was frequently expressed in the lining epithelia and peribiliary glands of normal livers (0, 0 and 0 case; 1+, 0 case and 5 cases; 2+, 5 and 14 cases; 3+, 27 and 13 cases, respectively) and hepatolithiatic livers (0, 0 and 0 case; 1+, 0 case and 2 cases; 2+, 1 case and 12 cases; 3+, 21 and 8 cases, respectively). Its expression was slightly higher in the lining epithelia of hepatolithiatic livers; C: CD44s expression varied in the lining epithelia and peribiliary glands of normal livers (0, 22 and 19 cases; 1+, 3 and 5 cases; 2+, 6 and 8 cases; 3+, 1 and 0 case, respectively) and hepatolithiatic livers (0, 3 and 10 case; 1+, 7 and 4 cases; 2+, 11 and 5 cases; 3+, 1 case and 3 cases, respectively). The expression of CD44s in the lining epithelia and peribiliary glands was higher in hepatolithiatic livers than in normal livers and a significant difference was observed in the lining epithelia ($P < 0.01$); D: The expression of chemokine receptor type 4 (CXCR4) was rare in the lining epithelia and peribiliary glands of normal livers (0, 30 and 30 cases; 1+, 0 and 1 case; 2+, 2 cases and 1 case; 3+, 0 and 0 cases, respectively), but was not infrequent in hepatolithiatic livers (0, 11 and 12 cases; 1+, 7 and 7 cases; 2+, 4 and 3 cases; 3+, 0 and 0 case, respectively). A significant difference in its expression in the lining epithelia was observed between normal and hepatolithiatic livers ($P < 0.01$).

potential to become pancreatic cells and the biliary tract can be regarded as an incomplete pancreas[1]. Gerber *et al*[19] proposed one hypothesis in which glandular cells within the peribiliary glands could migrate via their conduits and renew or replace the lining epithelia of the bile ducts. Sutton *et al*[20] also showed that peribiliary glands renewed the biliary lining epithelia as a repair process. Taken together, glandular cells in the peribiliary glands may lose PDX1 expression but gain HES1 expression during their migration toward the lining layer. Interestingly, this unique expression pattern of PDX1 and HES1 in the lining epithelia and peribiliary glands was generally retained in hepatolithiatic livers, suggesting that the above-mentioned renewal process may be maintained in hepatolithiasis.

Recent studies have shown multipotent, endoderm S/P cells to be present in human peribiliary glands at all ages[11-13]. In the studies using cell cultures and tissue explants, isolated cells of the peribiliary glands expanded *in vitro* and these cells readily and efficiently showed cell lineages differentiating into liver, the biliary tree or pancreatic cells[11-13]. It was found in this study that the markers of endodermal S/P cells such as EpCAM, CD44s, CXCR4 and SOX9[13,21-23] were variably expressed in the peribiliary glands and also in the lining epithelium of hilar bile ducts, supporting that peribiliary glands are a niche for endodermal S/P cells[11-13]. The different expression patterns of endoderm S/P cell markers may reflect heterogeneous S/P cell components within the peribiliary glands.

Mucin hypersecretion is a frequent finding in cases of hepatolithiasis[15,16]. Mucin secreted from the bile ducts is central to calcium bilirubinate stones. According to our previous studies, gel-forming mucins such as MUC2 and MUC5AC were shown to be important for the development of calcium bilirubinate stones and these mucins were detected in the glands showing the intestinal and gastric metaplasia in the bile ducts and peribiliary glands with hepatolithiasis[14,15]. The increased expression of

endodermal S/P cell markers in the lining epithelia and peribiliary glands in hepatolithiatic livers may be involved in these metaplastic processes[24].

Interestingly, the peribiliary glands were shown to be dense at the hepatic hilar regions, the cystic duct of gallbladder, and the periampullary region, where cholangiocarcinomas (CCs) are likely to occur. Furthermore, CCs and precursor lesions, such as biliary intraepithelial neoplasm and intraductal papillary neoplasm of bile duct (IPNB), are known to develop in the stone-containing bile ducts in hepatolithiasis[25]. This study showed that the expression of these S/P cell markers tended to be higher in the peribiliary glands and also lining epithelia of hepatolithiatic livers than in those of normal livers. CD44s-positive cells were also increased in the lining epithelia and peribiliary glands in hepatolithiasis, while CD44s was not typically expressed in the normal biliary tract. It was reported that CD44s was expressed in CCs[26]. CXCR4 is a chemokine receptor, its interaction with its ligand was reportedly involved in cholangiocarcinogenesis[27] and the frequent expression of CXCR4 in the lining epithelia and peribiliary glands in hepatolithiasis was shown in this study. Taken together, this study suggested that CD44s and CXCR4 may also be related to the neoplastic changes in bile ducts with hepatolithiasis. Our previous study showed that PDX1 expressed in preneoplastic and neoplastic biliary epithelia was related to their proliferative activities; therefore, it also seems likely that PDX1 expression in the peribiliary glands of hepatolithiasis may be related to their proliferation and neoplastic process[6,7,14].

The increased expression of pancreatic and endodermal S/P cell markers in the lining epithelium and peribiliary glands may be also related to the unique features of the neoplastic processes of the biliary tract[28]. Our recent study showed that hilar cholangiocarcinomas shared features with pancreatic duct adenocarcinomas (PDAC)[29]. Furthermore, IPNB, which is known to develop in hepatolithiasis, often shows intestinal or gastric phenotypes[24]. Therefore, it is possible that the lining epithelia and peribiliary glands expressing pancreatic and endodermal S/P cell markers may be related to the similarities of hilar cholangiocarcinoma to PDAC and may be also involved in the intestinal or gastric metaplasia of IPNB.

In conclusion, the peribiliary glands frequently and extensively expressed PDX1 but focally expressed HES1 and this unique expression pattern may be involved in the biliary tract maintenance. The expression of endoderm S/P cell markers in the peribiliary glands and also lining epithelia may be involved in the intestinal and gastric metaplasia occurring in these glands in hepatolithiasis. The lining epithelia and peribiliary glands expressing pancreatic and endodermal S/P cell markers in hepatolithiasis may be also involved in cholangiocarcinogenesis with pancreatic and gastrointestinal phenotypes. Further studies are needed to clarify the exact roles of the peribiliary glands expressing endodermal S/P cell markers in the pathophysiologies of biliary diseases in hepatolithiasis.

COMMENTS

Background

Pancreatic duodenal homeobox factor 1 (PDX1), a transcription factor crucial for the development of the pancreas and a marker of pancreatic progenitor cells, was shown to be expressed in the fetal biliary tract. In contrast, hairy and enhancer of split 1 (HES1), which is also a fetal transcription factor that represses pancreatic exocrine and endocrine differentiation, is also important for the development and differentiation of the biliary tract.

Research frontiers

PDX1 was infrequently and focally expressed in the lining epithelia of normal livers, but was extensively expressed in the peribiliary glands. In contrast, HES1 was extensively expressed in the lining epithelia, although infrequently and usually focally expressed in the peribiliary glands. Taken together, it seems possible that the peribiliary glands expressing PDX1 in adults retain potential properties for the promotion of pancreatic development as seen in the biliary epithelia of the fetal bile duct, while the lining epithelia of the bile duct expressing HES1 keep properties for the inhibition of pancreatic development.

Innovations and breakthroughs

In this study, the authors examined the expression of fetal pancreatic markers (PDX1 and HES1) and endodermal stem/progenitor (S/P) cell markers in the lining epithelia and peribiliary glands immunohistochemically, using 32 normal livers and 22 hepatolithiatic livers, and then tried to evaluate the roles and significance of these markers in the biliary tract pathophysiologies in hepatolithiasis.

Applications

The study's unique combination was also seen in hepatolithiatic livers. The expression of these markers, particularly CD44s and chemokine receptor type 4, increased in the peribiliary glands and lining epithelia in hepatolithiatic livers. This increased expression of endodermal S/P cell markers may be related to the increased production of intestinal and gastric mucin and also to the biliary neoplasia associated with the gastric and intestinal phenotypes reported in hepatolithiasis.

Peer review

The authors have investigated the pathophysiologies of peribiliary glands (currently known to be stem cell niches of pancreas and the biliary tree) in hepatolithiasis by performing immunohistochemistry using a set of fetal pancreatic markers and endoderm S/P cell markers. The present work clearly demonstrated the roles of peribiliary glands as biliary epithelial renewal and metaplasia and supported the previous findings as stem cell niches. It also provides interesting insights into the roles of peribiliary glands in inflammation-associated pancreatic duct cancer and cholangiocarcinoma.

REFERENCES

1 **Nakanuma Y**. A novel approach to biliary tract pathology based on similarities to pancreatic counterparts: is the biliary tract an incomplete pancreas? *Pathol Int* 2010; **60**: 419-429 [PMID: 20518896 DOI: 10.1111/j.1440-1827.2010.02543.x]

2 **Fukuda A**, Kawaguchi Y, Furuyama K, Kodama S, Horiguchi M, Kuhara T, Koizumi M, Boyer DF, Fujimoto K, Doi R, Kageyama R, Wright CV, Chiba T. Ectopic pancreas formation in Hes1 -knockout mice reveals plasticity of endodermal progenitors of the gut, bile duct, and pancreas. *J Clin Invest* 2006; **116**: 1484-1493 [PMID: 16710472 DOI: 10.1172/JCI27704]

3 **Gerrish K**, Gannon M, Shih D, Henderson E, Stoffel M, Wright CV, Stein R. Pancreatic beta cell-specific transcription of the pdx-1 gene. The role of conserved upstream control regions and their hepatic nuclear factor 3beta sites. *J Biol Chem* 2000; **275**: 3485-3492 [PMID: 10652343 DOI: 10.1074/jbc.275.5.3485]

4 **Igarashi S**, Matsubara T, Harada K, Ikeda H, Sato Y, Sasaki M, Matsui O, Nakanuma Y. Bile duct expression of pancreatic and duodenal homeobox 1 in perihilar cholangiocarcinogenesis. *Histopathology* 2012; **61**: 266-276 [PMID: 22594685 DOI: 10.1111/j.1365-2559.2012.04218.x]

5 **Sumazaki R**, Shiojiri N, Isoyama S, Masu M, Keino-Masu K, Osawa M, Nakauchi H, Kageyama R, Matsui A. Conversion of biliary system to pancreatic tissue in Hes1-deficient mice.

Nat Genet 2004; **36**: 83-87 [PMID: 14702043 DOI: 10.1038/ng1273]
6 **Nakanuma Y**, Hoso M, Sanzen T, Sasaki M. Microstructure and development of the normal and pathologic biliary tract in humans, including blood supply. *Microsc Res Tech* 1997; **38**: 552-570 [PMID: 9330346]
7 **Nakanuma Y**, Zen Y, Portman BC. Diseases of the bile ducts. In: Burt AD, Portman BC, Ferrell LD. MacSween's Pathology of the Liver. Edinburg: Churchill Livingstone, 2011: 491- 562
8 **Terada T**, Nakanuma Y, Ohta G. Glandular elements around the intrahepatic bile ducts in man; their morphology and distribution in normal livers. *Liver* 1987; **7**: 1-8 [PMID: 3553822 DOI: 10.1111/j.1600-0676.1987.tb00308.x]
9 **Tsuneyama K**, Kono N, Yamashiro M, Kouda W, Sabit A, Sasaki M, Nakanuma Y. Aberrant expression of stem cell factor on biliary epithelial cells and peribiliary infiltration of c-kit-expressing mast cells in hepatolithiasis and primary sclerosing cholangitis: a possible contribution to bile duct fibrosis. *J Pathol* 1999; **189**: 609-614 [PMID: 10629565]
10 **Lim JH**, Zen Y, Jang KT, Kim YK, Nakanuma Y. Cyst-forming intraductal papillary neoplasm of the bile ducts: description of imaging and pathologic aspects. *AJR Am J Roentgenol* 2011; **197**: 1111-1120 [PMID: 22021503 DOI: 10.2214/AJR.10.6363]
11 **Cardinale V**, Wang Y, Carpino G, Cui CB, Gatto M, Rossi M, Berloco PB, Cantafora A, Wauthier E, Furth ME, Inverardi L, Dominguez-Bendala J, Ricordi C, Gerber D, Gaudio E, Alvaro D, Reid L. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. *Hepatology* 2011; **54**: 2159-2172 [PMID: 21809358 DOI: 10.1002/hep.24590]
12 **Carpino G**, Cardinale V, Onori P, Franchitto A, Berloco PB, Rossi M, Wang Y, Semeraro R, Anceschi M, Brunelli R, Alvaro D, Reid LM, Gaudio E. Biliary tree stem/progenitor cells in glands of extrahepatic and intraheptic bile ducts: an anatomical in situ study yielding evidence of maturational lineages. *J Anat* 2012; **220**: 186-199 [PMID: 22136171 DOI: 10.1111/j.1469-7580.2011.01462.x]
13 **Semeraro R**, Carpino G, Cardinale V, Onori P, Gentile R, Cantafora A, Franchitto A, Napoli C, Anceschi M, Brunelli R, Bosco D, Torrice A, Reid L, Gaudio E, Alvaro D. Multipotent stem/progenitor cells in the human foetal biliary tree. *J Hepatol* 2012; **57**: 987-994 [PMID: 22820480 DOI: 10.1016/j.jhep.2012.07.013]
14 **Nakanuma Y**, Yamaguchi K, Ohta G, Terada T. Pathologic features of hepatolithiasis in Japan. *Hum Pathol* 1988; **19**: 1181-1186 [PMID: 3169727 DOI: 10.1016/S0046-8177(88)80150-3]
15 **Sasaki M**, Ikeda H, Nakanuma Y. Expression profiles of MUC mucins and trefoil factor family (TFF) peptides in the intrahepatic biliary system: physiological distribution and pathological significance. *Prog Histochem Cytochem* 2007; **42**: 61-110 [PMID: 17616258 DOI: 10.1016/j.proghi.2007.02.001]
16 **Terada T**, Nakanuma Y. Pathological observations of intrahepatic peribiliary glands in 1,000 consecutive autopsy livers. II. A possible source of cholangiocarcinoma. *Hepatology* 1990; **12**: 92-97 [PMID: 2165033 DOI: 10.1002/hep.1840120115]
17 **Terada T**, Kida T, Nakanuma Y. Extrahepatic peribiliary glands express alpha-amylase isozymes, trypsin and pancreatic lipase: an immunohistochemical analysis. *Hepatology* 1993; **18**: 803-808 [PMID: 8406353 DOI: 10.1002/hep.1840180409]
18 **Terada T**, Nakanuma Y, Kakita A. Pathologic observations of intrahepatic peribiliary glands in 1000 consecutive autopsy livers. Heterotopic pancreas in the liver. *Gastroenterology* 1990; **98**: 1333-1337 [PMID: 2182371]
19 **Gerber MA**, Thung SN. Liver stem cells and development. *Lab Invest* 1993; **68**: 253-254 [PMID: 8450645]
20 **Sutton ME**, op den Dries S, Koster MH, Lisman T, Gouw AS, Porte RJ. Regeneration of human extrahepatic biliary epithelium: the peribiliary glands as progenitor cell compartment. *Liver Int* 2012; **32**: 554-559 [PMID: 22171992 DOI: 10.1111/j.1478-3231.2011.02721.x]
21 **Furuyama K**, Kawaguchi Y, Akiyama H, Horiguchi M, Kodama S, Kuhara T, Hosokawa S, Elbahrawy A, Soeda T, Koizumi M, Masui T, Kawaguchi M, Takaori K, Doi R, Nishi E, Kakinoki R, Deng JM, Behringer RR, Nakamura T, Uemoto S. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. *Nat Genet* 2011; **43**: 34-41 [PMID: 21113154 DOI: 10.1038/ng.722]
22 **Turner R**, Lozoya O, Wang Y, Cardinale V, Gaudio E, Alpini G, Mendel G, Wauthier E, Barbier C, Alvaro D, Reid LM. Human hepatic stem cell and maturational liver lineage biology. *Hepatology* 2011; **53**: 1035-1045 [PMID: 21374667 DOI: 10.1002/hep.24157]
23 **Antoniou A**, Raynaud P, Cordi S, Zong Y, Tronche F, Stanger BZ, Jacquemin P, Pierreux CE, Clotman F, Lemaigre FP. Intrahepatic bile ducts develop according to a new mode of tubulogenesis regulated by the transcription factor SOX9. *Gastroenterology* 2009; **136**: 2325-2333 [PMID: 19403103 DOI: 10.1053/j.gastro.2009.02.051]
24 **Ikeda H**, Sasaki M, Ishikawa A, Sato Y, Harada K, Zen Y, Kazumori H, Nakanuma Y. Interaction of Toll-like receptors with bacterial components induces expression of CDX2 and MUC2 in rat biliary epithelium in vivo and in culture. *Lab Invest* 2007; **87**: 559-571 [PMID: 17417665 DOI: 10.1038/labinvest.3700556]
25 **Zen Y**, Fujii T, Itatsu K, Nakamura K, Minato H, Kasashima S, Kurumaya H, Katayanagi K, Kawashima A, Masuda S, Niwa H, Mitsui T, Asada Y, Miura S, Ohta T, Nakanuma Y. Biliary papillary tumors share pathological features with intraductal papillary mucinous neoplasm of the pancreas. *Hepatology* 2006; **44**: 1333-1343 [PMID: 17058219 DOI: 10.1002/hep.21387]
26 **Ashida K**, Terada T, Kitamura Y, Kaibara N. Expression of E-cadherin, alpha-catenin, beta-catenin, and CD44 (standard and variant isoforms) in human cholangiocarcinoma: an immunohistochemical study. *Hepatology* 1998; **27**: 974-982 [PMID: 9537436 DOI: 10.1002/hep.510270412]
27 **Ohira S**, Sasaki M, Harada K, Sato Y, Zen Y, Isse K, Kozaka K, Ishikawa A, Oda K, Nimura Y, Nakanuma Y. Possible regulation of migration of intrahepatic cholangiocarcinoma cells by interaction of CXCR4 expressed in carcinoma cells with tumor necrosis factor-alpha and stromal-derived factor-1 released in stroma. *Am J Pathol* 2006; **168**: 1155-1168 [PMID: 16565491 DOI: 10.2353/ajpath.2006.050204]
28 **Kokuryo T**, Yokoyama Y, Nagino M. Recent advances in cancer stem cell research for cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* 2012; **19**: 606-613 [PMID: 22907641 DOI: 10.1007/s00534-012-0542-6]
29 **Gandou C**, Harada K, Sato Y, Igarashi S, Sasaki M, Ikeda H, Nakanuma Y. Hilar cholangiocarcinoma and pancreatic ductal adenocarcinoma share similar histopathologies, immunophenotypes, and development-related molecules. *Hum Pathol* 2013; **44**: 811-821 [PMID: 23134772 DOI: 10.1016/j.humpath.2012.08.004]

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BRIEF ARTICLE

Can non-invasive measurements aid clinical assessment of volume in patients with cirrhosis?

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Abstract

AIM: To evaluate the non-invasive assessments of volume status in patients with cirrhosis.

METHODS: Echocardiography and multifrequency bioimpedance analysis measurements and short synacthen tests were made in 20 stable and 25 acutely decompensated patients with cirrhosis.

RESULTS: Both groups had similar clinical assessments, cortisol response and total body water (TBW), however the ratio of extracellular water (ECW)/TBW was significantly greater in the trunk (0.420 ± 0.004 *vs* 0.404 ± 0.005), and limbs (R leg 0.41 ± 0.003 *vs* 0.398 ± 0.003, $P < 0.05$, and L leg 0.412 ± 0.003 *vs* 0.399 ± 0.003) with decompensated cirrhosis compared to stable cirrhotics, $P < 0.05$). Echocardiogram derived right atrial and ventricular filling and end diastolic pressures and presence of increased left ventricular end diastolic volume and diastolic dysfunction were similar in both groups. The decompensated group had lower systemic blood pressure, mean systolic 101.8 ± 4.3 *vs* 122.4 ± 5.3 and diastolic 58.4 ± 4.1 mmHg *vs* 68.8 ± 3.1 mmHg respectively, $P < 0.01$, and serum albumin 30 (27-33) *vs* 32 (31-40.5) g/L, $P < 0.01$.

CONCLUSION: Decompensated cirrhotics had greater leg and truncal ECW expansion with lower serum albumin levels consistent with intravascular volume depletion and increased vascular permeability.

Key words: Cirrhosis; Bioimpedance; Echocardiography; Extracellular water; Ascites; Cortisol

Core tip: Despite peripheral oedema and ascites patients with cirrhosis may be intravascularly volume deplete and require parenteral fluids to prevent acute kidney injury. We assessed whether non-invasive measurements with multifrequency bioimpedance and echocardiography aided clinical assessment of volume status. Multifrequency bioimpedance showed that patients with decompensated cirrhosis had similar total body water to stable cirrhotics, but with an expanded extracellular volume, suggesting increased vascular permeability. Echocardiography was not helpful in assessing volume status in the two groups, and neither echocardiography nor multifrequency bioimpedance could aid assessment of intravascular volume.

