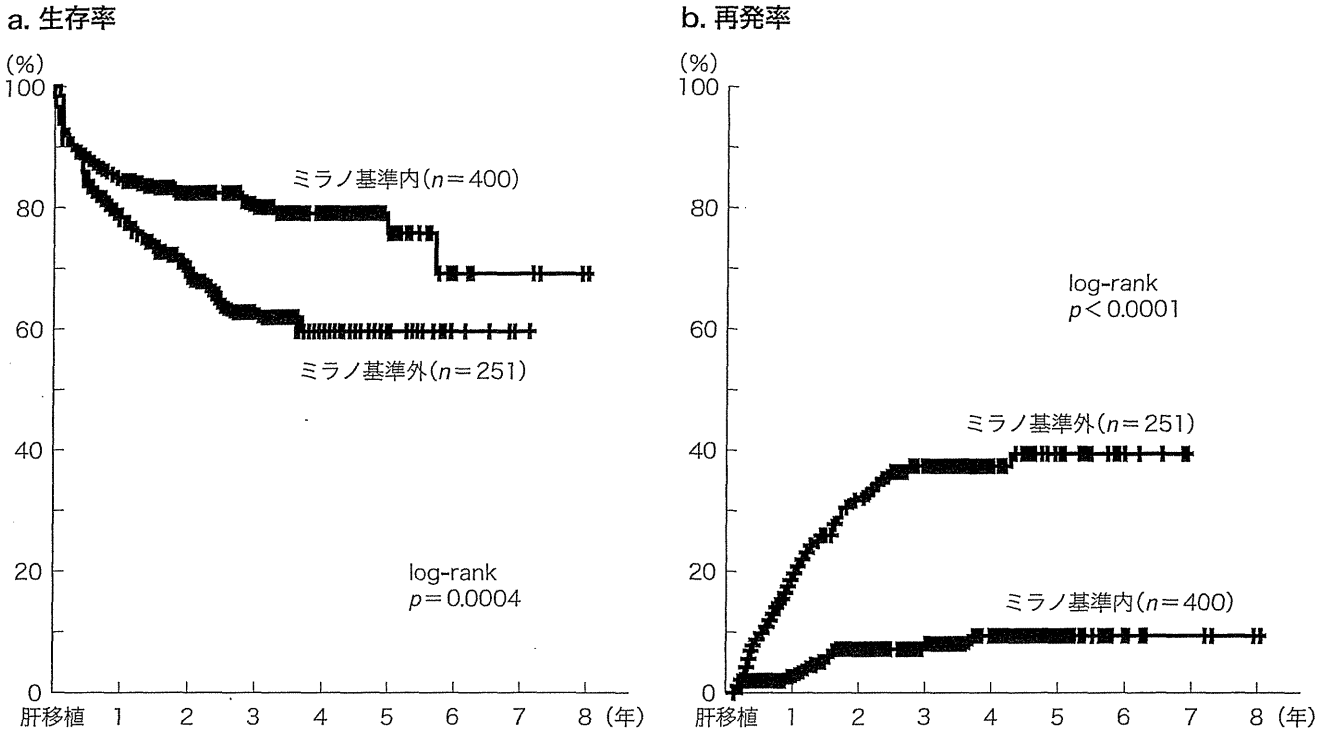

Fig. 2. 肝細胞癌に対する生体肝移植のミラノ基準内外別生存曲線と再発率

［文献 6)より引用，改変］

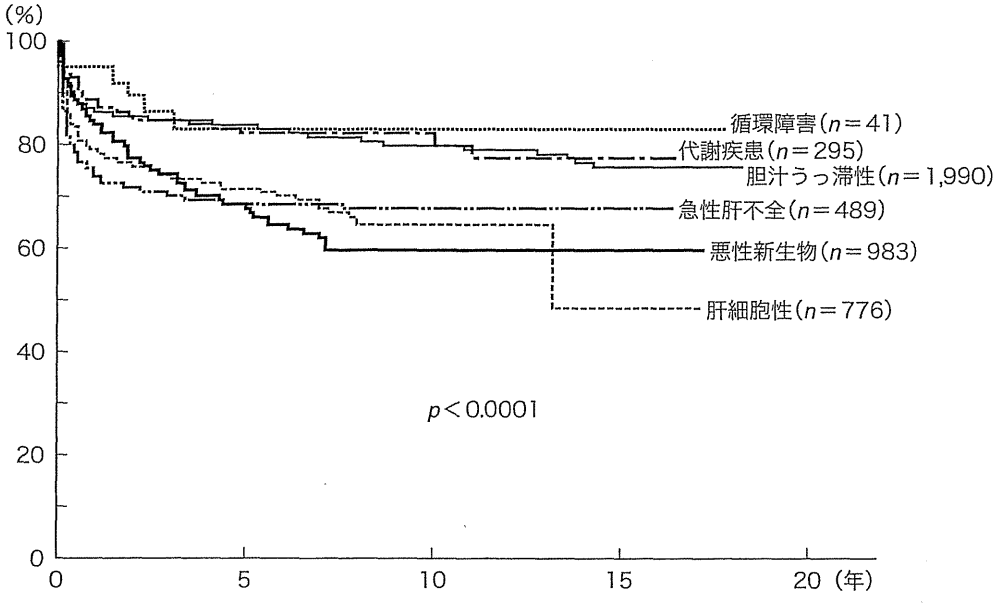

Fig. 3. 日本肝移植研究会の生体肝移植の生存曲線

［文献 7)より引用，改変］

が望める背景肝を有する肝癌は，局所療法が優先するという理論を展開したい．最近の抗ウイルス療法の進歩により，たとえばC型肝炎ウイルス(HCV)陽性慢性肝疾患に出現した肝癌に対して，初回局所療法施行後に抗ウイルス療法を施行し，有意に肝癌の再発率を抑制したという報告が散見される．これは局所再発を制御するのでなく，多中心性発癌を抑制すると解釈される．一方，B型

肝炎ウイルス(HBV)陽性慢性肝疾患から出現する肝癌に対しては,副作用が低率であることより,より積極的に核酸アナログ製剤などの抗ウイルス療法が施行されている．これら投与により，肝癌の再発率は抑制されつつあるが，統計学的には有意な差は今までのところ示されていないがその傾向はある．したがって，初発肝癌に対して，たとえばHCV陽性の場合，年齢，ウイルスの遺伝子型，背景肝の線維化の程度，ウイルス量，ホスト因子のIl28βのmajor alleleを有するなどにより抗ウイルス療法が効果的と考えられる場合は，その再発率抑止の観点から局所療法を第一選択とする価値が十分あるといえる．

そして，その場合，1度目の再発時点で肝移植の適応となるとしたほうが理解はされやすいと思う．それは肝癌の再発は局所制御後，複数回みられる．再発が繰り返されれば繰り返されるほど再発率は高まることが知られている．また，肝移植の成績からみると，肝癌の肝移植術前の治療回数が多いほど，その成績がわるいことが知られている．したがって，局所療法をなしえて，かつ術後に抗ウイルス療法がなされる条件の肝癌でも一度再発すれば，その時点で肝移植の適応が妥当であると考える．

結　　論