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INTRODUCTION

Cirrhotic patients with progressive liver disease typically develop a hyper-dynamic circulation characterised by increased cardiac output, with reduced systemic vascular resistance with a normal or even low systemic blood pressure[1], and may have an associated cardiomyopathy[2]. Optimizing intravascular volume is essential in managing patients with cirrhosis to avoid acute kidney injury induced by hypovolaemia, and also reduce the risk of developing hepatorenal syndrome (HRS)[3]. Intravascular volume expansion, which is often necessary to treat these patients, can potentially lead to worsening of ascites, pleural effusion or heart failure.

Clinical assessment of volume status in patients with cirrhosis and progressive liver disease may be difficult as patients with ascites and peripheral oedema may still be relatively under filled in terms of intravascular volume, as some 40%-50% of the extracellular fluid volume can be in the microcirculation.

In addition, central venous pressure and pulmonary capillary wedge pressure often used to measuring static haemodynamics are not reliable markers of circulatory volume[4,5].

Other techniques for assessing volume status, include, inferior vena caval diameter and cardiac end diastolic volumes as measured by echocardiography[6-8], although experience with these static monitoring measurements of volume have not been generally translated into daily clinical practice[9].

Recently multifrequency bioelectrical impedance analysis (MF-BIA) has become available, which measures total body water and compartmental volumes by passing a series of different electrical currents and electrical frequencies through the body. We therefore compared volume assessment of patients with standard 2-dimensional transthoracic echocardiography with MF-BIA.

MATERIALS AND METHODS

Patients

Twenty patients with cirrhosis with chronic decompensation but stable liver function being assessed for potential liver transplant work up or transjugular intrahepatic porto-systemic shunting (TIPS) were evaluated along with 25 patients with acute decompensation on a background of cirrhosis, who had been admitted to hospital as acute emergencies, due to acute variceal haemorrhage, spontaneous bacterial peritonitis, sepsis and hypovolaemia secondary to diarrhoea. Plasma cortisol was measured prior to and at 30 min following 250 μg of synacthen.

Methods

All patients had MF-BIA, where assessments were made in the supine position, using an eight hand and feet tactile electrode system (Biospace in body 720, Seoul, South Korea)[10,11]. No patient had a peripheral amputation, or cardiac pacemaker/defibrillator, and no female patient was pregnant. Height was measured by a standard wall mounted measure (Sigmeas 1, Doherty signature range, www.mediclick.co.uk), and weight by calibrated scales (MPSS250, Marsden, Henley on Thames, United Kingdom). MF-BIA measurements were repeated three times over 30 min to determine reliability of measurements.

Standard 2-dimensional transthoracic echocardiograms (Philips IE33, Philips Medical Systems, Eindhoven, the Netherlands) with measurement of inferior vena cava width and collapsibility were recorded and analysed offline by a single experienced observer. Left ventricular volumes and ejection fraction were estimated using Simpson's modified biplane method[12]. Ethical approval was granted by the local ethical committee as audit and clinical service development.

Statistical analysis

Statistical analysis was by student's *t* test for normally distributed data and Mann Whitney *U* test for nonparametric data (GraphPad Prism version 6.0, San Diego, United States). In addition χ^2 analysis with correction for small numbers and one way anova with Tukey post analysis correction were also performed using SPSS software for Windows version 15.0 (SPSS Inc., Univ Chicago, Illinois, United States), and agreement of repeated MF-BIAs by Bland Altman analysis and Pearson correlation (Analyse It, Leeds, United Kingdom). Data are expressed as mean ± SE of the mean, median and inter-quartile range, or percentages. Statistical significance was taken at or below the 5% level.

RESULTS

Twenty patients, mean age 53.4 ± 1.5 years, 60% male with compensated cirrhosis (9 hepatitis C, 6 alcohol related, 2 primary biliary cirrhosis, 1 each of hepatitis B, non-alcoholic steatosis and cryptogenic) of whom additionally 8 had primary hepatocellular carcinoma being assessed for liver transplant or TIPS insertion, had their volume assessed clinically and also by 2 dimensional echocardiography and MF-BIA (Table 1). We compared these volume assessments with those from 25 acutely decompensated cirrhotic patients (underlying chronic liver disease due to 13 alcohol, 6 hepatitis C, 4 non-alcoholic steatosis, and 1 each of autoimmune and primary biliary cirrhosis), mean age 53.9 ± 2.7 years, 64% male. Fourteen of these patients had decompensation precipitated by acute variceal haemorrhage, 6 spontaneous bacterial peritonitis, 3 with other sources of sepsis and 2 with acute dehydration and hypovolaemia secondary to diarrhoea. Ten/twenty five were encephalopathic at presentation. However, clinical grading of encephalopathy was similar for both the decompensated and compensated groups, median 2 (1-2) *vs* 2 (1-2.5) respectively. Similarly clinical assessment of ascites was similar for both groups, 65% of the compensated group and 62% of the decompensated group, with both groups having a median ascites grading of 2 (1-3) *vs* 2 (1-3). Twenty six percent of the decompensated

Table 1 Demographics and standard biochemistry and haematology results in the stable cirrhotic and the acutely decompensated cirrhotic patients

	Compensated	Decompensated
Age (yr)	53.4 ± 1.5	53.9 ± 2.7
Male sex	60%	64%
Weight (kg)	75.2 ± 1.9	76.1 ± 3.2
Sodium (mmol/L)	135.8 ± 1.4	135.9 ± 1.3
Urea (mmol/L)	4.8 (3.4-6.7)	6.1 (2.9-13.4)
Creatinine (μmol/L)	70 (48-81)	65 (45-116)
Albumin (g/L)	32 (31-40.5)	30 (27-33)[b]
Bilirubin (μmol/L)	36.5 (21-98)	41 (27.5-100.5)
ALT (U/L)	44 (26-55)	31 (19-51)
AST (U/L)	65 (44-98)	63 (46-112)
GGT (U/L)	63 (30-188)	118 (41-263)
ALP (U/L)	117.8 ± 9.4	132.7 ± 14.8
Haemoglobin (g/L)	119 ± 6	101 ± 2[a]
WBC ($\times 10^9$/L)	5.31 ± 0.4	9.5 ± 1.5[a]
Platelets ($\times 10^9$/L)	92.5 (57.5-134)	84 (53.5-143)
INR	1.58 ± 0.11	1.72 ± 0.08
aPTT s	36.7 ± 2.9	36.3 ± 1.6
Cortisol (μmol/L)	361 (180-483)	361 (208-611)
Synacthen 30 min cortisol (μmol/L)	614 (411-719)	518 (399-767)

Results expressed as mean ± SE, or as percentage, or median (interquartile range). [a]$P < 0.05$, [b]$P < 0.01$ *vs* compensated cirrhotic group. ALT: Alanine aminotranferease; AST: Aspartate aminotransferase; GGT: Gamma glutamyl transpeptidase; ALP: Alkaline phosphatase; WBC: White blood cells; INR: International normalized ratio.

Table 2 Blood pressure and echocardiographic findings in stable cirrhotic and the acutely decompensated cirrhotic patients

	Compensated	Decompensated
Systolic blood pressure (mmHg)	122.4 ± 5.3	101.8 ± 4.3[b]
Diastolic blood pressure (mmHg)	68.8 ± 3.1	58.4 ± 4.1[a]
Heart rate min^{-1}	74.7 ± 3.4	100.3 ± 6.7[b]
Right atrial pressure (mmHg)	6.1 ± 0.5	5.0 ± 0.1
Ejection fraction (%)	59.1 ± 1.0	59.6 ± 0.9
RVESP (mmHg)	31.1 ± 2.3	32.2 ± 2.5
PVFV (m/s)	1.24 ± 0.1	1.22 ± 0.1
LVEDV (mL)	165.5 ± 10.3	149.9 ± 12.9
Vaortic (m/s)	1.54 ± 0.06	1.60 ± 0.09

Right ventricular end systolic pressure (RVESP) normal, 25 mmHg, pulmonary valve flow velocity (PVFV), left ventricular end diastolic volume (LVEDV) normal 78-128 mL, aortic valve flow velocity (Vaortic). Results expressed as mean ± SE, [a]$P < 0.05$, [b]$P < 0.01$ *vs* compensated cirrhotic group.

group had clinical signs of peripheral oedema, although 65% were thought to be hypovolemic, whereas the compensated group were recorded as being euvolaemic. Fifty five percent of patients in the compensated group were prescribed diuretics, 8 spironolactone and 8 loop diuretics, compared to 45% of the decompensated group had been prescribed diuretics, 9 spironolactone and 5 loop diuretics. Ten patients in the decompensated group were on inotropic support (5 terlipressin, 5 norepinephrine).

The MELD and Child Turcotte Pugh scores were greater in the decompensated group, median 18.0 (14.5-23.5) *vs* 12.5 (8.5-24.5) and 9.6 ± 0.4 *vs* 8.4 ± 0.4, but not statistically significantly different. Thirty five percent of those with stable chronic liver had Child Turcotte Pugh scores 4-6, compared to 8% of the decompensated group, 30% had scores of 7-9, compared to 40% of the decompensated group, 30% had scores of 10-12 compared to 44% of the decompensated group, and 5% had a score of > 12, compared to 8% in the decompensated group. Serum albumin was lower in the decompensated group, as was haematocrit and peripheral total white cell count was greater. Despite inotropic support blood pressure was lower and heart rate increased in the decompensated group (Table 2). Baseline plasma cortisol levels were not different (Table 1), and 53% of both groups had a baseline cortisol of < 280 μmol/L[11]. Following a short synacthen test, the 30 min cortisol was not different (Table 1), with a similar rise in both the compensated, 190 (170-361) μmol/L and decompensated groups, 188 (125-239) μmol/L, with a rise of < 250 μmol/L in 63% of the compensated group and 84% in the decompensated group ($\chi^2 = 1.22$, $P = 0.269$).

In keeping with clinical assessment of the jugular venous pulse wave transthoracic echocardiographic measured right atrial filling pressures were not elevated. Whereas the right atrium was mildly dilated in the majority of the compensated group, all patients in the decompensated group had a dilated right atrium, with most having moderate to severe dilatation. 85% of the compensated group, and 64% of the decompensated group had evidence of diastolic dysfunction on echocardiography with an early to late atrial filling ratio (E/A) of less then one. Inferior vena cava width was < 1.5 cm in 70% of the compensated group, and between 1.5 and 2.5 cm in the remainder, whereas it was less than 1.5 cm in all those measured in the decompensated group, but in all cases moved normally with respiration, showing normal collapse.

MF-BIA showed that both groups of patients had excess total body water, with mean ratio of ECW/TBW in the decompensated group above the 95% confidence limit for a healthy population. However segmental analysis showed normal hydration status in the arms, but increased extracellular fluid in the trunk and legs. In addition although total body water was not different between the groups, the ratio of extracellular water (ECW) to total body water (TBW) was greater for the decompensated group, in particular for both the trunk and legs (Table 3). Repeated MF-BIA were very reproducible with minimum differences on repeated measurements (Table 4).

We then compared those patients who on clinical examination were thought to have ascites and those with moderate to severe ascites. There was no difference in patient weights, or total body water, intracellular or extracellular water measured by MF-BIA (Table 5). Similarly there was no difference in the ratio of ECW in the arms to total ECW, but patients with ascites had greater amounts of ECW in the trunk and legs (Table 5). Subdividing the decompensated group into those with moderate to severe ascites and those with no or mild ascites,

Table 3 Multi-frequency bioelectrical impedance analysis data in the stable cirrhotic and the acutely decompensated cirrhotic patients

	Compensated	Decompensated
Total body water ICW (L)	39.2 ± 1.9	40.7 ± 1.9
ICW (L)	23.6 ± 1.2	23.9 ± 1.1
ECW (L)	15.7 ± 0.7	16.8 ± 0.8
Total body ECW/TBW	0.399 ± 0.004	0.412 ± 0.003
R arm ECW/TBW	0.385 ± 0.001	0.388 ± 0.002
L arm ECW/TBW	0.386 ± 0.002	0.387 ± 0.003
Trunk ECW/TBW	0.404 ± 0.005	0.420 ± 0.004[a]
R leg ECW/TBW	0.398 ± 0.003	0.410 ± 0.003[a]
L leg ECW/TBW	0.399 ± 0.003	0.412 ± 0.003[a]

Mean extracellular water (ECW)/total body water (TBW) ratio for total body, limb and trunk value in normal healthy humans 0.38 (95% confidence limits 0.36-0.4). Results expressed as mean ± SE, [a]$P < 0.05$ *vs* compensated cirrhotic group. ICW: Intracellular water.

Table 4 Reliability of multi-frequency bioelectrical impedance assessments

Assessment	1st	2nd	3rd
%ECW/TBW	40.7 ± 0.26	40.8 ± 0.21	40.8 ± 0.22
Bias (95%CI)	0.14 (-0.16-0.44)	0.12 (-0.23-0.46)	-0.03 (-0.16-0.00)
Pearson *r*	0.81	0.77	0.96
Pearson *P*	< 0.001	< 0.001	< 0.001

Results expressed as mean ± SE, comparison by Bland Altman bias with 95%CI and Pearson correlation. 1st assessment *vs* 2nd and 3rd, and 2nd *vs* 3rd. ECW: Extracellular water; TBW: Total body water.

then although the ECW/TBW ratio for those with moderate to severe ascites, was greater (total 0.416 ± 0.004 *vs* 0.405 ± 0.005, trunk 0.425 ± 0.004 *vs* 0.409 ± 0.007, right leg 0.413 ± 0.003 *vs* 0.403 ± 0.004, and left leg 0.414 ± 0.004 *vs* 0.408 ± 0.005), with these smaller patient groups these values were no significant ($P = 0.27$ to 0.063).

We also divided the decompensated group into those in whom decompensation was primarily following variceal haemorrhage or dehydration, and those in whom decompensation was primarily due to infection (spontaneous bacterial peritonitis and pneumonia). The mean ECW/TBW on admission in the variceal haemorrhage group was 0.417 ± 0.005, which was lower but not statistically different from the sepsis group, 0.422 ± 0.006.

DISCUSSION

Clinical examination of the two groups was not significantly different in terms of ascites and jugular venous pulse wave, although the majority of the decompensated group were thought to be clinically hypovolemic. Although peripheral systolic and diastolic blood pressure was lower and heart rate greater in the decompensated group, both groups had similar basal cortisol levels, and also following a synacthen challenge. It has been suggested that for critically ill patients that the baseline cortisol should be > 280 μmol/L, and an appropriate response > 250 μmol/L[13]. In our cohort around 53% of both

Table 5 Multi-frequency bioelectrical impedance analysis measurements in those patients with no clinically detectable ascites those patients with moderate to severe ascites judged clinically

	No ascites	Moderate/severe ascites
Weight (kg)	75.1 ± 3.9	75.4 ± 3.0
Total body water (L)	39.4 ± 1.8	37.9 ± 1.9
ICW (L)	23.71 ± 0.66	22.29 ± 1.12
ECW (L)	15.66 ± 0.66	15.65 ± 0.76
Total body ECW/TBW	0.399 ± 0.004	0.413 ± 0.003[a]
R arm ECW/TBW	0.384 ± 0.002	0.386 ± 0.002
L arm ECW/TBW	0.385 ± 0.0021	0.385 ± 0.003
Trunk ECW/TBW	0.403 ± 0.005	0.421 ± 0.003[a]
R leg ECW/TBW	0.398 ± 0.004	0.410 ± 0.003[a]
L leg ECW/TBW	0.400 ± 0.004	0.413 ± 0.003[a]

Results expressed as mean ± SE, [a]$P < 0.05$ *vs* No ascites group. ICW: Intracellular water; ECW: Extracellular water; TBW: Total body water.

groups had a relatively low baseline cortisol, and an inappropriate response to synacthen in 63% and 84%. Thus it would appear that the differences in blood pressure were not due to steroid deficiency. Previous reports have suggested a relationship between cardiac function and severity of liver disease[14], characterised by cardiac dilatation, with increased left atrial diameter, left ventricular end diastolic volume, and increased cardiac output and aortic flow[15]. In our study both groups had a normal mean right atrial pressure and cardiac ejection fraction of > 55%, although some studies have reported reduced cardiac output[16]. As with previous reports of diastolic dysfunction in patients with cirrhosis the majority of both groups had an E/A ratio of < 1.0[17], although no patient had grade 2 diastolic dysfunction. Although an E/A ratio of < 1.0 can be considered a normal finding in the older patient, both groups had a mean age of less than 55 years. Right ventricular end systolic pressure was greater than normal (> 30 mmHg) in 50% of the compensated group and 75% of those measured in the acutely decompensated cirrhotic group, with normal pulmonary valve flow velocity in all cases. Similarly left ventricular end diastolic volumes were greater than normal (> 128 mL) in 80% and 55% respectively, with normal aortic valve flow velocities. Thus the main changes in echocardiography were found estimating right sided cardiac function with the decompensated group having modestly lower right atrial pressures, but with increased atrial dilatation and mildly increased right ventricular end systolic pressures. Whereas, although left ventricular diastolic dysfunction was prominent in both groups, it was not different.

Clinical examination for ascites was similar in both groups, as was total body water, extracellular and intracellular volumes as measured by MF-BIA. Bioimpedance works by passing an electrical current through the body and measuring both the resistance to flow and reactance, and has developed from single current and frequency devices[18,19], to those using multiple currents and frequencies, and from devices which simply record total body values, to those with eight electrodes which can provide

compartmental assessments[20,21], as used in this study. As the resistance to the passage of electricity depends upon circuit length, the majority of resistance occurs in the arms and legs, with much less for the trunk, and earlier reports suggested that intra-abdominal fluid had little effect on bioimpedance measurements[22]. We found that MF-BIA results were highly reproducible and using MF-BIA we could detect significant segmental differences. We found that those patients with moderate to severe ascites had greater ECW volumes in both the trunk and legs. Thus compared to previous reports using single frequency bioimpedance machines, MF-BIA with segmental analysis could show increased ascitic fluid[23]. Similarly both the compensated and decompensated groups had normal hydration status of the upper limbs, however the ratio of extracellular to total body water, a marker of extracellular fluid excess was increased in the trunk and both legs compared to reported data from healthy controls, and was significantly greater for those patients with decompensated cirrhosis, more so for the trunk than the legs. Thus although total body water was similar in both groups, the decompensated group had more fluid in the trunk and legs. This redistribution of fluid is in keeping the clinical assessments which suggested that the majority of decompensated patients were hypovolemic, whereas the compensated group were thought to be euvolaemic. The whole body ratio of ECW/TBW was lower in those patients who had decompensated due to variceal haemorrhage, in keeping with the clinical assessment of volume status, but was not statistically different from those who had decompensated secondary to sepsis. This was a small pilot study and as such the number of subjects many have been too small to show any statistical effect.

Thus patients with both compensated and decompensated cirrhosis had evidence of increased fluid retention in the trunk and legs, despite normal right atrial filling pressures and lack of clinically detectable peripheral oedema, presumably due to increased interstitial fluid formation or reduced removal. Serum albumin concentrations were lower in the decompensated cirrhotic group, and although this could be secondary to reduced synthesis, increased passage of albumin into the extracellular fluid compartment, due to increased vascular permeability could be an alternative explanation, as described in other chronic disease states, such as chronic kidney disease[24-27]. This increased leak and increased extracellular fluid was not associated with changes in cortisol. In keeping with a hyperdynamic circulation there was dilatation of the right atrium, increased end systolic right atrial pressure and increased left ventricular end diastolic volume, but derived right atrial pressure and inferior vena cava diameter were not increased. Single transthoracic echocardiography assessments could not differentiate those with stable chronic liver disease from those acutely admitted to hospital with decompensated cirrhosis, whereas MF-BIA showed that although both groups had similar overall TBW, the decompensated group had increased ECW/TBW, particularly in the legs and trunk, suggesting that plasma volume was decreased. However none of these techniques reliably predicted intravascular volume, and clinical assessment remains a crucial element in the examination of patients with chronic liver disease to determine volume assessment.

COMMENTS

Background

Cirrhotic patients with progressive liver disease typically develop a hyper-dynamic circulation characterised by increased cardiac output, with reduced systemic vascular resistance with a normal or even low systemic blood pressure, and may have an associated cardiomyopathy. Optimizing intravascular volume is essential in managing patients with cirrhosis to avoid acute kidney injury induced by hypovolaemia, and also reduce the risk of developing hepatorenal syndrome. Intravascular volume expansion, which is often necessary to treat these patients, can potentially lead to worsening of ascites, pleural effusion or heart failure.

Research frontiers

Clinical assessment of volume status in patients with cirrhosis and progressive liver disease may be difficult as patients with ascites and peripheral oedema may still be relatively under filled in terms of intravascular volume, as some 40%-50% of the extracellular fluid volume can be in the microcirculation. In addition, central venous pressure and pulmonary capillary wedge pressure often used to measuring static haemodynamics are not reliable markers of circulatory volume. Other techniques for assessing volume status, include, inferior vena caval diameter and cardiac end diastolic volumes as measured by echocardiography, although experience with these static monitoring measurements of volume have not been generally translated into daily clinical practice.

Innovations and breakthroughs

The authors assessed whether non-invasive measurements with multifrequency bioimpedance and echocardiography aided clinical assessment of volume status. Multifrequency bioimpedance showed that patients with decompensated cirrhosis had similar total body water to stable cirrhotics, but with an expanded extracellular volume, suggesting increased vascular permeability. Echocardiography was not helpful in assessing volume status in the two groups, and neither echocardiography nor multifrequency bioimpedance could aid assessment of intravascular volume.

Applications

Decompensated cirrhotics had greater leg and truncal extracellular water expansion with lower serum albumin levels consistent with intravascular volume depletion and increased vascular permeability.

Peer review

This manuscript is very interesting. This paper describes the results of assessment of volume status in patients with cirrhosis.

REFERENCES

1 **Abelmann WH**. Hyperdynamic circulation in cirrhosis: a historical perspective. *Hepatology* 1994; **20**: 1356-1358 [PMID: 7927272]

2 **Møller S**, Henriksen JH. Cirrhotic cardiomyopathy: a pathophysiological review of circulatory dysfunction in liver disease. *Heart* 2002; **87**: 9-15 [PMID: 11751653]

3 **Wong F**, Nadim MK, Kellum JA, Salerno F, Bellomo R, Gerbes A, Angeli P, Moreau R, Davenport A, Jalan R, Ronco C, Genyk Y, Arroyo V. Working Party proposal for a revised classification system of renal dysfunction in patients with cirrhosis. *Gut* 2011; **60**: 702-709 [PMID: 21325171 DOI: 10.1136/gut.2010.236133]

4 **Davenport A**, Ahmad J, Al-Khafaji A, Kellum JA, Genyk YS, Nadim MK. Medical management of hepatorenal syndrome. *Nephrol Dial Transplant* 2012; **27**: 34-41 [PMID: 22287700 DOI: 10.1093/ndt/gfr736]

5 **Marik PE**, Baram M, Vahid B. Does central venous pressure predict fluid responsiveness? A systematic review of the literature and the tale of seven mares. *Chest* 2008; **134**: 172-178

[PMID: 18628220 DOI: 10.1378/chest.07-2331]
6 **Mintz GS**, Kotler MN, Parry WR, Iskandrian AS, Kane SA. Reat-time inferior vena caval ultrasonography: normal and abnormal findings and its use in assessing right-heart function. *Circulation* 1981; **64**: 1018-1025 [PMID: 7285290]
7 **Diebel LN**, Wilson RF, Tagett MG, Kline RA. End-diastolic volume. A better indicator of preload in the critically ill. *Arch Surg* 1992; **127**: 817-821; discussion 821-822 [PMID: 1524482]
8 **Renner J**, Gruenewald M, Brand P, Steinfath M, Scholz J, Lutter G, Bein B. Global end-diastolic volume as a variable of fluid responsiveness during acute changing loading conditions. *J Cardiothorac Vasc Anesth* 2007; **21**: 650-654 [PMID: 17905268]
9 **Singh S**, Kuschner WG, Lighthall G. Perioperative intravascular fluid assessment and monitoring: a narrative review of established and emerging techniques. *Anesthesiol Res Pract* 2011; **2011**: 231493 [PMID: 21785588]
10 **Kumar S**, Khosravi M, Massart A, Potluri M, Davenport A. Changes in upper limb extracellular water content during hemodialysis measured by multi-frequency bioimpedance. *Int J Artif Organs* 2013; **36**: 203-207 [PMID: 23404642 DOI: 10.5301/IJAO.5000190]
11 **Papakrivopoulou E**, Booth J, Pinney J, Davenport A. Comparison of volume status in asymptomatic haemodialysis and peritoneal dialysis outpatients. *Nephron Extra* 2012; **2**: 48-54 [PMID: 22619667 DOI: 10.1159/000337338]
12 **Schiller NB**, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr* 1989; **2**: 358-367 [PMID: 2698218]
13 **Marik PE**, Pastores SM, Annane D, Meduri GU, Sprung CL, Arlt W, Keh D, Briegel J, Beishuizen A, Dimopoulou I, Tsagarakis S, Singer M, Chrousos GP, Zaloga G, Bokhari F, Vogeser M. Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: consensus statements from an international task force by the American College of Critical Care Medicine. *Crit Care Med* 2008; **36**: 1937-1949 [PMID: 18496365 DOI: 10.1097/CCM.0b013e31817603ba]
14 **Nadim MK**, Kellum JA, Davenport A, Wong F, Davis C, Pannu N, Tolwani A, Bellomo R, Genyk YS. Hepatorenal syndrome: the 8th International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2012; **16**: R23 [PMID: 22322077 DOI: 10.1186/cc11188]
15 **Sun FR**, Meng YM, Wang BY, Liu YF, Liu CX, Xie DW, Ding YY, Li JP, Ma L. [Correlations between MELD score and left ventricular function in patients with end-stage liver disease]. *Zhonghua Ganzangbing Zazhi* 2010; **18**: 758-762 [PMID: 21059293 DOI: 10.3760/cma.j.issn.1007-3418.2010.10.009]
16 **Kazankov K**, Holland-Fischer P, Andersen NH, Torp P, Sloth E, Aagaard NK, Vilstrup H. Resting myocardial dysfunction in cirrhosis quantified by tissue Doppler imaging. *Liver Int* 2011; **31**: 534-540 [PMID: 21382164 DOI: 10.1111/j.1478-3231.2011.02468.x]
17 **Rabie RN**, Cazzaniga M, Salerno F, Wong F. The use of E/A ratio as a predictor of outcome in cirrhotic patients treated with transjugular intrahepatic portosystemic shunt. *Am J Gastroenterol* 2009; **104**: 2458-2466 [PMID: 19532126 DOI: 10.1038/ajg.2009.321]
18 **Patel RV**, Matthie JR, Withers PO, Peterson EL, Zarowitz BJ. Estimation of total body and extracellular water using single- and multiple-frequency bioimpedance. *Ann Pharmacother* 1994; **28**: 565-569 [PMID: 8068989]
19 **Davenport A**, Willicombe M. Comparison of fluid status in patients treated by different modalities of peritoneal dialysis using multi-frequency bioimpedance. *Int J Artif Organs* 2009; **32**: 779-786 [PMID: 20020409]
20 **Fürstenberg A**, Davenport A. Comparison of multifrequency bioelectrical impedance analysis and dual-energy X-ray absorptiometry assessments in outpatient hemodialysis patients. *Am J Kidney Dis* 2011; **57**: 123-129 [PMID: 20692749 DOI: 10.1053/j.ajkd.2010.05.022]
21 **Fürstenberg A**, Davenport A. Assessment of body composition in peritoneal dialysis patients using bioelectrical impedance and dual-energy x-ray absorptiometry. *Am J Nephrol* 2011; **33**: 150-156 [PMID: 21293116 DOI: 10.1159/000324111]
22 **Davenport A**. Does peritoneal dialysate affect body composition assessments using multi-frequency bioimpedance in peritoneal dialysis patients? *Eur J Clin Nutr* 2013; **67**: 223-225 [PMID: 23249878 DOI: 10.1038/ejcn.2012.205]
23 **Selberg O**, Selberg D. Norms and correlates of bioimpedance phase angle in healthy human subjects, hospitalized patients, and patients with liver cirrhosis. *Eur J Appl Physiol* 2002; **86**: 509-516 [PMID: 11944099]
24 **Davenport A**, Willicombe MK. Hydration status does not influence peritoneal equilibration test ultrafiltration volumes. *Clin J Am Soc Nephrol* 2009; **4**: 1207-1212 [PMID: 19556380 DOI: 10.2215/CJN.01060209]
25 **Booth J**, Pinney J, Davenport A. N-terminal proBNP--marker of cardiac dysfunction, fluid overload, or malnutrition in hemodialysis patients? *Clin J Am Soc Nephrol* 2010; **5**: 1036-1040 [PMID: 20507952]
26 **Kumar S**, Khosravi M, Massart A, Davenport A. Is there a role for N-terminal probrain-type natriuretic peptide in determining volume status in haemodialysis patients? *Nephron Clin Pract* 2012; **122**: 33-37 [PMID: 23548328 DOI: 10.1159/000348510]
27 **Davenport A**. Changes in N-terminal pro-brain natriuretic peptide correlate with fluid volume changes assessed by bioimpedance in peritoneal dialysis patients. *Am J Nephrol* 2012; **36**: 371-376 [PMID: 23051933 DOI: 10.1159/000343286]

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BRIEF ARTICLE

Noninvasive assessment of liver damage in chronic hepatitis B

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Abstract

AIM: To evaluate the efficacy of the aspartate aminotransferase/platelet ratio index (APRI) and neutrophil-lymphocyte (N/L) ratio to predict liver damage in chronic hepatitis B (CHB).

METHODS: We analyzed 89 patients diagnosed with CHB by percutaneous liver biopsy and 43 healthy subjects. Liver biopsy materials were stained with hematoxylin-eosin and Masson's trichrome. Patients' fibrosis scores and histological activity index (HAI) were calculated according to the Ishak scoring system. Fibrosis score was recognized as follows: F0-1 No /early-stage fibrosis, F2-6 significant fibrosis, F0-4 non-cirrhotic and F5-6 cirrhotic. Significant liver fibrosis was defined as an Ishak score of $\geqslant$ 2. APRI and N/L ratio calculation was made by blood test results.

RESULTS: The hepatitis B and control group showed no difference in N/L ratios while there was a significant difference in terms of APRI scores ($P < 0.001$). Multiple logistic regression analysis revealed that the only independent predictive factor for liver fibrosis in CHB was platelet count. APRI score was significantly higher in cirrhotic patients than in non-cirrhotic patients. However, this significance was not confirmed by multiple logistic regression analysis. The optimum APRI score cut-off point to identify patients with cirrhosis was 1.01 with sensitivity, specificity, positive predictive value and negative predictive value of 62% (36%-86%), 74% (62%-83%), 29% (13%-49%) and 92% (82%-97%), respectively. In addition, correlation analyses revealed that N/L ratio has a negative and significant relationship with HAI ($r = -0.218$, $P = 0.041$).

CONCLUSION: N/L ratio was negatively correlated with HAI. APRI score may be useful to exclude cirrhosis in CHB patients.

Key words: Chronic hepatitis B; Fibrosis; Liver cirrhosis; Noninvasive; Serum markers

Core tip: Due to the limitations of liver biopsy, the use of non-invasive markers has emerged in recent years. The aspartate aminotransferase/platelet ratio index (APRI) is used to determine chronic hepatitis C patients with advanced fibrosis. Neutrophil-lymphocyte (N/L) ratio is higher in patients with advanced fibrosis and considered as a novel non-invasive marker to pre-

dict advanced disease in non-alcoholic steatohepatitis. This study showed that N/L ratio is negatively correlated with HAI in chronic hepatitis B (CHB). APRI score may be useful to exclude cirrhosis in CHB patients.

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INTRODUCTION

Hepatitis B virus (HBV) infection is a major health problem all over the world, and is thought to affect 350-400 million people. Disease can be found in a wide range from inactive carrier state to cirrhosis and hepatocellular carcinoma (HCC)[1]. Disease morbidity and mortality in chronic hepatitis B (CHB) depends on the continuation of viral replication and progression of the disease to cirrhosis and HCC[2]. The goal of treatment is to prevent progression of the disease to advanced stages like cirrhosis and HCC. Establishing the status of hepatic fibrosis is important to decide the treatment[2]. Liver biopsy gives more accurate results about liver damage and fibrosis stage. Low patient compliance because of the invasive nature of liver biopsy, the occurrence of bleeding and pain, as a result of faulty sampling and missing pathological evaluation, differences between pathologists in the evaluation of biopsies and the limited use of biopsy in the monitoring of treatment are the limitations of liver biopsy[3,4]. For these reasons, non-invasive tests are needed to determine liver damage and fibrosis in CHB.

The aspartate aminotransferase/platelet ratio index (APRI) has been used to determine chronic hepatitis C (CHC) patients with advanced fibrosis[5]. APRI also predicts significant fibrosis in CHB[6]. The neutrophil-lymphocyte (N/L) ratio can be calculated easily from complete blood counts and is an easily accessible marker which indicates the state of inflammation in the body. It is considered to evaluate disease prognosis in HCC[7,8]. Alkhouri *et al*[9] found that the N/L ratio is higher in patients with non-alcoholic steatohepatitis (NASH) and advanced fibrosis. They also suggested that the N/L ratio can be used as a novel non-invasive marker to predict advanced disease in NASH. In our study, we evaluated APRI and the N/L ratio, which are cheap and easily accessible markers, to determine hepatic damage and fibrosis in patients with CHB. This study aimed to evaluate the efficacy of the N/L ratio to predict significant fibrosis in CHB for the first time in the literature.

MATERIALS AND METHODS

Study population

This study was conducted between January 2007 and November 2008 at Erciyes University Medical Faculty in the Department of Gastroenterology. We retrospectively analyzed 89 patients diagnosed with CHB by percutaneous liver biopsy. Inclusion criteria were accepted as follows: positive surface antigen of HBV for at least 6 months, HBV DNA $\geqslant$ 2.000 IU/mL, patients with pre-treatment liver biopsies, the lack of HIV, HCV and hepatitis D virus infections, the lack of other liver diseases, lack of HCC and lack of alcohol use. The control group consisted of 43 individuals with normal liver tests without systemic disease. All cases were evaluated for clinical and medical background. Our study was conducted in accordance with the principles of the Helsinki Declaration. Erciyes University's Medical Faculty Ethics Committee approved the study.

Calculation of indirect fibrosis markers

APRI and N/L ratio calculation is made by blood test results at least 1 mo prior to liver biopsy. The APRI score was calculated with the formula (AST/40)/platelet (10^9/L) $\times$ 100[5]. The N/L ratio was calculated using the values of neutrophils and lymphocytes obtained from the patients complete blood counts.

Histopathological assessment

Liver biopsy materials were stained with hematoxylin-eosin and Masson's trichrome. All of the liver biopsies were examined by experienced pathologists. Patients' fibrosis scores and histological activity index (HAI) were calculated according to the Ishak scoring system[10]. Fibrosis score was recognized as follows: F0-1 No/early-stage fibrosis, F2-6 significant fibrosis, F0-4 non-cirrhotic and F5-6 cirrhotic. Significant liver fibrosis was defined as an Ishak score of $\geqslant$ 2. This score is also defined as a histologic indication of treatment[11].

Statistical analysis

MedCalc (Version 9.2.0.1) and IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, United States) softwares were used for all analyses. The Shapiro-Wilk's test was used and histogram and q-q plots were examined to assess the data normality. Accordingly, either an independent samples *t* test or Mann-Whitney *U* tests were used to compare the differences of continuous variables between groups. χ^2 analyses were used to compare the differences of categorical variables. Results are expressed as frequencies and percentages, mean ± SD or median (25^{th} and 75^{th} percentiles). Moreover, univariate and multivariate logistic regression analyses were performed and ORs with 95%CI were calculated in order to indentify the risk factors of significant fibrosis and cirrhosis. Significant variables at a $P < 0.10$ level in univariate analysis were taken to multivariate analysis and backward stepwise elimination was used at a $P < 0.10$ stringency level to identify the independent risk factors of significant fibrosis and cirrhosis. Receiver operating characteristic (ROC) curves were plotted for the N-L ratio and APRI score to detect significant fibrosis and cirrhosis. The areas also, cut-offs were determined for each variable. Sensitivity, specifity, positive predictive rate, negative predictive rate and accuracy rate diagnostic measures were calculated and Kappa

Table 1 Comparison of clinical and laboratory parameters between control and hepatitis B patients groups

Variable	Control (n = 43)	Hepatitis B patients (n = 89)	P value
Gender (female/male)	31 (72.1)/12 (27.9)	39 (43.8)/50 (56.2)	0.004
Age (yr)	35.4 ± 12.64	41.5 ±1 3.02	0.012
Platelet count (10^3 μL)	272.0 (242-342)	179.0 (147-231)	< 0.001
Total bilirubin (mg/dL)	0.6 (0.4-0.8)	0.8 (0.6-1)	0.009
AST (IU/L)	17.0 (14-20)	45.0 (30-68)	< 0.001
ALT (IU/L)	16.0 (11-19)	56.0 (36-111)	< 0.001
AP (IU/L)	69.0 (53-79)	72.0 (61-90)	0.086
GGT (IU/L)	17.0 (14-27)	29.0 (21-50)	< 0.001
Neutrophil count	4.3 (3.4-4.9)	3.4 (2.9-4.7)	0.004
Lymphocyte count	2.1 (1.6-2.4)	2.0 (1.6-2.4)	0.355
N/L	2.1 (1.5-2.8)	1.9 (1.4-2.3)	0.123
APRI score	0.1 (0.1-0.1)	0.6 (0.3-1.1)	< 0.001

Values are expressed as n (%), mean ± SD or median (25^{th}-75^{th} percentiles). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AP: Alkaline phosphatase; GGT: Gamma glutamyl transferase; N/L: Neutrophil-lymphocyte ratio; APRI: Aspartate aminotransferase/platelet ratio index.

tests were performed for the N/L ratio and APRI score for the given cut-off value. Speaman's rank test was used for correlation analysis. A $P < 0.05$ probability level was considered statistically significant.

RESULTS

In this study 89 patients with hepatitis B and 43 healthy subjects with no systemic disease were included as a control group. There were 31 (72%) females and 12 (28%) males in the control group and also 39 (44%) females and 50 (56%) males in the patient group. The demographic and laboratory data of the hepatitis B and control group are summarized in Table 1.

The hepatitis B and control group showed no difference in N/L ratios while there was a significant difference in terms of APRI scores ($P < 0.001$). In addition, as expected, platelet count, AST, ALT and GGT values were significantly different from those of the control group ($P < 0.001$). While platelet count was lower, AST, ALT and GGT levels were higher in the patient group (Table 1).

In CHB patients, when significant fibrosis was compared with early-stage fibrosis, a significant difference in platelet count and INR values was found ($P < 0.05$). Multiple logistic regression analysis revealed that the only independent predictive factor for liver fibrosis in CHB was platelet count. The APRI score was found to be higher in CHB with significant fibrosis but this increase was not found to be statistically significant (Table 2).

Cirrhotic patients were found to be more elderly compared to the non-cirrhotic patients ($P < 0.05$). The APRI score was significantly higher in cirrhotic patients than in non-cirrhotic patients ($P < 0.05$). However, this significance was not confirmed by multiple logistic regression analysis (Table 3).

ROC curve analysis suggested that the optimum APRI score cut-off point to identify patients with cirrhosis was 1.01 with sensitivity, specificity, positive predictive value and negative predictive value of 62% (36%-86%), 74% (62%-83%), 29% (13-49) and 92% (82-97) respectively (Figure 1, Table 4). In general, the accuracy of the APRI score to determine patients with cirrhosis is 72%. In addition, correlation analyses revealed that the N/L ratio has a negative and significant relationship with HAI (r = -0.218, P = 0.041).

DISCUSSION

In CHB patients with cirrhosis the APRI score was significantly higher but this significance was not confirmed by multiple logistic regression analysis. The APRI score was higher in significant fibrosis but it was not statistically significant. While the N/L ratio was not related with significant fibrosis and cirrhosis, it was found to be negatively correlated with HAI in patients with CHB.

Liver biopsy may give valuable data to assess liver histology in CHB disease

Due to the limitations of liver biopsy, the use of non-invasive markers has emerged in recent years[12,13]. In these studies, positive results were obtained with Fibrotest and Fibroscan to determine advanced fibrosis and cirrhosis in patients with CHB and CHC. Studies have conflicting results with regard to the use of APRI score to predict significant fibrosis in CHB patients. In their study Wai *et al*[14] suggest that APRI score, which is used to predict significant fibrosis and cirrhosis in CHC, was not suitable for patients with CHB. They explain this by the presence of a fluctuating course with acute attacks in CHB patients while the progression of fibrosis in CHC is more quiet. Yilmaz *et al*[15] also confirmed this and concluded that in patients with CHC the APRI score showed good accuracy for the assessment of liver fibrosis, but not in those with CHB. In contrast to these findings, Shin *et al*[6] studied a large number of CHB patients and suggested a strong positive linear correlation between fibrosis and APRI. Kim *et al*[16] also concluded that APRI score correlated significantly to fibrosis stage. Güzelbulut *et al*[17] found that the areas under the ROC curves of the APRI score to predict significant fibrosis and cirrhosis were 0.77 and 0.78, respectively. They also mentioned that APRI score is more accurate in the prediction of the absence of both significant fibrosis and cirrhosis with negative predictive values of over 90%. In a recent meta-analysis, Jin *et al*[18] suggested that APRI score showed limited value in predicting CHB related significant fibrosis and cirrhosis and the areas under the ROC curves of APRI score were 0.79 and 0.75, respectively. In our study, we did not find statistically significant relation with APRI score and significant fibrosis. APRI score was significantly higher in cirrhotic patients and the accuracy of APRI score to determine patients with cirrhosis was 72%. In our study, as in that of Güzelbulut *et al*[17], the accuracy of the APRI score in the prediction of the absence of cirrhosis was high with negative predictive values of over 90%. Our study results also showed a statistical association between age and cirrhosis (P = 0.022).

Table 2 Between group comparisons and logistic regression results in chronic hepatitis B patients according to fibrosis stage

Variable	Between group comparisons			Logistic regression analysis	
	No/mild fibrosis (n = 34)	Significant fibrosis (n = 55)	P value	Univariate OR (95%CI)	Multivariate OR (95%CI)
Gender (female/male)	16 (47.1)/18 (52.9)	23 (41.8) /32 (58.2)	0.792	1.2 (0.5-2.9)	-
Age (yr)	40.2 ± 11.7	42.2 ± 13.7	0.473	1.01 (0.9-1.05)	-
HGB	14.4 ± 2.08	14.6 ± 1.8	0.735	1.04 (0.8-1.3)	-
Platelet count (10^3 μL)	203 (176-232)	171 (115-227)	0.010	0.9 (0.9-1)	0.9 (0.9-1)
INR	1.07 ± 0.1	1.13 ± 0.1	0.045	34.5 (1.01-1183.1)	-
Albumine	4.06 ± 0.3	4.01 ± 0.3	0.466	0.6 (0.1-2.3)	-
Total bilirubin (mg/dL)	0.7 (0.5-0.9)	0.8 (0.6-1.1)	0.110	3.05 (0.8-10.8)	-
AST (IU/L)	41.5 (27-73)	47 (32-68)	0.447	1 (0.9-1.01)	-
ALT (IU/L)	57 (33-132)	54 (36-106)	0.866	1 (0.9-1.01)	-
AP (IU/L)	71.5 (62-89)	73 (61-100)	0.630	1.01 (0.9-1.02)	-
GGT (IU/L)	27 (19-48)	36 (23-52)	0.119	1.01 (0.9-1.03)	-
HBV DNA	346 (1.9-1000)	75.7 (1.8-1000)	0.838	1 (0.9-1.01)	-
HBeAg (negative/positive)	30 (88.2)/4 (11.8)	45 (81.8)/10 (18.2)	0.611	1.6 (0.4-5.8)	-
Neutrophil count	3.2 (2.7-4.8)	3.5 (3.04-4.7)	0.569	0.9 (0.6-1.2)	-
Lymphocyte count	1.9 (1.4-2.3)	2 (1.6-2.4)	0.630	0.9 (0.5-1.7)	-
N/L	1.9 (1.3-2.5)	1.8 (1.5-2.2)	0.859	0.8 (0.5-1.3)	-
APRI score	0.5 (0.3-0.9)	0.7 (0.3-1.4)	0.060	1.2 (0.7-2.1)	-

Values are expressed as n (%), mean ± SD or median(25^{th}-75^{th} percentiles). HGB: Hemoglobin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AP: Alkaline phosphatase; GGT: Gamma glutamyl transferase; HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; N/L: Neutrophil-lymphocyte ratio; APRI: Aspartate aminotransferase/platelet ratio index.

Table 3 Between group comparisons and logistic regression results in chronic hepatitis B patients according to cirrhosis

Variable	Between group comparisons			Logistic regression analysis	
	Non-cirrhotic (n = 76)	Cirrhotic (n = 13)	P value	Univariate OR (95%CI)	Multivariate OR (95%CI)
Gender (female/male)	33 (43.4)/43 (56.6)	6 (46.2)/7 (53.8)	0.999	1.1 (0.3-3.6)	-
Age (yr)	40.2 ± 12.3	49.08 ± 15.04	0.022	1.06 (1.01-1.1)	1.06 (1-1.11)
HGB	14.5 ± 2.03	14.6 ± 1.37	0.891	1.0 (0.7-1.39)	-
Platelet Count (103 μL)	190.5 (152-233.5)	152 (117-175)	0.051	0.9 (0.9-1)	-
INR	1.1 ± 0.1	1.1 ± 0.09	0.250	6.3 (0.09-478.1)	-
Albumine	4.04 ± 0.3	3.9 ± 0.4	0.210	0.2 (0.04-2.01)	-
Total Bilirubin (mg/dL)	0.8 (0.6-1)	1 (0.6-1.3)	0.389	2.09 (0.5-8.7)	-
AST (IU/L)	41.5 (28-65)	66 (40-79)	0.078	1 (0.9-1.01)	-
ALT (IU/L)	54 (33-124)	69 (51-97)	0.419	1 (0.9-1.01)	-
AP (IU/L)	72 (61-89.5)	82 (62-116)	0.225	1.02 (1-1.03)	1.02 (1-1.04)
GGT (IU/L)	28 (20-50.5)	47 (26-50)	0.189	1.01 (1-1.02)	-
HBV DNA	269.5 (2-1000)	10.1 (0.4-1000)	0.339	1 (0.9-1.01)	-
HbeAg (negative/positive)	65 (85.5)/11 (14.5)	10 (76.9)/3 (23.1)	0.423	1.7 (0.4-7.4)	-
Neutrophil count	3.4 (2.8-4.7)	3.8 (3.04-4.1)	0.468	1.05 (0.7-1.5)	-
Lymphocyte count	2 (1.6-2.4)	2 (1.5-2.2)	0.493	0.6 (0.2-1.5)	-
N/L	1.8 (1.3-2.2)	2.04 (1.6-2.8)	0.160	1.3 (0.8-2.07)	-
APRI score	0.5 (0.3-1.08)	1.1 (0.7-1.7)	0.047	1.2 (0.8-2.05)	-

Values are expressed as n (%), mean ± SD or median(25^{th}-75^{th} percentiles). HGB: Hemoglobin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AP: Alkaline phosphatase; GGT: Gamma glutamyl transferase; HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; N/L: Neutrophil-lymphocyte ratio; APRI: Aspartate aminotransferase/platelet ratio index.

This is an expecting result because the liver damage increases gradually in proportion to the exposure to HBV infection. Patients with CHB above 40 years can be at increased risk of mortality because of liver disease. This can be explained by the increased cirrhosis rates with older age as a host risk factor[19].

With recent evidence, the APRI score, which is used to predict significant fibrosis and cirrhosis in CHC, did not seem as effective in determining fibrosis and cirrhosis in patients with CHB. This can be attributed to differences in the histopathological findings and course of disease. Regenerative nodules are wider in CHB than in CHC[16]. Piecemeal necrosis is more localized and less severe in CHC than in CHB[16]. Hepatic steatosis is an important factor in CHC histology[20]. Disease progression shows a fluctuating course with acute attacks in CHB patients while the progression of fibrosis in CHC is more quiet[16]. For all these reasons, non-invasive markers shown to be effective in CHC should be validated in CHB before use.

The prognosis of patients, who are infected with HBV, depends on the patient's immune response[21]. The hepatitis B virus can be eliminated with a moderate immune response, whereas an excessive response may result in liver damage. HBV persists in the body due to the low-grade immune response. N/L ratio, which is a cheap and easily accessible marker, shows the body's immune response[9].

Table 4 Statistical diagnostic measures and Kappa test results of neutrophil-lymphocyte and aspartate aminotransferase/platelet ratio score in the detection of significant fibrosis and cirrhosis

Variable	Diagnostic measures					Kappa test	
	SEN (95%CI)	SPE (95%CI)	PPR (95%CI)	NPR (95%CI)	AR (95%CI)	κ	*P* value
Significant fibrosis							
N/L (≤ 2.18)	0.73 (0.59-0.84)	0.41 (0.25-0.59)	0.67 (0.53-0.78)	0.48 (0.29-0.67)	0.61 (0.50-0.71)	0.143	0.174
APRI (> 0.56)	0.65 (0.51-0.78)	0.56 (0.38-0.73)	0.71 (0.56-0.83)	0.50 (0.33-0.67)	0.62 (0.51-0.72)	0.209	0.048
Cirrhosis							
N/L (> 2.58)	0.38 (0.14-0.68)	0.87 (0.77-0.64)	0.33 (0.12-0.62)	0.89 (0.80-0.95)	0.80 (0.70-0.88)	0.238	0.024
APRI (> 1.01)	0.62 (0.36-0.86)	0.74 (0.62-0.83)	0.29 (0.13-0.49)	0.92 (0.82-0.97)	0.72 (0.61-0.81)	0.238	0.011

SEN: Sensitivity; SPE: Specifity; PPR: Positive predictive rate; NPR: Negative predictive rate; AR: Accuracy rate; APRI: Aspartate aminotransferase/platelet ratio index; N/L: Neutrophil-lymphocyte ratio.

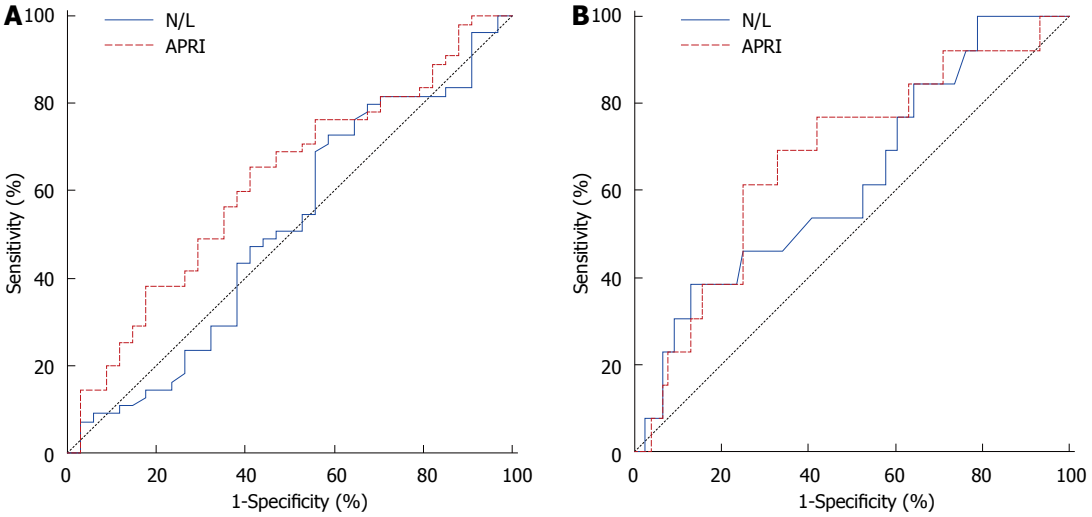

Figure 1 Comparison of receiver operating characteristic curves of neutrophil-lymphocyte ratio and aspartate aminotransferase/platelet ratio index values in identifying significant fibrosis (A) and cirrhosis (B). For significant fibrosis area under receiver operating characteristic (ROC) curves were 0.51 (0.40-0.62) and 0.62 (0.51-0.72) respectively and the differences between two areas were not statistically significant. For cirrhosis, area under ROC curves were 0.62 (0.51-0.72) and 0.67 (0.57-0.77) respectively and the differences between two areas were not statistically significant. N/L: Neutrophil-lymphocyte ratio; APRI: Aspartate aminotransferase/platelet ratio index.

This ratio provides information about two important immune pathways like neutrophils responsible for ongoing inflammation and lymphocytes which have a regulatory role in immune response. Lymphocytes have an impact on liver fibrosis in CHB[22,23]. Alkhouri *et al*[9] showed a relation between N/L ratio and advanced fibrosis in patients with non-alcoholic steatohepatitis. Consequently, N/L ratio may be considered as an important non-invasive marker of liver damage in response to HBV infection.

To our knowledge, our study is the first to evaluate the N/L ratio in CHB disease. In our study, we found a negative and significant relationship between HAI with N/L ratio. This negative relationship demonstrates the important role of lymphocytes in liver damage in CHB. According to our findings fibrosis stage and cirrhosis were not associated with N/L ratio.

All the spectra of biopsies of patients with CHB give rise to the study of the relationship between histological findings with the APRI score and the N/L ratio. The case-control nature of the present study and the number of cases were the limitations of this study.

As with other non-invasive markers APRI and N/L ratio are readily available and inexpensive tests. However, APRI and N/L ratio were not adequate tests to determine either significant fibrosis or cirrhosis in CHB according to our study. For the first time in the literature, this study showed that N/L ratio was negatively correlated with HAI. APRI score may be useful to exclude cirrhosis in CHB patients. Comprehensive and prospective studies are needed to determine the diagnostic value of non-invasive tests for liver damage in CHB.

COMMENTS

Background

Liver biopsy is the standard method to assess liver histology in chronic hepatitis B (CHB) disease. Due to the limitations of liver biopsy, the use of non-invasive markers has emerged in recent years. The aspartate aminotransferase/platelet ratio index (APRI) is used to determine chronic hepatitis C (CHC) patients with advanced fibrosis. Neutrophil-lymphocyte (N/L) ratio is higher in patients with advanced fibrosis and considered as a novel non-invasive marker to predict advanced disease in non-alcoholic steatohepatitis. But up to now, no study evaluated the efficacy of N/L ratio to predict liver damage in CHB.

Research frontiers

The APRI has been used to determine CHC patients with advanced fibrosis. APRI also predicts significant fibrosis in CHB. The N/L ratio can be calculated

easily from complete blood counts and is an easily accessible marker which indicates the state of inflammation in the body. The N/L ratio is higher in patients with non-alcoholic steatohepatitis (NASH) and advanced fibrosis. Also the N/L ratio may be used as a novel non-invasive marker to predict advanced disease in NASH. The research hotspot is to evaluate the N/L ratio to determine hepatic damage and fibrosis in patients with CHB and compare its effectiveness with APRI.

Innovations and breakthroughs

In the present study, the APRI score was significantly higher in CHB patients with cirrhosis. The APRI score was higher in significant fibrosis but it was not statistically significant. While the N/L ratio was not related with significant fibrosis and cirrhosis, it was found to be negatively correlated with HAI in patients with CHB.

Applications

The study results suggest that the N/L ratio is negatively correlated with HAI. APRI score may be useful to exclude cirrhosis in CHB patients.

Terminology

The APRI score was calculated with the formula (AST/40)/platelet (10^9/L) × 100. The N/L ratio was calculated using the values of neutrophils and lymphocytes obtained from the patients complete blood counts.

Peer review

The authors provide an interesting and potentially important manuscript describing noninvasive assessment of liver Fibrosis in CHB. The authors showed that the platelet count is a unique independent predictive factor for liver fibrosis in CHB.

REFERENCES

1 **Franco E**, Bagnato B, Marino MG, Meleleo C, Serino L, Zaratti L. Hepatitis B: Epidemiology and prevention in developing countries. *World J Hepatol* 2012; **4**: 74-80 [PMID: 22489259 DOI: 10.4254/wjh.v4.i3.74]

2 **European Association For The Study Of The Liver**. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; **50**: 227-242 [PMID: 19054588 DOI: 10.1016/j.jhep.2008.10.001]

3 **Cadranel JF**, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). *Hepatology* 2000; **32**: 477-481 [PMID: 10960438]

4 **Standish RA**, Cholongitas E, Dhillon A, Burroughs AK, Dhillon AP. An appraisal of the histopathological assessment of liver fibrosis. *Gut* 2006; **55**: 569-578 [PMID: 16531536 DOI: 10.1136/gut.2005.084475]

5 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]

6 **Shin WG**, Park SH, Jang MK, Hahn TH, Kim JB, Lee MS, Kim DJ, Jun SY, Park CK. Aspartate aminotransferase to platelet ratio index (APRI) can predict liver fibrosis in chronic hepatitis B. *Dig Liver Dis* 2008; **40**: 267-274 [PMID: 18055281 DOI: 10.1016/j.dld.2007.10.011]

7 **Chen L**, Zhang Q, Chang W, Du Y, Zhang H, Cao G. Viral and host inflammation-related factors that can predict the prognosis of hepatocellular carcinoma. *Eur J Cancer* 2012; **48**: 1977-1987 [PMID: 22325840 DOI: 10.1016/j.ejca.2012.01.015]

8 **Halazun KJ**, Hardy MA, Rana AA, Woodland DC, Luyten EJ, Mahadev S, Witkowski P, Siegel AB, Brown RS, Emond JC. Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma. *Ann Surg* 2009; **250**: 141-151 [PMID: 19561458 DOI: 10.1097/SLA.0b013e3181a77e59]

9 **Alkhouri N**, Morris-Stiff G, Campbell C, Lopez R, Tamimi TA, Yerian L, Zein NN, Feldstein AE. Neutrophil to lymphocyte ratio: a new marker for predicting steatohepatitis and fibrosis in patients with nonalcoholic fatty liver disease. *Liver Int* 2012; **32**: 297-302 [PMID: 22097893 DOI: 10.1111/j.1478-3231.2011.02639.x]

10 **Ishak K**, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696-699 [PMID: 7560864 DOI: 10.1016/0168-8278(95)80226-6]

11 **Papatheodoridis GV**, Manesis EK, Manolakopoulos S, Elefsiniotis IS, Goulis J, Giannousis J, Bilalis A, Kafiri G, Tzourmakliotis D, Archimandritis AJ. Is there a meaningful serum hepatitis B virus DNA cutoff level for therapeutic decisions in hepatitis B e antigen-negative chronic hepatitis B virus infection? *Hepatology* 2008; **48**: 1451-1459 [PMID: 18924246 DOI: 10.1002/hep.22518]

12 **Castera L**, Pinzani M. Non-invasive assessment of liver fibrosis: are we ready? *Lancet* 2010; **375**: 1419-1420 [PMID: 20417845 DOI: 10.1016/S0140-6736(09)62195-4]

13 **Poynard T**, Ngo Y, Munteanu M, Thabut D, Ratziu V. Noninvasive Markers of Hepatic Fibrosis in Chronic Hepatitis B. *Curr Hepat Rep* 2011; **10**: 87-97 [PMID: 21654911 DOI: 10.1007/s11901-011-0096-0]

14 **Wai CT**, Cheng CL, Wee A, Dan YY, Chan E, Chua W, Mak B, Oo AM, Lim SG. Non-invasive models for predicting histology in patients with chronic hepatitis B. *Liver Int* 2006; **26**: 666-672 [PMID: 16842322]

15 **Yilmaz Y**, Yonal O, Kurt R, Bayrak M, Aktas B, Ozdogan O. Noninvasive assessment of liver fibrosis with the aspartate transaminase to platelet ratio index (APRI): Usefulness in patients with chronic liver disease: APRI in chronic liver disease. *Hepat Mon* 2011; **11**: 103-106 [PMID: 22087126]

16 **Kim BK**, Kim SA, Park YN, Cheong JY, Kim HS, Park JY, Cho SW, Han KH, Chon CY, Moon YM, Ahn SH. Noninvasive models to predict liver cirrhosis in patients with chronic hepatitis B. *Liver Int* 2007; **27**: 969-976 [PMID: 17696936 DOI: 10.1111/j.1478-3231.2007.01519.x]

17 **Güzelbulut F**, Sezıklı M, Akkan-Çetınkaya Z, Yaşar B, Özkara S, Kurdaş-Övünç AO. AST-platelet ratio index in the prediction of significant fibrosis and cirrhosis in patients with chronic hepatitis B. *Turk J Gastroenterol* 2012; **23**: 353-358 [PMID: 22965506 DOI: 10.4318/tjg.2012.0348]

18 **Jin W**, Lin Z, Xin Y, Jiang X, Dong Q, Xuan S. Diagnostic accuracy of the aspartate aminotransferase-to-platelet ratio index for the prediction of hepatitis B-related fibrosis: a leading meta-analysis. *BMC Gastroenterol* 2012; **12**: 14 [PMID: 22333407 DOI: 10.1186/1471-230X-12-14]

19 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]

20 **Sebastiani G**, Vario A, Guido M, Alberti A. Sequential algorithms combining non-invasive markers and biopsy for the assessment of liver fibrosis in chronic hepatitis B. *World J Gastroenterol* 2007; **13**: 525-531 [PMID: 17278217]

21 **Lohse AW**, Weiler-Normann C, Tiegs G. Immune-mediated liver injury. *J Hepatol* 2010; **52**: 136-144 [PMID: 19913936 DOI: 10.1016/j.jhep.2009.10.016]

22 **Jin Z**, Sun R, Wei H, Gao X, Chen Y, Tian Z. Accelerated liver fibrosis in hepatitis B virus transgenic mice: involvement of natural killer T cells. *Hepatology* 2011; **53**: 219-229 [PMID: 21140473 DOI: 10.1002/hep.23983]

23 **Mohamadkhani A**, Bastani F, Sotoudeh M, Sayehmiri K, Shahnazari P, Montazeri G, Poustchi H. Influence of B cells in liver fibrosis associated with hepatitis B virus harboring basal core promoter mutations. *J Med Virol* 2012; **84**: 1889-1896 [PMID: 23080492 DOI: 10.1002/jmv.23410]

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BRIEF ARTICLE

Serum complement C4a and its relation to liver fibrosis in children with chronic hepatitis C

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Abstract

AIM: To evaluate serum complement C4a and its relation to liver fibrosis in children with chronic hepatitis C virus (HCV) infection.

METHODS: The study included 30 children with chronic HCV infection before receiving antiviral therapy. Chronic HCV infection was defined by positive anti-HCV, a positive polymerase chain reaction for HCV-RNA for more than 6 mo with absence of any associated liver disease. A second group of 30 age- and sex-matched healthy children served as controls. Serum C4a levels were measured by enzyme-linked immunosorbent assay. Liver fibrosis stage and inflammatory grade were assessed using Ishak scoring system. Serum C4a levels were compared according to different clinical, laboratory and histopathological parameters. Statistical significance for quantitative data was tested by Mann-Whitney *U* non-parametric tests. For qualitative data, significance between groups was tested by χ^2 test. Correlation was tested by Spearman's test. Results were considered significant if P value $\leqslant 0.05$.

RESULTS: The age of the patients ranged from 3.5 to 18 years and that of controls ranged from 4 to 17 years. C4a mean levels were merely lower in patients (153.67 ± 18.69 mg/L) than that in the controls (157.25 ± 11.40 mg/L) with no statistical significance (P = 0.378). It did not differ significantly in patients with elevated *vs* those with normal transaminases (152.25 ± 16.62 *vs* 155.36 ± 21.33; P = 0.868) or with different HCV viremia (P = 0.561). Furthermore, there was no statistical significant difference in serum levels between those with no/mild fibrosis and those with moderate fibrosis (154.65 ± 20.59 *vs* 152.97 ± 17.72; P = 0.786) or minimal and mild activity (155.1 ± 21.93 *vs* 152.99 ± 17.43; P = 0.809). Though statistically not significant, C4a was highest in fibrosis score 0 (F0), decreasing in F1 and F2 to be the lowest in F3. When comparing significant fibrosis (Ishak score $\geqslant 3$) *vs* other stages, C4a was significantly lower in F3 compared to other fibrosis scores (143.55 ± 2.33 mg/L *vs* 155.26 ± 19.64 mg/L; P = 0.047) and at a cutoff value of less than 144.01 mg/L, C4a could discriminate F3 with 76.9% sensitivity and 75% specificity from other stages of fibrosis.

CONCLUSION: Serum complement C4a did not correlate with any of transaminases, HCV viremia or with the histopathological scores. Although C4a decreased with higher stages of fibrosis, this change was not significant enough to predict individual stages of fibrosis. Yet, it could predict significant fibrosis with acceptable clinical performance.

Key words: Children; Hepatitis C virus; Complement C4a; Liver biopsy; Liver fibrosis

Core tip: Non-invasive prediction of liver fibrosis is a challenging issue especially in pediatric population. Complement C4a was found, by proteomic analysis, to be associated with liver fibrosis and therefore proposed as a candidate for fibrosis prediction. In adults, serum C4 was found to decrease in hepatitis C virus (HCV) patients with moderate fibrosis and cirrhosis compared to healthy controls. In addition, in advanced HCV-induced liver fibrosis, the net production of C4a was found to be down-regulated. Furthermore, it was found to correlate negatively with alanine transaminase and the histological activity index of the Knodell scoring system. The issue has never been investigated in children before.

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INTRODUCTION

Hepatitis C virus (HCV) is a serious health problem affecting more than 170 million people worldwide[1]. It establishes a chronic infection in up to 85% of cases[2]. Estimates range from less than 1.0% in northern Europe to more than 2.9% in northern Africa[3]. In children younger than 11 years, worldwide seroprevalence of HCV is 0.2% and in those older than 11 years it is 0.4%[1]. Egypt reports the highest prevalence worldwide ranging from 8.7% in upper Egypt to 24.3% in lower Egypt with an average of 13.8%[2,4]. The main (90%) HCV genotype is type 4. Studies of the magnitude of HCV infection in Egyptian children revealed a prevalence of 3% in upper Egypt and 9% in lower Egypt[5].

HCV causes intrahepatic lobular inflammation resulting in fibrosis and eventually cirrhosis[6]. Fibrosis prediction is an essential part of the assessment and management of patients with chronic HCV, worsening of which is probably the best surrogate marker for progression of chronic liver disease[7]. Liver biopsy represents the gold-standard for evaluating fibrosis; however, developing non-invasive tests that can predict liver fibrosis, especially in pediatric population, represents a growing medical need[8].

Conventional biochemical and serological tests are of little value for diagnosis of the degree of liver fibrosis. However, a liver biopsy is sometimes of questionable value because of the heterogeneous distribution of pathological changes in the liver[9]. Blood-based biomarkers offer a number of advantages including safety, cost-savings and widespread accessibility. Although liver biopsy is the gold-standard, it can have life-threatening complications in both adults and children[10,11].

Current serum biomarkers of fibrosis include indirect measures of fibrosis (such as transaminases and platelet count) or direct measures of fibrinogenesis or fibrinolysis (such as hyaluronic acid)[12]. The serum also contains all tissue proteins as leakage markers. Since the liver makes many serum proteins, it is logical to expect that the serum proteome may reflect liver disease[13].

A recent study, using proteomic analysis of serum from adult patients with chronic HCV infection, revealed that complement C4a was a candidate to predict liver fibrosis[14]. Complement C4 is a polymorphic serum protein consisting of two isoforms, C4a and C4b. C4 is expressed primarily in the liver and in macrophages, and its expression is induced in response to acute inflammation or tissue injury[15]. In adults, serum C4 was found to decrease in HCV patients with moderate fibrosis and cirrhosis compared to healthy controls. But in advanced HCV-induced liver fibrosis, the net production of C4a was found to be down-regulated[16]. Furthermore, it was found to correlate negatively with alanine transaminase (ALT) and the histological activity index of the Knodell scoring system[17]. The issue was not investigated in children before.

We aimed to evaluate serum C4a and its relation to liver fibrosis in children with chronic HCV infection.

MATERIALS AND METHODS

Study population

The study included 30 children with chronic hepatitis C recruited from outpatients and inpatients of Pediatric Hepatology department, National Liver Institute, Menofiya University. Diagnosis was based on serological and virological tests; complete blood count (CBC), liver function tests (LFTs), prothrombin time, anti-HCV antibody (Ab), qualitative and quantitative polymerase chain reaction (PCR) for HCV-RNA. Histopathological findings in liver biopsies, the grade of inflammatory activity and the stage of the disease were also evaluated. A second group of 30 healthy children, served as controls. A signed informed consent was obtained from the parents of all the patients and controls before enrollment in the study. The study was approved by the Research Ethics Committee of the National Liver Institute.

Etiological diagnosis

Chronic HCV infection was defined by positive anti-HCV, a positive PCR for HCV-RNA for more than 6 mo, negative hepatitis B viral markers and absence of any associated liver disease. This was supported by the histopathological features of HCV infection in liver biopsy. Patients with decompensated liver disease or cirrhosis were excluded from the study. Control group were defined by apparently healthy individuals with no signs or symptoms of liver disease or any other diseases, normal liver transaminases and negative anti-HCV Ab.

Table 1 Clinical, laboratory and histopathological characteristics of the studied patients *n* (%)

Parameter	HCV patients (*n* = 30)
Clinical findings	
Jaundice	0 (0.0)
Hepatomegaly	4 (13.3)
Splenomegaly	1 (3.3)
Ascites	0 (0.0)
Liver function tests	
Total bilirubin (mg/dL)	1.23 ± 1.051
Direct bilirubin (mg/dL)	0.30 ± 0.26
Albumin (g/L)	43.17 ± 7.5
Alanine transaminase (U/L)	55.57 ± 126.16
Aspartate transaminase (U/L)	72.10 ± 131.97
Gamma glutamyl transpeptidase (U/L)	38.90 ± 21.92
Alkaline phosphatase (U/L)	253.48 ± 97.38
Complete blood count	
Hemoglobin (g/L)	113.6 ± 11.2
White blood cells (× 10^3/ L)	8.77 ± 7.52
Platelets (× 10^3/L)	383.57 ± 390.87
Fibrosis stage	
No (F0)	1 (3.3)
Mild (F1)	12 (40)
Moderate (F2-F3)	17 (56.7)
Activity grade	
Minimal	10 (33.3)
Mild	20 (66.7)
Steatosis	8 (26.7)

HCV: Hepatitis C virus; F0/1/2/3: Fibrosis score 0/1/2/3.

Laboratory investigations

Laboratory investigations, including LFTs, CBC, kidney function tests, serum autoantibodies (anti-nuclear antibodies, anti-smooth muscle antibodies and anti-mitochondrial antibodies) and prothrombin time were performed for all the patients. Viral markers were performed using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer instructions; HCV Ab (Innogenetics, Ghent, Belgium), hepatitis B virus surface antigen, hepatitis B virus core immunoglobulin (Ig)M and IgG Abs (all from Dia Sorin, Saluggia, Italy). Real-time PCR for HCV-RNA was performed using COBAS Ampliprep/COBAS TaqMan, Roche Molecular Systems, Inc., Branchburg, NJ 08876, United States (detection limit was 15 IU/mL). According to the viral load, viremia was classified arbitrarily into low ($< 2 \times 10^5$ IU/mL), moderate ($\geq 2 \times 10^5$-2×10^6 IU/mL), and high viremia ($\geq 2 \times 10^6$ IU/mL)[18]. Serum C4a levels were assayed using ELISA (WKEA Med Supplies Corp, NY 10123, United States) according to the manufacturer instructions. Serum samples of the patients were collected, maximally, within 6 mo of liver biopsy[19].

Liver biopsy and histopathological evaluation

Liver biopsy was performed using a true cut needle for all the patients. Specimens were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin, Masson's trichrome, reticulin and Perl's stains. Hepatic necroinflammatory activity and liver fibrosis were evaluated according to Ishak staging and grading score. Necroinflammatory activity was classified into minimal (score 1-3), mild (score 4-8), moderate (score 9-12), and severe (score 13-18)[20]. Fibrosis was classified into mild (stage 1), moderate (stages 2-3), and severe fibrosis or cirrhosis (stages 4-6)[5]. Significant fibrosis was defined as Ishak score of 3 or more (presence of bridging fibrosis)[21].

Statistical analysis

Descriptive results were expressed as mean ± SD or number (percentage) of individuals with a condition. For quantitative data, statistical significance was tested by Mann-Whitney *U* non-parametric tests. For qualitative data, significance between groups was tested by χ^2 test. Correlation was tested by Spearman's test. Results were considered significant if P value ≤ 0.05. The diagnostic performance was measured as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) and all were expressed as percentages. The cutoff values for optimal clinical performance was determined from the receiver-operating characteristic curve. Statistical analysis was performed using SPSS statistical package version 13 (SPSS Inc., Chicago, IL, United States).

RESULTS

Study population characteristics

The study included 30 children with chronic HCV infection. They were 12 females and 18 males. Their mean age was 11.12 ± 4.62 ranging from 3.5 to 18 years. A second group of 30 age- and sex-matched ($P > 0.05$ for both) healthy children served as controls. They were 14 females and 16 males. Their mean age was 10.73 ± 4.19 ranging from 4 to 17 years. The major possible modes of infection were male circumcision and family contact (60% each) followed by surgery (43.4%), blood transfusion (30%) and dental procedures (16.7%). Many children had more than one possible mode of infection. The majority of patients were asymptomatic. Four children had hepatomegaly, one child had splenomegaly and none had jaundice or ascites. Fibrosis stage ranged from F0 to F3 and activity grade ranged from A1 to A7. The majority had moderate fibrosis (56.7%) and mild activity (66.7%), while 8 out of 30 (26.7%) had steatosis (Table 1).

Histopathological findings in patients with normal vs elevated transaminases

All the patients (except one with F0) had mild to moderate fibrosis and minimal to mild activity in liver biopsy. Yet, nearly half of them had normal transaminases (41.7%, 47.1%, 50.0% and 45.0% for mild fibrosis, moderate fibrosis, minimal activity and mild activity respectively) (Table 2).

C4a did not differ significantly according to laboratory parameters or histopathological parameters

The mean value of serum C4a was lower in patients than in controls (153.67 ± 18.69 mg/L *vs* 157.25 ± 11.40 mg/L respectively) with no statistically significant difference ($P = 0.378$). Moreover, there was no statistically significant

Table 2 Histopathological findings in patients with normal *vs* elevated transaminases *n* (%)

Histopathology	Normal transaminases	Elevated transaminases
Fibrosis stage		
No fibrosis (n = 1)	1 (100)	
Mild fibrosis (n = 12)	5 (41.7)	7 (58.3)
Moderate fibrosis (n = 17)	8 (47.1)	9 (52.9)
Activity grade		
Minimal activity (n = 10)	5 (50)	5 (50)
Mild activity (n = 20)	9 (45)	11 (55)

Table 3 Serum complement C4a according to different transaminases levels, viral loads and histopathological findings

Parameter	C4a (mg/L)	*P* value
Fibrosis stage		0.786
No/Mild (n = 13)	154.65 ± 20.59	
Moderate (n = 17)	152.97 ± 17.72	
Activity grade		0.809
Minimal (n = 10)	155.1 ± 21.93	
Mild (n = 20)	152.99 ± 17.43	
Steatosis		0.186
Present (n = 8)	146.13 ± 3.32	
Absent (n = 22)	156.45 ± 21.19	
Normal transaminases (n = 14)	155.36 ± 21.33	0.868
Elevated transaminases (n = 16)	152.25 ± 16.62	
Viremia		0.561
Low viremia (n = 17)	156.37 ± 18.91	
Moderate viremia (n = 9)	152.43 ± 22.19	
High viremia (n = 4)	145.2 ± 3.96	

difference in C4a regarding sex in both the patients (M/F: 150.33 ± 16.22 mg/L *vs* 158.75 ± 21.64 mg/L; $P = 0.234$) and controls (M/F: 154.29 ± 8.60 mg/L *vs* 160.64 ± 13.46 mg/L; $P = 0.130$). There was no statistically significant difference in mean level of C4a when comparing patients with different fibrosis stages, different activity grades, different levels of viremia and patients with normal transaminases *vs* those with elevated transaminases ($P > 0.05$ for all; Table 3). Furthermore, there was no correlation between C4a and any of the studied laboratory parameters or with fibrosis stage and activity grade (Table 4).

Complement C4a according the individual fibrosis stage

Serum C4a, though not statistically significant, was inversely proportional to the stage of fibrosis. It was the highest (182.52 mg/L) in the patient with F0, decreasing in patients with F1 and F2 (152.33 ± 19.64 mg/L and 155.87 ± 19.46 mg/L respectively) and reaching the lowest level in F3 (143.55 ± 2.33 mg/L) (Figure 1).

Comparing C4a in significant fibrosis (Ishak score ⩾ 3) vs other fibrosis stages (F0-F2)

When comparing significant fibrosis with the other stages, C4a was significantly lower in F3 compared to other fibrosis scores (143.55 ± 2.33 mg/L *vs* 155.26 ± 19.64 mg/L; $P = 0.047$). C4a at a cutoff level of less than 144.01 mg/L could discriminate F3 with 76.9% sensitivity, 75% specificity, 95.24% PPV and 33.3% NPV from other stages of fibrosis (Figure 2).

Table 4 Correlation of complement C4a with laboratory and histopathological parameters in liver biopsy

Parameter	C4a (mg/L)	
	r	*P* value
Total bilirubin (mg/dL)	-0.022	0.910
Direct bilirubin (mg/dL)	-0.038	0.841
Albumin (g/L)	0.162	0.393
Alanine transaminase (U/L)	-0.148	0.332
Aspartate transaminase (U/L)	-0.026	0.891
Gamma glutamyl transpeptidase (U/L)	0.000	1.000
Alkaline phosphatase (U/L)	0.176	0.446
Hemoglobin (g/dL)	-0.100	0.599
White blood cells (× 10^3/L)	0.054	0.777
Platelets (× 10^3/L)	0.228	0.226
HCV-RNA (IU/mL)	-0.210	0.265
Fibrosis stage	-0.208	0.269
Activity grade	-0.114	0.548

HCV: Hepatitis C virus.

DISCUSSION

The natural history of chronic HCV infection in children differs from that in adults since HCV infection is relatively benign, induces mild changes in the liver with a low level of fibrosis and a low rate of progression and is rarely associated with severe or decompensate liver disease[22]. However, progressive fibrosis and early appearance of end-stage liver disease have been documented[23]. Bortolotti *et al*[24], reported that hepatitis C in children is usually asymptomatic. Clinically, most of our patients were asymptomatic (73.36%), 13.3% had hepatomegaly, and 3.3% had splenomegaly but none had jaundice or ascites. A similar finding was reported by El-Raziky *et al*[5], since soft enlargement of the liver was found in 2 (11%) children with HCV infection and none had splenomegaly.

In the current study, patients with normal transaminases (46.7%) had both mild (41.7%) to moderate (47.1%) fibrosis and minimal (50%) to mild (45%) activity on histopathological examination. It has been reported that ALT levels are elevated in half of the subjects and histological abnormalities are detectable in three quarters of HCV-RNA positive cases[5]. This means that liver enzymes in chronic HCV infection do not reflect histopathological abnormalities in the majority of cases and liver biopsy would be essential for evaluation of the disease state and extent of liver injury.

There is relatively little information on the histopathology of chronic hepatitis C in children. It has been shown that low ALT levels, low viral load and mild histological changes characterize chronic hepatitis C infection in children[25]. Goodman *et al*[26], reported that, in a cohort study, grading and staging of liver biopsies from 121 children ages 2 to 16 (mean, 9.8 years) infected with HCV revealed minimal, mild, moderate and severe inflammatory activity in 42%, 17%, 38%, 3% of patients respec-

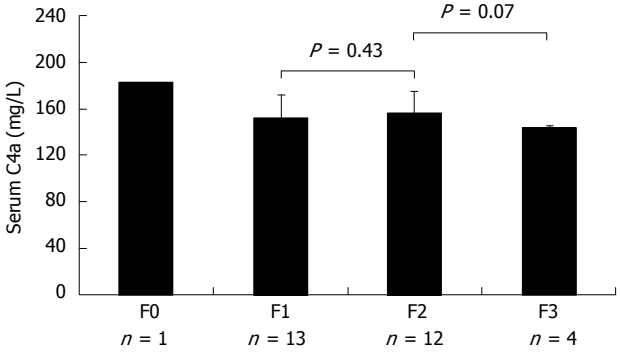

Figure 1 Complement C4a in the individual fibrosis stages.

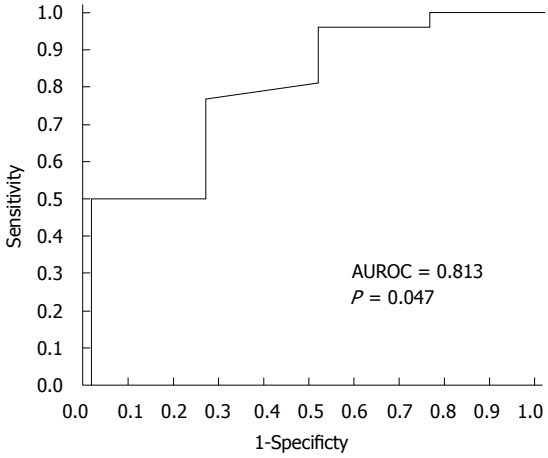

Figure 2 Clinical performance of C4a in discriminating significant fibrosis from other stages of fibrosis. AUROC: Area under the receiver operating characteristic.

tively. Five (4%) had bridging fibrosis and 2 (1.7%) had cirrhosis. In the current study, all the patients had liver biopsy. None of them had cirrhosis where fibrosis scores ranged from 0 to 3 and activity grades ranged from 1 to 7. Although universal screening for hepatitis C is not recommended, it is actually the only method to detect HCV in children because carriers are usually asymptomatic. Even transaminases are usually within normal range. Consequently, they would remain undiagnosed until the appearance of symptoms in adolescence or adulthood[27].

The main target of the current study was to evaluate serum complement C4a levels in children with chronic HCV infection and its relation to liver fibrosis. Complement represents a significant non-specific host defense system involved in the protection of the host from virus infection[28]. To escape this protection, viruses are able to express host-homologous proteins, or to borrow cell membrane proteins from the host with complement regulatory activity, protecting viral particles from neutralization by the complement[29]. C4 specific activity appears as a valuable parameter for predicting and monitoring interferon and ribavirin therapy[30]. Deficiencies of complement component C4 isotype *C4a* has been associated with various autoimmune, inflammatory or infectious diseases as well as with mental disorders and cancer survival[31]. Phenotypic C4 deficiencies are caused by increased protein consumption or genetic deficiencies[32].

Serum C4 levels were found to decrease in adult HCV patients compared to healthy controls as a result of altered transcriptional regulation[30]. In the present study, although there was no significant statistical difference in the mean serum C4a levels between patients and controls, it was lower in patients. Moreover, there was no significant statistical difference in complement C4a level according to different stages of fibrosis, grades of activity or presence or absence of steatosis ($P > 0.05$ for all). Nonetheless, C4a was in its highest value in F0 and decreased as fibrosis increased with its lowest level in F3. This finding is in agreement with that of Imakiire *et al*[33], who reported that C4a increases with HCV infection, but decreases with disease progression which reflects the development of an inflammatory process and, evidently, the higher secretion of complement C4a by stimulated macrophages[15]. In addition, we found that C4a was not correlated with any of CBC parameters, liver functions or HCV viremia. Imakiire *et al*[33] showed that the level of C4a in serum was higher in HCV carriers with persistently normal ALT compared to chronic HCV patients or healthy volunteers.

Buğdaci *et al*[17] showed a significant negative correlation of C4 with ALT (r = -0.368, P = 0.001) and histological activity index (r = -0.639, P = 0.001) by Knodell score. Such relation was not found in the present study as there was no significant difference in complement C4a levels between patients with normal transaminases and those with elevated ones (P = 0.868). This discrepancy may be due to the difference in the mean age of the studied population (53.88 ± 11.44 years in Buğdaci *et al*[17], and 11.12 ± 4.62 years in ours), or the smaller number of patients (n = 30) in our study compared to 70 patients in Buğdaci *et al*[17]. Another important difference is the grade of activity (A1 to A7) and stage of fibrosis (F0 to F3) in ours using Ishak score, compared to that in Buğdaci *et al*[17], (8 ± 2.75 and 1.66 ± 0.784 for activity and fibrosis respectively) using Knodell score. In agreement with our results, Dumestre-Perard *et al*[30] reported that there were no statistical significant correlations between specific C4 activity and each of HCV-RNA, ALT, Knodell score, Metavir histological fibrosis and Metavir histological activity (P = 0.29, 0.9, 0.48, 0.96 and 0.22 respectively).

In chronic HCV infection, patients with no or minimal fibrosis at presentation appear to progress slowly and treatment could possibly be delayed or withheld. On the other hand, patients with significant fibrosis (*i.e.*, septal or bridging fibrosis) progress almost invariably to cirrhosis over a 10- to 20- year period, so antiviral treatment should be strongly considered[34]. For that we compared C4a in F3 *vs* other stages of fibrosis. C4a was significantly lower in F3 compared to other fibrosis scores and at a cutoff value of less than 144.01 mg/L it could discriminate F3 with 76.9% sensitivity and 75% specificity (P = 0.047). Although it is accepted to assess serum markers of fibrosis if serum sample is taken within 6 mo of liver

biopsy[19], this might be a limitation in the study. For that, serum sampling in the same setting with liver biopsy would be preferred. Another limitation is the relatively small number of patients in the study.

In conclusion, our study demonstrated that complement C4a did not correlate with any of transaminases, HCV viral load or the histopathological scores of liver biopsy. Though C4a decreased in higher stages of fibrosis, this change was not statistically significant enough to predict individual stages of fibrosis. Yet, it could predict significant fibrosis with acceptable clinical performance.

COMMENTS

Background

The need for repetition of liver biopsy in patients with chronic hepatitis C virus (HCV), especially in assessing the degree of fibrosis and follow-up of treatment protocols, justifies an intensive search for non-invasive alternatives. Of these alternatives, serum C4a has been proposed. In adults, the reports are contradictory. In this study the authors evaluate C4a as a predictor of HCV-associated liver fibrosis in the pediatric age group.

Research frontiers

The natural course of HCV infection in children differs from that in adults since the infection is relatively benign and induces mild changes in the liver with a low level of fibrosis. Patients with no or minimal fibrosis at presentation appear to progress slowly and treatment could possibly be delayed or withheld. On the other hand, patients with significant fibrosis (*i.e.*, septal or bridging fibrosis) progress almost invariably to cirrhosis over a 10- to 20-year period so antiviral treatment should be strongly considered.

Innovations and breakthroughs

Complement C4a was proposed by proteomic analytical study in adults as a predictor of HCV-induced liver fibrosis. Further studies of serum level of C4a as a predictor for liver fibrosis were contradictory. Authors evaluated serum C4a as a predictor of HCV-induced liver fibrosis in the pediatric population. Although C4a decreased with higher stages of fibrosis, this change was not statistically significant enough to predict individual stages of fibrosis. Yet, it could predict significant fibrosis (Ishak score $\geqslant$ 3) with acceptable clinical performance.

Applications

Serum C4a can be used as a non-invasive marker to discriminate patients with significant liver fibrosis who are in need for critical consideration of antiviral therapy from those with no or minimal fibrosis for whom treatment could be delayed or deferred.

Terminology

Hepatic fibrosis is the final common path of liver injury in most chronic liver diseases and can lead to cirrhosis, which is responsible for the majority of clinical complications. Fibrosis is characterized by excess deposition of extracellular matrix components including different collagens and non-collagenous proteins such as laminin, fibronectin, undulin, cytokines and complement components. The serum contains such tissue proteins as leakage markers.

Peer review

The submitted manuscript investigates the significance of C4a as a surrogate marker for fibrosis in a children population with HCV infection. The article is interesting. The statistical analysis is good, but the number of patients is small and authors should mention in conclusion that the results were interpreted taking into consideration the small number of patients.

REFERENCES

1 **Yazigi N**, Balistreri W. Viral hepatitis. In: Nelson Textbook of pediatrics. 19th ed. Kliegman RM, Behrman RE, Jenson HB, Stanton BF, editors. Philadelphia (PA): Saunders, 2011: 1393-1400

2 **Welbourn S**, Pause A. The hepatitis C virus NS2/3 protease. *Curr Issues Mol Biol* 2007; **9**: 63-69 [PMID: 17263146]

3 **Webster DP**, Klenerman P, Collier J, Jeffery KJ. Development of novel treatments for hepatitis C. *Lancet Infect Dis* 2009; **9**: 108-117 [PMID: 19179226 DOI: 10.1016/S1473-3099(09)70020-9]

4 **Lehman EM**, Wilson ML. Epidemic hepatitis C virus infection in Egypt: estimates of past incidence and future morbidity and mortality. *J Viral Hepat* 2009; **16**: 650-658 [PMID: 19413698 DOI: 10.1111/j.1365-2893.2009.01115.x]

5 **El-Raziky MS**, El-Hawary M, Esmat G, Abouzied AM, El-Koofy N, Mohsen N, Mansour S, Shaheen A, Abdel Hamid M, El-Karaksy H. Prevalence and risk factors of asymptomatic hepatitis C virus infection in Egyptian children. *World J Gastroenterol* 2007; **13**: 1828-1832 [PMID: 17465475]

6 **Shackel NA**, McGuinness PH, Abbott CA, Gorrell MD, McCaughan GW. Novel differential gene expression in human cirrhosis detected by suppression subtractive hybridization. *Hepatology* 2003; **38**: 577-588 [PMID: 12939584 DOI: 10.1053/jhep.2003.50376]

7 **Ghany MG**, Kleiner DE, Alter H, Doo E, Khokar F, Promrat K, Herion D, Park Y, Liang TJ, Hoofnagle JH. Progression of fibrosis in chronic hepatitis C. *Gastroenterology* 2003; **124**: 97-104 [PMID: 12512034 DOI: 10.1053/gast.2003.50018]

8 **Valva P**, Casciato P, Lezama C, Galoppo M, Gadano A, Galdame O, Galoppo MC, Mullen E, De Matteo E, Preciado MV. Serum apoptosis markers related to liver damage in chronic hepatitis C: sFas as a marker of advanced fibrosis in children and adults while M30 of severe steatosis only in children. *PLoS One* 2013; **8**: e53519 [PMID: 23326448 DOI: 10.1371/journal.pone.0053519]

9 **Johansen JS**, Christoffersen P, Møller S, Price PA, Henriksen JH, Garbarsch C, Bendtsen F. Serum YKL-40 is increased in patients with hepatic fibrosis. *J Hepatol* 2000; **32**: 911-920 [PMID: 10898311 DOI: 10.1016/S0168-8278(00)80095-1]

10 **Scheimann AO**, Barrios JM, Al-Tawil YS, Gray KM, Gilger MA. Percutaneous liver biopsy in children: impact of ultrasonography and spring-loaded biopsy needles. *J Pediatr Gastroenterol Nutr* 2000; **31**: 536-539 [PMID: 11144439 DOI: 10.1097/00005176-200011000-00015]

11 **Oshrine B**, Lehmann LE, Duncan CN. Safety and utility of liver biopsy after pediatric hematopoietic stem cell transplantation. *J Pediatr Hematol Oncol* 2011; **33**: e92-e97 [PMID: 21317809 DOI: 10.1097/MPH.0b013e3182025236]

12 **Adams LA**. Biomarkers of liver fibrosis. *J Gastroenterol Hepatol* 2011; **26**: 802-809 [PMID: 21198831 DOI: 10.1111/j.1440-1746.2010.06612.x]

13 **Anderson NL**, Anderson NG. The human plasma proteome: history, character, and diagnostic prospects. *Mol Cell Proteomics* 2002; **1**: 845-867 [PMID: 12488461 DOI: 10.1074/mcp.R200007-MCP200]

14 **Yang L**, Rudser KD, Higgins L, Rosen HR, Zaman A, Corless CL, David L, Gourley GR. Novel biomarker candidates to predict hepatic fibrosis in hepatitis C identified by serum proteomics. *Dig Dis Sci* 2011; **56**: 3305-3315 [PMID: 21590334 DOI: 10.1007/s10620-011-1745-4]

15 **Galibert MD**, Boucontet L, Goding CR, Meo T. Recognition of the E-C4 element from the C4 complement gene promoter by the upstream stimulatory factor-1 transcription factor. *J Immunol* 1997; **159**: 6176-6183 [PMID: 9550420]

16 **White IR**, Patel K, Symonds WT, Dev A, Griffin P, Tsokanas N, Skehel M, Liu C, Zekry A, Cutler P, Gattu M, Rockey DC, Berrey MM, McHutchison JG. Serum proteomic analysis focused on fibrosis in patients with hepatitis C virus infection. *J Transl Med* 2007; **5**: 33 [PMID: 17625010 DOI: 10.1186/1479-5876-5-33]

17 **Buğdaci MS**, Karaca C, Alkim C, Kesıcı B, Bayraktar B, Sökmen M. Serum complement C4 in chronic hepatitis C: correlation with histopathologic findings and disease activity. *Turk J Gastroenterol* 2012; **23**: 33-37 [PMID: 22505377]

18 **Witthöft T**, Möller B, Wiedmann KH, Mauss S, Link R, Lohmeyer J, Lafrenz M, Gelbmann CM, Hüppe D, Niederau C, Alshuth U. Safety, tolerability and efficacy of peginterferon alpha-2a and ribavirin in chronic hepatitis C

in clinical practice: The German Open Safety Trial. *J Viral Hepat* 2007; **14**: 788-796 [PMID: 17927615 DOI: 10.1111/j.1365-2893.2007.00871.x]

19 **Brown KS**, Keogh MJ, Tagiuri N, Grainge MJ, Presanis JS, Ryder SD, Irving WL, Ball JK, Sim RB, Hickling TP. Severe fibrosis in hepatitis C virus-infected patients is associated with increased activity of the mannan-binding lectin (MBL)/MBL-associated serine protease 1 (MASP-1) complex. *Clin Exp Immunol* 2007; **147**: 90-98 [PMID: 17177967 DOI: 10.1111/j.1365-2249.2006.03264.x]

20 **Ishak K**, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696-699 [PMID: 7560864 DOI: 10.1016/0168-8278(95)80226-6]

21 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]

22 **Camarero C**, Ramos N, Moreno A, Asensio A, Mateos ML, Roldan B. Hepatitis C virus infection acquired in childhood. *Eur J Pediatr* 2008; **167**: 219-224 [PMID: 17464514 DOI: 10.1007/s00431-007-0472-5]

23 **Giacchino R**, Bortolotti F, Indolfi G, Verucchi G, Zancan L, Cammj C, D'Antiga L, Marazzi MG, Barbera C, Resti M, Guido M. Antiviral treatment of hepatitis C in Italian children. *Digest Liver Dis* 2009; **41**: A7-A8 [DOI: 10.1016/j.dld.2008.12.019]

24 **Bortolotti F**, Jara P, Diaz C, Vajro P, Hierro L, Giacchino R, de la Vega A, Crivellaro C, Camarena C, Barbera C. Post-transfusion and community-acquired hepatitis C in childhood. *J Pediatr Gastroenterol Nutr* 1994; **18**: 279-283 [PMID: 8057208]

25 **Wu TC**, Chuang WL, Dai CY, Huang JF, Hsieh MY, Hou NJ, Lee LP, Lin WY, Yang JF, Chiu CC, Chen SC, Hsieh MY, Chang WY, Yu ML. Hepatitis C virus infection among children in aboriginal areas in Taiwan. *Trans R Soc Trop Med Hyg* 2008; **102**: 935-938 [PMID: 18656216 DOI: 10.1016/j.trstmh.2008.06.012]

26 **Goodman ZD**, Makhlouf HR, Liu L, Balistreri W, Gonzalez-Peralta RP, Haber B, Jonas MM, Mohan P, Molleston JP, Murray KF, Narkewicz MR, Rosenthal P, Smith LJ, Robuck PR, Schwarz KB. Pathology of chronic hepatitis C in children: liver biopsy findings in the Peds-C Trial. *Hepatology* 2008; **47**: 836-843 [PMID: 18167062 DOI: 10.1002/hep.22094]

27 **Gerner P**, Wirth S, Wintermeyer P, Walz A, Jenke A. Prevalence of hepatitis C virus infection in children admitted to an urban hospital. *J Infect* 2006; **52**: 305-308 [PMID: 16473408 DOI: 10.1016/j.jinf.2005.04.004]

28 **Morgan B**, Harris C. Complement regulators and micro-organisms. Morgan BP, Harris CL, editors. Complement regulatory proteins. San Diego: Academic Press, 1999: 207-225

29 **Lubinski J**, Nagashunmugam T, Friedman HM. Viral interference with antibody and complement. *Semin Cell Dev Biol* 1998; **9**: 329-337 [PMID: 9665870 DOI: 10.1006/scdb.1998.0242]

30 **Dumestre-Perard C**, Ponard D, Drouet C, Leroy V, Zarski JP, Dutertre N, Colomb MG. Complement C4 monitoring in the follow-up of chronic hepatitis C treatment. *Clin Exp Immunol* 2002; **127**: 131-136 [PMID: 11882043 DOI: 10.1046/j.1365-2249.2002.01729.x]

31 **Mougey R**. A review of the Chido/Rodgers blood group. *Immunohematology* 2010; **26**: 30-38 [PMID: 20795316]

32 **Braun L**, Schneider PM, Giles CM, Bertrams J, Rittner C. Null alleles of human complement C4. Evidence for pseudogenes at the C4A locus and for gene conversion at the C4B locus. *J Exp Med* 1990; **171**: 129-140 [PMID: 2295875 DOI: 10.1084/jem.171.1.129]

33 **Imakiire K**, Uto H, Sato Y, Sasaki F, Mawatari S, Ido A, Shimoda K, Hayashi K, Stuver SO, Ito Y, Okanoue T, Tsubouchi H. Difference in serum complement component C4a levels between hepatitis C virus carriers with persistently normal alanine aminotransferase levels or chronic hepatitis C. *Mol Med Rep* 2012; **6**: 259-264 [PMID: 22614103 DOI: 10.3892/mmr.2012.924]

34 **Yano M**, Kumada H, Kage M, Ikeda K, Shimamatsu K, Inoue O, Hashimoto E, Lefkowitch JH, Ludwig J, Okuda K. The long-term pathological evolution of chronic hepatitis C. *Hepatology* 1996; **23**: 1334-1340 [PMID: 8675148 DOI: 10.1002/hep.510230607]

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CASE REPORT

Alcohol and tobacco misuse: Reducing aerodigestive cancer risk

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Abstract

Significant concerns over the health, social and economic burdens of the two most common, and frequently co-misused drugs of abuse, alcohol and tobacco, has encouraged focused but separate health promotion and disease prevention policies. However, this separation of focus means that while individuals who present with alcohol-related problems are increasingly supported to attain and maintain abstinence from alcohol they are not routinely assisted to refrain from smoking. This is tragically inopportune as alcohol and tobacco have an established "synergistic" effect on aerodigestive cancer risk. Moreover, even when patients successfully tackle their alcohol problems they remain at increased risk for developing these cancers, especially if they continue to smoke. A case series is presented together with a discussion on how service provision for co-misuse could be improved to obviate aerodigestive cancer risk. Given the prevalence of alcohol and tobacco use in the United Kingdom, these observations may have far reaching implications for the individual, health provider(s) and wider society.

Key words: Tobacco; Alcohol; Substance misuse; Co-dependence; Behavioural control; Early intervention; Preventive therapy; Aerodigestive cancer; Mortality

Core tip: What is already known? Most people who drink heavily also smoke; alcohol and smoking synergistically increase aerodigestive cancer risk; people with alcohol problems and/or liver injury, are supported to attain and maintain abstinence, from alcohol but much less effort is employed to help them achieve smoking cessation. What is the key message? Patients who maintain abstinence from alcohol remain at risk for aerodigestive cancers for several years, especially if they continue to smoke. How might it impact on future clinical practice? Smoking behaviour should be addressed in co-dependent individuals if the health benefits of long-term abstinence from alcohol are to be maximized.

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INTRODUCTION

Alcohol consumption and tobacco usage are associated with major health issues and are principle causes of preventable deaths in developed countries. The risks to health they pose are also interrelated. Thus, cigarette smoking is independently related to the risk of developing alcohol-related cirrhosis, with smokers of a pack or more per day at three times the risk compared with lifelong non-smokers[1]. Both behaviours also have a significant effect on cancer risk, particularly the development of aerodigestive tract cancers (*e.g.*, squamous

cell cancers (SCCs) of the oropharynx, larynx, and oesophagus), with the risks correlating to the levels of both alcohol consumption and tobacco usage[2,3]. Moreover, the risk associated with alcohol and tobacco co-misuse is multiplicative or at least greater than additive[4]. Also of significant importance is the fact that the risk of cancer development persists even when behaviours are moderated although with temporal differences. Thus, a favourable effect of smoking cessation is evident within a few years[5], whereas the risk may remain persistently high for several years following abstinence from alcohol[6,7]. This is of particular concern in patient who undergo orthotopic liver transplantation in whom the risk of *de novo* aerodigestive cancers is increased, independently of immunosuppressant use, particularly in those with alcohol-related cirrhosis, a long history of cigarette smoking and continuation of smoking post-transplantation[8-11].

Intervening in addictive behaviours may provide the most effective was of limiting both the health and economic burden of upper aerodigestive cancers. However, despite the fact that some 80% of individuals seeking treatment for alcohol problems also smoke the opportunity to simultaneously address smoking issues is rarely effectively taken. Examples of the consequences of these missed opportunities are presented.

CASE REPORT

Case 1

A 42-year-old Caucasian woman presented in 1993 with a long history of alcohol misuse; her liver biopsy showed cirrhosis. Subsequently she attained and maintained abstinence from alcohol apart from one brief relapse in 1996; her liver disease was well-compensated. She was a long-standing, heavy smoker but declined referral for smoking cessation, only briefly engaging with these services in 2000. In 2001, aged 50 and still smoking, she presented with hoarseness and throat swelling; a diagnosis of SCC of the uvula and soft-palate was made. She underwent curative radical surgery and radiotherapy but was left with significant nasal regurgitation of food. Post-surgery, despite self-reported periods of smoking cessation, she never successfully quit. In 2009, aged 58, she developed anaemia and reflux symptoms secondary to a new mid-oesophageal SCC (T3N1M0). She was not a candidate for surgery due to advanced emphysema, large gastric varices and upper aerodigestive radiation damage that would have made oesophageal resection and anastomosis hazardous. She underwent her first cycle of palliative chemoradiotherapy but died 9-wk from cancer diagnosis with aspiration pneumonia and neutropaenic sepsis complicating endoscopic gastrostomy placement (Table 1).

Case 2

A 50-year-old Anglo-Indian man presented in 2001 with a history of ongoing mental health issues, previous intravenous drug misuse, current marijuana use and alcohol dependence. He had evidence of decompensated cirrhosis secondary to alcohol and newly diagnosis hepatitis C virus (HCV) infection, complicated by the development of subacute bacterial peritonitis (SBP). Following an initial stormy inpatient stay he eventually achieved stable disease with maintained abstinence from alcohol. However, despite efforts to encourage engagement with smoking cessation services he continued to smoke and to use marijuana occasionally. He initially declined antiviral therapy, but in 2007 was eventually and successfully treated for HCV with pegylated (PEG)-interferon and ribavirin. In 2010, aged 59, while still abstinent from alcohol, he presented with hoarseness, peri-tonsillar swelling and cervical lymphadenopathy due to a posterior hypopharyngeal SCC (T4M0N0). This was successfully treated with chemoradiotherapy; he independently quit smoking and remains well with no evidence of recurrence on positron emission tomography (PET)-scanning.

Case 3

A 55-year-old Caucasian man presented in 2000 with alcohol dependence and decompensated alcohol-related cirrhosis complicated by SBP. He was a lifelong heavy smoker and user of marijuana and although he subsequently maintained abstinence from alcohol and moderated his use of marijuana he repeatedly refused to consider smoking cessation. In 2007, aged 62 years, he presented with a cough and worsening shortness of breath; a diagnosis of early small-cell lung carcinoma was made and successfully treated with chemoradiotherapy with no evidence of recurrence to date. In 2011, a solitary small hepatocellular carcinoma was diagnosed on surveillance ultrasound and treated with radiofrequency ablation with no evidence of recurrence to date. Although he remains abstinent from alcohol he continues to smoke.

Case 4

A 46-year-old Caucasian woman presented in 1997 with a history of alcohol misuse, jaundice and ascites; she had cirrhosis on liver biopsy. Her initial hospitalisation was complicated by torrential haemorrhaging from a posterior, penetrating duodenal ulcer requiring gastroduodenal artery embolisation. Following discharge she maintained abstinence from alcohol and her liver disease stabilized with normalization of her liver function tests. However, she remained a heavy smoker despite medical advice to quit. In 2008, aged 57, she presented to her general practitioner with a history of painful neck swelling, otalgia and hoarseness. This failed to resolve with repeated courses of antibiotics but she was not referred for specialist review until she attended for her routine hepatology surveillance some 7 mo later. A diagnosis of laryngeal SCC (T1M0N0) was made and she underwent 2 mo of local radiotherapy. She independently quit smoking at cancer diagnosis but a year later was found, on surveillance imaging, to have a bronchogenic cancer (T1N0M0) and underwent further radiotherapy. In 2010 she developed faecal peritonitis secondary to a perforated sigmoid diverticulum and required a right hemicolectomy

Table 1 Details of the drinking and smoking behaviours of six patients with alcohol-related cirrhosis who developed aerodigestive cancers

Case	DoB	Sex	Alcohol misuse		Tobacco use		Age at diagnosis		Cancer	Misuse intervention					Cancer
											Smoking		Alcohol		
			Years	Units/wk	Pack years	cpd	Cirrhosis	Cancer		Provided yes/no	Quit (yr)	Relapsed (yr)	Abstinent (yr)	Recidivism	
1	1951	F	25	120	40	20	42	50	Uvula/soft palate SCC	Yes	0.3	Yes	7	No	Died
								58	Oesophageal SCC						
2	1951	M	30	100	18	10	50	59	Hypopharyngeal SCC	No	No	NA	8	No	Remission
3	1945	M	35	250	65	35	55	62	Small-cell lung cancer	No	No	NA	7	No	Remission
								65	HCC						Remission
4	1951	F	29	100	19	30	46	57	Laryngeal SCC	No	No	NA	12	No	Remission
								58	Bronchial carcinoma						Remission
5	1947	M	37	70	15	10	57	60	Posterior triangle neck SCC (unknown primary)	Yes	6	2	1	No	Remission
6	1947	F	22	200	63	40	52	64	Oesophageal SCC	Yes	No	NA	14	No	Remission

DoB: Date of birth; cpd: Cigarettes per day; HCC: Hepatocellular carcinoma; SCC: Squamous cell carcinoma; NA: Not applicable; F: Female; M: Male.

with colostomy. She is now well with no evidence of persistent or recurrent laryngeal or bronchial malignancy at follow-up. Of interest, her older sister, who had also been a heavy smoker and drinker, died from bronchial carcinoma five years after a successful liver transplant for alcohol-related cirrhosis.

Case 5

A 57-year-old Caucasian man initially presented in 2004 with a long history of alcohol and tobacco misuse, severe lethargy and abnormal liver function tests. He was found to have HCV infection secondary to prior drug misuse; his liver biopsy was consistent with cirrhosis secondary to both alcohol and HCV. In 2006 he was admitted for medically assisted withdrawal from alcohol and after 6 mo abstinence from alcohol was started on treatment for his HCV with PEG-interferon and ribavirin. He achieved an early viral response but at 3 mo he developed tender, rubbery lymph glands in his right neck diagnosed as a SCC of unknown origin (TxM0N3); the primary was never discovered despite extensive investigations including MRI and PET scanning and bronchoscopy. The antiviral therapy was stopped; he underwent radical block dissection of his right neck, a bilateral tonsillectomy and local radiotherapy. He stopped smoking at cancer diagnosis, although he admitted to occasional marijuana use. In 2008, despite support, he became depressed and returned to drinking, albeit at a lower level than previously. There are no sign of cancer reoccurrence, to date.

Case 6

A 50-year-old Caucasian woman of Polish origin was referred to our dermatology service in 1997 with spider naevi; on enquiry she admitted to a 22-year history of heavy drinking although she had stopped some 8 mo prior to presentation. Her initial liver biopsy showed severe hepatic fibrosis but by 1999 this had evolved to cirrhosis despite continued abstinence from alcohol. She smoked 40 cigarettes per day and did not wish to stop. In July 2011, having been abstinent from alcohol for 14 years, she presented with a 2.5 mo history of increasing dysphagia and a diagnosis of a mid-oesophageal carcinoma was made. Her cirrhosis was well-compensated with no clinical, radiological or endoscopic evidence of portal hypertension. Nevertheless she was not felt to be a candidate for surgery as she had limiting smoking-related emphysema. She underwent 3 mo of chemoradiotherapy and, when last reviewed, was in remission. She stopped smoking after cancer diagnosis.

DISCUSSION

Over the last 40 years the incidence of upper aerodigestive cancers has increased while mortality rates have shown little improvement despite earlier intervention with chemoradiotherapy and/or surgery[12]. Alcohol consumption and tobacco usage are the two most important risk factors for the development of these cancers and they are frequently co-misused.

Tobacco smoking is by far the most important risk factor for cancer in the United Kingdom and in 2010, was responsible for 61000 cases, equivalent to 19.4% of all new cancer diagnoses. This included 4500 oropharyngeal, 5600 oesophageal and 1700 laryngeal cancers, in addition to 34600 lung cancers[13,14]. The risk of developing upper aerodigestive cancers increases in relation to the amount and duration of tobacco use[2,4,7]. Thus, the RR of developing oral cancers increases threefold from 5.3 to 18.0 when usage increases from < 15 to 40 cigarettes per day.

Alcohol consumption is the fourth most important cause of cancer in the United Kingdom and in 2010 was responsible for 12500 cases of cancer equivalent to 4.0% of all new cancer diagnoses, including around 2100 oropharyngeal, 1760 oesophageal and 540 laryngeal cancers[13,14]. The risk of developing aerodigestive cancers, as with tobacco, increases with the amount and duration of exposure; thus increasing daily consumption from 25 to 100 g trebles the RR from 1.7 to 6.0[15]. Significantly, the risk of developing upper aerodigestive cancers with co-dependent tobacco and alcohol use is multiplicative and may increase by as much as 300-fold[16].

Currently, in the United Kingdom, 22% of men and 21% of women are smokers[14], while 33% of men and 16% of women regularly drink in excess of recommended guidelines in excess of recommended guidelines[17]. Thus, a substantial number of individuals are at risk for these essentially preventable cancers. Although the risks decrease with abstinence from alcohol and smoking cessation these patients remain at risk for several years and, as illustrated in the present cases, the multiplicative risk may not decrease substantially if one of the behaviours is retained.

Co-misuse and carcinogenesis

Alcohol itself is not a carcinogen, but its first endogenous metabolite, acetaldehyde, is genotoxic and causes DNA damage due to N^2-ethyl-2'-deoxyguanosidyne stable adduct formation[18,19]. Many microbes in the oral flora possess alcohol dehydrogenase (ADH) activity and are able to oxidize ethanol to acetaldehyde. However, their ability to remove it is limited, thus potentiating its local carcinogenic activity. Furthermore, human oral and oesophageal mucosa has been shown to possess high Km ADH activity but to lack low Km aldehyde dehydrogenase activity favouring the accumulation of acetaldehyde in the saliva and upper digestive tract in the presence of alcohol. Tobacco smoke is a direct source of acetaldehyde; its concentration is more than 1000 times greater than that of the other well known constituent carcinogens, *e.g.*, polycyclic aromatic hydrocarbons (PAH) or tobacco-specific nitrosamines. Chronic smoking modifies the oral flora favouring greater production of acetaldehyde from ethanol. Thus, upper digestive tract exposure to acetaldehyde increases further with concomitant alcohol and tobacco usage.

Alcohol, when taken habitually in high amounts, activates the cytochrome P450 enzymes system. As many of the pro-carcinogens in tobacco smoke require enzymatic activation to exert their carcinogenic activity alcohol may change the carcinogen activation pathway related to these substances. In addition, the solvent properties of ethanol can also facilitate the contact of substances dispersed in smoke with the mucosa.

Locally, alcohol can also affects cellular membrane permeability and acts as a liquid phase into which carcinogens diffuse, thereby facilitating their penetration into the intracellular domain of mucosal epithelial cells in the mouth. The increased membrane permeability causes changes in the diffusion/uptake patterns of other substances for which ethanol can also act as solvent.

Three of the six patients in this series (cases 2, 3 and 5) were also heavy marijuana users. Inhaled marijuana smoke contains various carcinogens some, including PAH and acetaldehyde, in concentrations up to 50% higher than in tobacco smoke[20]. Thus, the use of marijuana has been implicated in cancer development by direct DNA damage. Conversely, marijuana smoke also contains cannabinoids which have been shown to have anti-neoplastic properties[21]. Little is known about the effects of these competing factors. However, in a recent case-control study[22], marijuana smokers with consumption histories of between 10 and 20 years of continuous use demonstrated a significantly reduced risk for the development of head and neck SCC, compared to nonusers. Drinkers and tobacco users who also smoked marijuana retained a high but attenuated risk for the development of these neoplasms. Little is known, however, about the cellular and molecular pathways affected by these complex associations[20].

Intervening in co-misuse

Multiple environmental pressures act on genetic factors to alter addictive behaviour. In twin studies there is an approximately 60% likelihood of alcohol or tobacco misuse in those with a family history of dependence[23]. Both substances modify the activity of the mesolimbic dopamine system and this may explain features such as mutual cravings, cross-tolerance[24], and the reinforcement and reward effects. The frequency of co-dependence attests also to a degree of interdependence[25].

It has been shown that smoking cessation improves alcohol-related outcomes, with a possible 25% increase in the likelihood of long-term abstinence, most likely due to more intensive clinical contact, reduced triggers for misuse, behavioural skills practice, and a healthier lifestyle[26]. Nevertheless, smoking behaviour is frequently ignored

in individuals presenting with alcohol-related problems for a variety of reasons including: a lack of resources, professional apathy, poor training, a perception that this approach is ineffectual or simply that quitting alcohol and tobacco simultaneously is too difficult.

However, despite the acknowledged difficulties, smoking behaviour should be address in co-dependent individuals if the health benefits of long-term abstinence from alcohol are to be maximized.

Sequential vs simultaneous smoking cessation programs

Ellingstad *et al*[27], reported that over three quarters of alcohol abusers who also smoked would be willing to consider smoking cessation during or after treatment for their alcohol problems.

The merits of simultaneous tobacco treatment *vs* sequential treatment for alcohol-dependent patients are unclear. Seidner *et al*[28], found that those who accepted, or would consider concurrent treatment for smoking and substance use were more confident in their ability to stop smoking, had more smoking quit attempts, were more likely to believe that quitting smoking would benefit the resolution of their substance abuse problem, and were more likely to believe that the best time to quit smoking was during treatment or in the following 6 mo. However, some argue that concurrent treatment may be of limited impact, or even detrimental to achieving abstinence from alcohol[29-31]. Thus, Joseph *et al*[30], randomized 499 patients undergoing intensive treatment for alcohol abuse/dependence to smoking intervention in the form of individual behavioural counselling and nicotine replacement, delivered either concurrently or else delayed for 6 mo. Participants in the concurrent treatment group were significantly more likely to participate in smoking treatment than the delayed group (79% *vs* 65%). However, abstinence rates in the concurrent group were significantly lower at both 1 mo (51% *vs* 64%) and 6 mo (41% *vs* 56%). Nonetheless, there were no significant differences in smoking cessation rates (approximately 12%-14%) or in abstinence rates (41% *vs* 48%) between the two groups at 18 mo[30].

The concern that smoking cessation interventions might compromise sobriety were not confirmed in a systematic review and meta-analysis of 19 randomised controlled trials which showed that combined intervention was associated with a 25% increased likelihood of long-term abstinence from alcohol; this effect was observed independently of long-term smoking cessation[26]. Smoking cessation interventions were successful in the short term whether provided simultaneously or sequentially. Nevertheless, at 6 mo there was no significant treatment effect irrespective of when they were delivered. However, the strikingly low overall quit rate (3%) among smokers assigned to control groups suggests that few participants will likely attempt cessation on their own[26]. Also noticeable was the fact that treatment success rates were noticeably higher in programmes providing nicotine replacement therapy[26]. Overall these finding support the provision of smoking cessation interventions without fear that sobriety will be detrimentally affected.

Management of nicotine addiction in patients with alcohol misuse

It is estimated that 80% to 90% of individuals with alcohol problems smoke cigarettes[25,27]. Current evidence suggests that public health interventions that discourage both smoking and drinking are likely to be more beneficial than addressing the problems individually[7]. Failure to address smoking issues when dealing with patients with alcohol-related problems might convey the message that smoking cessation is not a priority for recovery or health. It is, however, particularly difficult for individuals with a history of alcohol misuse to maintain smoking cessation in the longer term[32]. Standard treatment with 8 to 12 wk of counselling and nicotine replacement therapy is better than no treatment. However, the success rates in this population are low and more sustained and intensive regimens are likely to be needed. Pharmacotherapy, particularly with the newer agents such as bupropion and varenicline needs to be used with caution particularly in patients who may have sustained alcohol-related liver or brain damage[33]. There is, therefore, still a need to identify: (1) the optimal timing and method for engaging alcohol misusers into smoking treatments; (2) effective treatment strategies for this population, including motivational, cognitive and behavioural, and pharmacological interventions; (3) methods for integrating smoking cessation interventions within treatments for alcohol misuse/dependence; and (4) the feasibility of staff providing alcohol treatment services also providing smoking cessation interventions.

In conclusion, alcohol consumption and smoking act synergistically to increase the risk of upper aerodigestive cancers. This risk could be significantly reduced with timely intervention and treatment for both addictions. There is a need to raise awareness amongst healthcare professionals of the impact of not addressing the issues of multiple drug addictions and the need to create more focused treatment and prevention strategies.

REFERENCES

1 **Klatsky AL**, Armstrong MA. Alcohol, smoking, coffee, and cirrhosis. *Am J Epidemiol* 1992; **136**: 1248-1257 [PMID: 1476147]

2 **Franceschi S**, Talamini R, Barra S, Barón AE, Negri E, Bidoli E, Serraino D, La Vecchia C. Smoking and drinking in relation to cancers of the oral cavity, pharynx, larynx, and esophagus in northern Italy. *Cancer Res* 1990; **50**: 6502-6507 [PMID: 2208109]

3 **Pelucchi C**, Gallus S, Garavello W, Bosetti C, La Vecchia C. Cancer risk associated with alcohol and tobacco use: focus on upper aero-digestive tract and liver. *Alcohol Res Health* 2006; **29**: 193-198 [PMID: 17373408]

4 **Talamini R**, Bosetti C, La Vecchia C, Dal Maso L, Levi F, Bidoli E, Negri E, Pasche C, Vaccarella S, Barzan L, Franceschi S. Combined effect of tobacco and alcohol on laryngeal cancer risk: a case-control study. *Cancer Causes Control* 2002;

13: 957-964 [PMID: 12588092]
5 **Bosetti C**, Gallus S, Peto R, Negri E, Talamini R, Tavani A, Franceschi S, La Vecchia C. Tobacco smoking, smoking cessation, and cumulative risk of upper aerodigestive tract cancers. *Am J Epidemiol* 2008; **167**: 468-473 [PMID: 18056925 DOI: 10.1093/aje/kwm318]
6 **Franceschi S**, Levi F, Dal Maso L, Talamini R, Conti E, Negri E, La Vecchia C. Cessation of alcohol drinking and risk of cancer of the oral cavity and pharynx. *Int J Cancer* 2000; **85**: 787-790 [PMID: 10709096]
7 **Ansary-Moghaddam A**, Huxley RR, Lam TH, Woodward M. The risk of upper aero digestive tract cancer associated with smoking, with and without concurrent alcohol consumption. *Mt Sinai J Med* 2009; **76**: 392-403 [PMID: 19642154 DOI: 10.1002/msj.20125]
8 **Jiménez C**, Rodríguez D, Marqués E, Loinaz C, Alonso O, Hernández-Vallejo G, Marín L, Rodríguez F, García I, Moreno E. De novo tumors after orthotopic liver transplantation. *Transplant Proc* 2002; **34**: 297-298 [PMID: 11959293]
9 **Jiménez C**, Marqués E, Loinaz C, Romano DR, Gómez R, Meneu JC, Hernández-Vallejo G, Alonso O, Abradelo M, Garcia I, Moreno E. Upper aerodigestive tract and lung tumors after liver transplantation. *Transplant Proc* 2003; **35**: 1900-1901 [PMID: 12962840 DOI: 10.1016/S0041-1345(03)00641-9]
10 **Jiménez C**, Manrique A, Marqués E, Ortega P, Loinaz C, Gómez R, Meneu JC, Abradelo M, Moreno A, López A, Moreno E. Incidence and risk factors for the development of lung tumors after liver transplantation. *Transpl Int* 2007; **20**: 57-63 [PMID: 17181654]
11 **Jiménez-Romero C**, Manrique A, Marqués E, Calvo J, Sesma AG, Cambra F, Abradelo M, Sterup RM, Olivares S, Justo I, Colina F, Moreno E. Switching to sirolimus monotherapy for de novo tumors after liver transplantation. A preliminary experience. *Hepatogastroenterology* 2011; **58**: 115-121 [PMID: 21510297]
12 **Garavello W**, Bertuccio P, Levi F, Lucchini F, Bosetti C, Malvezzi M, Negri E, La Vecchia C. The oral cancer epidemic in central and eastern Europe. *Int J Cancer* 2010; **127**: 160-171 [PMID: 19882710 DOI: 10.1002/ijc.25019]
13 **Parkin DM**. 2. Tobacco-attributable cancer burden in the UK in 2010. *Br J Cancer* 2011; **105** Suppl 2: S6-S13 [PMID: 22158323 DOI: 10.1038/bjc.2011.475]
14 **Parkin DM**. 3. Cancers attributable to consumption of alcohol in the UK in 2010. *Br J Cancer* 2011; **105** Suppl 2: S14-S18 [PMID: 22158312 DOI: 10.1038/bjc.2011.476]
15 **Bagnardi V**, Blangiardo M, La Vecchia C, Corrao G. A meta-analysis of alcohol drinking and cancer risk. *Br J Cancer* 2001; **85**: 1700-1705 [PMID: 11742491 DOI: 10.1054/bjoc.2001.2140]
16 **Boyce P**, Gray N, Henningfield J, Seffrin J, Zatonski W. Tobacco: science, policy and public health. Oxford: Oxford University Press, 2004: pp. 399-432
17 Adult Psychiatric Morbidity Survey, 2007. Health and Social Care Information Centre, 2009. Available from: URL: http://www.hscic.gov.uk/pubs/psychiatricmorbidity07
18 **Boffetta P**, Hashibe M. Alcohol and cancer. *Lancet Oncol* 2006; **7**: 149-156 [PMID: 16455479]
19 **Salaspuro M**. Interactions of alcohol and tobacco in gastrointestinal cancer. *J Gastroenterol Hepatol* 2012; **27** Suppl 2: 135-139 [PMID: 22320931 DOI: 10.1111/j.1440-1746.2012.07017.x]
20 **Lopes CF**, de Angelis BB, Prudente HM, de Souza BV, Cardoso SV, de Azambuja Ribeiro RI. Concomitant consumption of marijuana, alcohol and tobacco in oral squamous cell carcinoma development and progression: recent advances and challenges. *Arch Oral Biol* 2012; **57**: 1026-1033 [PMID: 22727410 DOI: 10.1016/j.archoralbio.2012.05.006]
21 **Preet A**, Ganju RK, Groopman JE. Delta9-Tetrahydrocannabinol inhibits epithelial growth factor-induced lung cancer cell migration in vitro as well as its growth and metastasis in vivo. *Oncogene* 2008; **27**: 339-346 [PMID: 17621270 DOI: 10.1038/sj.onc.1210641]
22 **Liang C**, McClean MD, Marsit C, Christensen B, Peters E, Nelson HH, Kelsey KT. A population-based case-control study of marijuana use and head and neck squamous cell carcinoma. *Cancer Prev Res* (Phila) 2009; **2**: 759-768 [PMID: 19638490 DOI: 10.1158/1940-6207.CAPR-09-0048]
23 **Grucza RA**, Bierut LJ. Co-occurring risk factors for alcohol dependence and habitual smoking: update on findings from the Collaborative Study on the Genetics of Alcoholism. *Alcohol Res Health* 2006; **29**: 172-178 [PMID: 17373405]
24 **Funk D**, Marinelli PW, Lê AD. Biological processes underlying co-use of alcohol and nicotine: neuronal mechanisms, cross-tolerance, and genetic factors. *Alcohol Res Health* 2006; **29**: 186-192 [PMID: 17373407]
25 **Hurt RD**, Offord KP, Croghan IT, Gomez-Dahl L, Kottke TE, Morse RM, Melton LJ. Mortality following inpatient addictions treatment. Role of tobacco use in a community-based cohort. *JAMA* 1996; **275**: 1097-1103 [PMID: 8601929 DOI: 10.1001/jama.1996.03530380039029.]
26 **Prochaska JJ**, Delucchi K, Hall SM. A meta-analysis of smoking cessation interventions with individuals in substance abuse treatment or recovery. *J Consult Clin Psychol* 2004; **72**: 1144-1156 [PMID: 15612860 DOI: 10.1037/0022-006X.72.6.1144]
27 **Ellingstad TP**, Sobell LC, Sobell MB, Cleland PA, Agrawal S. Alcohol abusers who want to quit smoking: implications for clinical treatment. *Drug Alcohol Depend* 1999; **54**: 259-265 [PMID: 10372799 DOI: 10.1016/S0376-8716(98)00180-X]
28 **Seidner AL**, Burling TA, Gaither DE, Thomas RG. Substance-dependent inpatients who accept smoking treatment. *J Subst Abuse* 1996; **8**: 33-44 [PMID: 8743767 DOI: 10.1016/S0899-3289(96)90067-X]
29 **Bowman JA**, Walsh RA. Smoking intervention within alcohol and other drug treatment services: a selective review with suggestions for practical management. *Drug Alcohol Rev* 2003; **22**: 73-82 [PMID: 12745361]
30 **Joseph AM**, Willenbring ML, Nugent SM, Nelson DB. A randomized trial of concurrent versus delayed smoking intervention for patients in alcohol dependence treatment. *J Stud Alcohol* 2004; **65**: 681-691 [PMID: 15700504]
31 **Kodl M**, Fu SS, Joseph AM. Tobacco cessation treatment for alcohol-dependent smokers: when is the best time? *Alcohol Res Health* 2006; **29**: 203-207 [PMID: 17373410]
32 **Kalman D**, Kim S, DiGirolamo G, Smelson D, Ziedonis D. Addressing tobacco use disorder in smokers in early remission from alcohol dependence: the case for integrating smoking cessation services in substance use disorder treatment programs. *Clin Psychol Rev* 2010; **30**: 12-24 [PMID: 19748166 DOI: 10.1016/j.cpr.2009.08.009]
33 Smoking cessation services in primary care, pharmacies, local authorities and workplaces, particularly for manual working groups, pregnant women and hard to reach communities. NICE PH10, 2008. Available from: URL: http://www.nice.org.uk/PH010

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CASE REPORT

A novel alpha1-antitrypsin null variant (*PiQ0Milano*)

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Abstract

Alpha1-antitrypsin deficiency is an autosomal recessive disease characterized by reduced serum levels of alpha1-antitrypsin (AAT) due to mutations in the *SERPINA1* gene causing early onset pulmonary emphysema and, occasionally, chronic liver disease. We report an incidental finding of a novel null *AAT* allele, Q0Milano, consisting of a 17 nucleotides deletion in exon 3 of *SERPINA1* gene, in an Italian child with persistently increased liver enzymes, a mild decrease in circulating AAT levels and without any pulmonary disease. Q0Milano variant results in an unfunctional protein lacking of AAT active site, as the resultant protein is truncated near PiS locus involved in AAT protein stability.

Key words: Alpha1-antitrypsin deficiency; Rare variant; Alpha1-antitrypsin null mutation; Liver disease

Core tip: We report an incidental finding of a novel null alpha1-antitrypsin (*AAT*) allele, Q0Milano, consisting of a 17 nucleotides deletion in exon 3 of *SERPINA1* gene, in an Italian child with persistently increased in liver enzymes and a mild decrease in circulating AAT levels. Q0Milano variant results in an unfunctional protein lacking of AAT active site, as the resultant protein is truncated near PiS locus involved in AAT protein stability.

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INTRODUCTION

Alpha1-antitrypsin deficiency (AATD) is an autosomal recessive disease characterized by reduced serum levels of alpha1-antitrypsin (AAT, *SERPINA1*), a 52 kDa glycoprotein functioning as the main extracellular protease inhibitor. AAT is mainly produced by liver, which releases about 2 g of AAT daily into the circulation under physiological conditions. The normal serum concentration may range between 1.5-3.5 g/L (or 20-48 μmol/L)[1]. AATD is associated with early onset pulmonary emphysema and, occasionally, with chronic liver disease in childhood, hepatocellular carcinoma and/or cirrhosis in adulthood[2]. AAT functions as neutrophil elastase inhibitor, playing a key role in the protection of the lower respiratory tract. AAT serum levels below 11 μmol/L are not sufficient to inhibit elastase *in vivo*, permitting progressive destruction of alveoli culminating in emphysema[3-5]. The pathophysiology of liver disease related to AATD is less well understood, but some deficient variants accumulate in endoplasmic reticulum of hepatocytes and are inefficiently secreted, leading to protein aggregation and culminating

Table 1 Sequence of primers used in Alpha1-antitrypsin coding sequence amplification and sequencing

	Primers forward 5′→3′	Primers reverse 5′→3′
AAT Ex2A	CCCCCCATCTCTGTCTTGC	GAGGAGTTCCTGGAAGCCTT
AAT Ex2B	ATGAAATCCTGGAGGGCCTG	CAGGCTGGTTGAGCAACCTT
AAT Ex3	CCCACCTTCCCCTCTCTCC	CACCCTCAGGTTGGGGAATC
AAT Ex4	CTTGAATTTCTTTTCTGCACGAC	AAGGTCGTCAGGGTGATCTC
AAT Ex5	GTCTCTGCTTCTCTCCCCTC	AGGGACCAGCTCAACCCTTC

AAT: Alpha1-antitrypsin; Ex: Exon.

in hepatocytes injury and liver disease[6].

AATD is caused by mutations in *SERPINA1*, a highly polymorphic genetic locus located on the distal long arm of chromosome 14. More than 100 alleles have been identified. They can be classified according to AAT serum levels and protein functionality: (1) normal variants, all common M types, accounting for 95% of those found in Caucasian individuals, and characterized by normal plasma levels (more than 20 μmol/L); (2) deficient variants associated with reduced AAT serum levels, lower than 20 μmol/L; (3) null variants determining undetectable serum levels; and (4) dysfunctional variants characterized by normal serum levels of dysfunctional AAT protein[7].

The firstly described, and most common cause of AATD, associated with very low serum concentration of the protein, is homozygosity for the PiZ mutation, the most severe AAT deficient variant known with plasma levels among homozygotes of about 5-6 μmol/L, resulting in the development of lung and liver disease[8]. It became later clear that AATD is a heterogeneous disease, caused by several gene defects expressed codominantly, mostly determining reduced serum AAT levels.

The most common deficient alleles are *PiS* and *PiZ*, with an allelic frequency of 2%-4% and 1%-2% respectively in Caucasian population, and are both caused by missense mutations responsible for intracellular protein accumulation and degradation. The *PiZ* mutation leads to a conformational change of AAT reactive site into a β-sheet polymer which forms characteristic periodic acid-Schiff-positive inclusions and can be isolated for liver of AAT PiZZ subjects. Several studies have shown that *PiMZ* in heterozygous state may lead to chronic liver disease, cryptogenic cirrhosis, and chronic active hepatitis[9-11], while the PiS variant is associated to liver disease only if carried in compound heterozygosity with the *PiZ* allele[12]. Null alleles are very rare (frequency < 0.001, 13% of AATD subjects in Italy[13]) and derived from nucleotide deletion, insertion, or non-sense mutations, causing premature stop codons and producing structurally unstable and truncated protein. Individuals with null-null AAT phenotype are not affected by liver disease, because of the lack of aggregation of mutant proteins in the endoplasmic reticulum, but are associated with an increased risk of emphysema.

In this study we report a novel AAT allele in a child with reduced protein levels.

CASE REPORT

An 11-years-old male child was referred to our centre for a persistent increase in liver enzymes (aspartate aminotransferase and alanine aminotransferase spanning from 58-239 UI/L, and 98-114 UI/L respectively). All common causes of liver disease were excluded, but mildly decreased AAT serum levels were detected (76 mg/dL). The subject did not show abnormalities in pulmonary function.

Sequencing of the proband revealed a novel null mutation in *AAT* gene (Table 1), g.9752-9768del ($PiQ0_{Milano}$) (gene ID: 5265, official name SERPINA1, genomic sequence number: NC_000014.8; NCBI Reference Sequence: NG_008290.1). This variant, localized in exon 3 near PiS locus (p.Glu264Val), consists in a 17 bp deletion (AAA CTA CAG CAC CTG GA), resulting in a frameshift causing a new stop codon downstream the deletion site (Figure 1), which leads to a premature termination of protein translation at amino acid 259. The truncated protein lacks of AAT active site centred around Met^{358}-Ser^{359}.

The mutation arose in *PiM3 AAT* allele, which the proband inherited from his mother, whose genotype was $PiM1A/Q0_{Milano}$ and had normal liver and pulmonary function, whereas his father, who had normal genotype *PiM1V*/*PiM3*, showed nonalcoholic steatohepatitis associated with hyperferritinemia (Figure 2). Liver biopsy of the proband showed aspecific findings, unrelated to AATD.

DISCUSSION

The g.9752-9768del mutation ($Q0_{Milano}$) occurs in a key functional region of AAT gene where several other deficiency variants (P_{Lowell}/P_{Duarte} at codon 256, $Q0_{Cairo}$ at codon 259, T/S at codon 264) have been reported[14-18].

Several mechanisms are responsible for AAT deficiency, including: gene deletion, mRNA degradation, intracellular protein accumulation and degradation, and production of dysfunctional proteins. Only mutations causing intracellular protein accumulation and polymerization of the newly synthesized protein are associated with increased risk of liver disease, as in PiZ and in PiM_{Malton} variants[19]. Many previously described null mutations (Pi_{Duarte}, $Pi_{Hong\ Kong}$, $Pi_{Granite\ Falls}$) have been associated with intra-reticular accumulation of unfunctional AAT proteins, which are immediately degraded without any

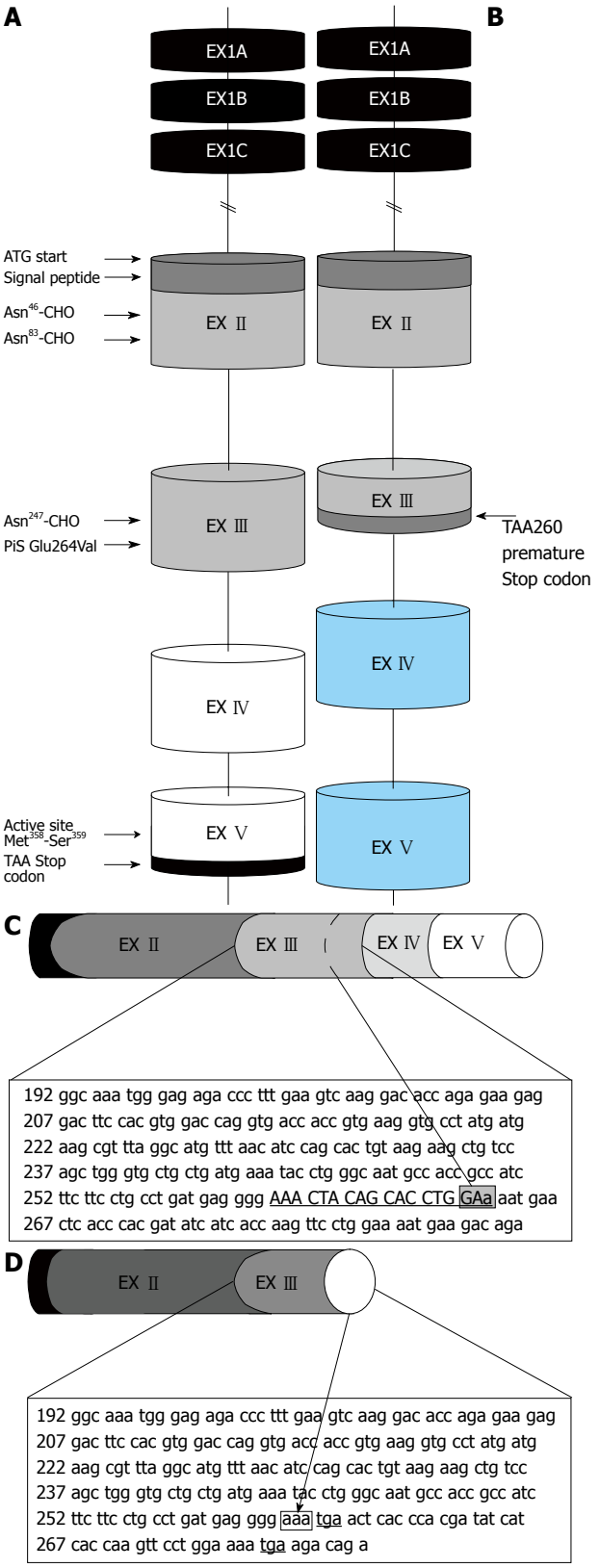

Figure 1 Schematic representation of wild type and mutant Alpha1-antitrypsin gene and protein. A: Alpha1-antitrypsin (*AAT*) wild type gene; B: *AAT* mutated gene: Bule region represents out of frame sequence. Untranslated regions are shown in black; C: AAT wild type protein: Exon 3 wild-type sequence is indicated in square; the deleted nucleotides are underlined; PiS locus is highlighted; D: AAT truncated unfunctional protein; Exon 3 deleted sequence is indicated below; premature stops codons resulting from frameshift were underlined.

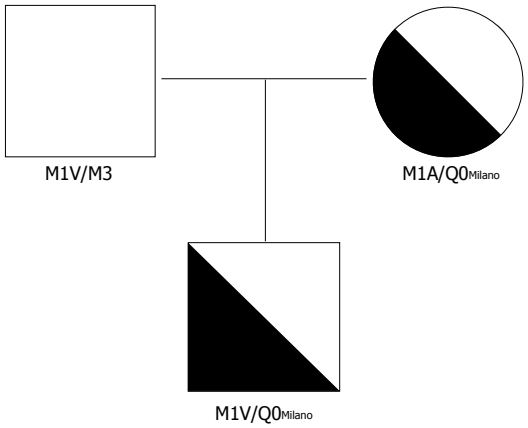

Figure 2 Pedigree of the Q0Milano proband's family. Alpha1-antitrypsin genotypes are listed below.

liver damage[20-22]. Heterozygosity for this novel null mutation is consistent with the lower AAT serum levels (76 mg/dL) measured in the proband, which collocates the patient in intermediate deficiency condition comparable to those of individuals carrying PiMZ genotype[23].

However, it is unlikely that this genetic variant explained liver disease in the proband, as it was carried in heterozygous state, and it does not affected liver function tests of the mother. Moreover, the absence of periodic acid-Schiff-positive inclusions revealed in liver biopsy excluded hepatic AAT protein accumulation. Thus, the novel null *AAT* variant was not responsible for liver damage because of the lack of hepatic protein polymerization.

It is likely that other hepatotoxic insults, as non alcoholic steatohepatitis associated with hyperferritinemia, a strongly heritable condition reported in the father of proband[24,25], were involved in the development of liver disease.

In conclusion, in this study we report a novel *AAT* null variant (*Q0Milano*) generated by a 17 nucleotides deletion in exon 3 of *AAT*, which leads to a premature stop codon.

REFERENCES

1 **Fagerhol MK**, Laurell CB. The polymorphism of "prealbumins" and alpha-1-antitrypsin in human sera. *Clin Chim Acta* 1967; **16**: 199-203 [PMID: 4166396]
2 **Crystal RG**. Alpha 1-antitrypsin deficiency, emphysema, and liver disease. Genetic basis and strategies for therapy. *J Clin Invest* 1990; **85**: 1343-1352 [PMID: 2185272]
3 **Gadek JE**, Fells GA, Zimmerman RL, Rennard SI, Crystal RG. Antielastases of the human alveolar structures. Implications for the protease-antiprotease theory of emphysema. *J Clin Invest* 1981; **68**: 889-898 [PMID: 6169740]
4 **Wewers MD**, Casolaro MA, Sellers SE, Swayze SC, McPhaul KM, Wittes JT, Crystal RG. Replacement therapy for alpha 1-antitrypsin deficiency associated with emphysema. *N Engl J Med* 1987; **316**: 1055-1062 [PMID: 3494198]
5 **Ferrarotti I**, Thun GA, Zorzetto M, Ottaviani S, Imboden M, Schindler C, von Eckardstein A, Rohrer L, Rochat T, Russi EW, Probst-Hensch NM, Luisetti M. Serum levels and genotype distribution of α1-antitrypsin in the general

population. *Thorax* 2012; **67**: 669-674 [PMID: 22426792 DOI: 10.1136/thoraxjnl-2011-201321]
6 **Carrell RW**, Lomas DA. Alpha1-antitrypsin deficiency--a model for conformational diseases. *N Engl J Med* 2002; **346**: 45-53 [PMID: 11778003]
7 **Zaimidou S**, van Baal S, Smith TD, Mitropoulos K, Ljujic M, Radojkovic D, Cotton RG, Patrinos GP. A1ATVar: a relational database of human SERPINA1 gene variants leading to alpha1-antitrypsin deficiency and application of the VariVis software. *Hum Mutat* 2009; **30**: 308-313 [PMID: 19021233 DOI: 10.1002/humu.20857]
8 **Laurell CB**, Eriksson S. The electrophoretic α1-globulin pattern of serum in α1-antitrypsin deficiency. *COPD* 2013; **10** Suppl 1: 3-8 [PMID: 23527532 DOI: 10.1080/00365516309051324]
9 **Hodges JR**, Millward-Sadler GH, Barbatis C, Wright R. Heterozygous MZ alpha 1-antitrypsin deficiency in adults with chronic active hepatitis and cryptogenic cirrhosis. *N Engl J Med* 1981; **304**: 557-560 [PMID: 6969850]
10 **Graziadei IW**, Joseph JJ, Wiesner RH, Therneau TM, Batts KP, Porayko MK. Increased risk of chronic liver failure in adults with heterozygous alpha1-antitrypsin deficiency. *Hepatology* 1998; **28**: 1058-1063 [PMID: 9755243]
11 **Fischer HP**, Ortiz-Pallardó ME, Ko Y, Esch C, Zhou H. Chronic liver disease in heterozygous alpha1-antitrypsin deficiency PiZ. *J Hepatol* 2000; **33**: 883-892 [PMID: 11131449]
12 **Chan CH**, Steer CJ, Vergalla J, Jones EA. Alpha1-antitrypsin deficiency with cirrhosis associated with the protease inhibitor phenotype SZ. *Am J Med* 1978; **65**: 978-986 [PMID: 217266]
13 **Ferrarotti I**, Baccheschi J, Zorzetto M, Tinelli C, Corda L, Balbi B, Campo I, Pozzi E, Faa G, Coni P, Massi G, Stella G, Luisetti M. Prevalence and phenotype of subjects carrying rare variants in the Italian registry for alpha1-antitrypsin deficiency. *J Med Genet* 2005; **42**: 282-287 [PMID: 15744045]
14 **Faber JP**, Weidinger S, Goedde HW, Ole K. The deficient alpha-I-antitrypsin phenotype PI P is associated with an A-to-T transversion in exon III of the gene. *Am J Hum Genet* 1989; **45**: 161-163 [PMID: 2787118]
15 **Graham A**, Kalsheker NA, Newton CR, Bamforth FJ, Powell SJ, Markham AF. Molecular characterisation of three alpha-1-antitrypsin deficiency variants: proteinase inhibitor (Pi) nullcardiff (Asp256----Val); PiMmalton (Phe51----deletion) and PiI (Arg39----Cys). *Hum Genet* 1989; **84**: 55-58 [PMID: 2606478]
16 **Hildesheim J**, Kinsley G, Bissell M, Pierce J, Brantly M. Genetic diversity from a limited repertoire of mutations on different common allelic backgrounds: alpha 1-antitrypsin deficiency variant Pduarte. *Hum Mutat* 1993; **2**: 221-228 [PMID: 8364590]
17 **Zorzetto M**, Ferrarotti I, Campo I, Balestrino A, Nava S, Gorrini M, Scabini R, Mazzola P, Luisetti M. Identification of a novel alpha1-antitrypsin null variant (Q0Cairo). *Diagn Mol Pathol* 2005; **14**: 121-124 [PMID: 15905697]
18 **Curiel DT**, Chytil A, Courtney M, Crystal RG. Serum alpha 1-antitrypsin deficiency associated with the common S-type (Glu264----Val) mutation results from intracellular degradation of alpha 1-antitrypsin prior to secretion. *J Biol Chem* 1989; **264**: 10477-10486 [PMID: 2567291]
19 **Eriksson S**, Carlson J, Velez R. Risk of cirrhosis and primary liver cancer in alpha 1-antitrypsin deficiency. *N Engl J Med* 1986; **314**: 736-739 [PMID: 3485248]
20 **Lieberman J**, Gaidulis L, Klotz SD. A new deficient variant of alpha1-antitrypsin (MDUARTE). Inability to detect the heterozygous state by antitrypsin phenotyping. *Am Rev Respir Dis* 1976; **113**: 31-36 [PMID: 1082282]
21 **Nukiwa T**, Takahashi H, Brantly M, Courtney M, Crystal RG. alpha 1-Antitrypsin nullGranite Falls, a nonexpressing alpha 1-antitrypsin gene associated with a frameshift to stop mutation in a coding exon. *J Biol Chem* 1987; **262**: 11999-12004 [PMID: 3040726]
22 **Sifers RN**, Brashears-Macatee S, Kidd VJ, Muensch H, Woo SL. A frameshift mutation results in a truncated alpha 1-antitrypsin that is retained within the rough endoplasmic reticulum. *J Biol Chem* 1988; **263**: 7330-7335 [PMID: 3259232]
23 **Zorzetto M**, Russi E, Senn O, Imboden M, Ferrarotti I, Tinelli C, Campo I, Ottaviani S, Scabini R, von Eckardstein A, Berger W, Brändli O, Rochat T, Luisetti M, Probst-Hensch N. SERPINA1 gene variants in individuals from the general population with reduced alpha1-antitrypsin concentrations. *Clin Chem* 2008; **54**: 1331-1338 [PMID: 18515255 DOI: 10.1373/clinchem.2007.102798]
24 **Dongiovanni P**, Anstee QM, Valenti L. Genetic Predisposition in NAFLD and NASH: Impact on Severity of Liver Disease and Response to Treatment. *Curr Pharm Des* 2013; **19**: 5219-5238 [PMID: 23394097]
25 **Dongiovanni P**, Fracanzani AL, Fargion S, Valenti L. Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. *J Hepatol* 2011; **55**: 920-932 [PMID: 21718726 DOI: 10.1016/j.jhep.2011.05.008]

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13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23243641.

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

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