基本的には高癌化状態から見出された肝癌は局所療法後，高頻度に再発を認める．このことを考えると高癌化状態を正常の肝臓に置換することは臨床的に意義深いと考えると，肝移植が第一選択肢となるのは当然のことである．しかも，今までの脳死，生体肝移植の成績をみても，ミラノ基準を遵守している肝癌は逸脱している肝癌より再発率も生存率も明らかに異なるため，高癌化状態から肝癌が比較的早期に見出された場合は，局所療法でなく肝移植が第一選択肢として考慮すべきと考える．高癌化状態とはHCVあるいはHBV陽性の慢性肝炎，肝硬変であり，これらは術後の再感染予防を積極的に行うことでグラフトを損なうことなく治療効果が高まり，肝切除などの局所療法を選択した場合に比べて，明らかにその生存率が高いことが判明している．

その場合，今まで必要としていた肝予備力や肝障害度は考慮せず，慢性肝炎の段階でも肝移植を第一選択肢として何ら不思議なことではなくなるであろう．しかし，正常肝臓，肝炎ウイルス以外の原因による肝癌の場合は，肝炎ウイルス陽性の場合に比して，肝癌の再発率はきわめて低率であることが知られていることより，この場合は局所療法が第一選択肢になるであろう．したがって，すべての肝癌が肝移植の第一選択肢のなりうることはないことは明記しておく．

すなわち，高癌化状態からの肝癌は肝移植が第一選択肢になるという理論が成り立つわけである．

今後の展望

ミラノ基準は10年前の画像診断により1結節であれば最大径5cm未満，3結節の場合は最大径3cm未満で，いずれも脈管浸潤の存在が否定できているものとされている．はたして，この基準を現在社会に当てはめてよいものであろうか．

脈管浸潤を画像で同定することはVP1までで，microinvasionを画像で描出ことは困難である．さらに，最近の画像診断の進歩から，より細かい腫瘍結節が見出されるようになり，それらをミラノ基準に厳格に当てはめると，多くの例で適応外になる恐れがある．

さらに，ミラノ基準外の生体肝移植の肝癌に対する成績をみても，約40%は再発を認めるが残りの約60%はミラノ基準を逸脱しても再発を認めていない[6](Fig. 2)．このことは，大きさや個数だけで判断するのでなく，肝癌の有するmalignant potentialもしくは脈管浸潤能を的確に判断することのほうがより合理的と考える．すなわち，何らかのバイオマーカーが必要になるであろう．現在，われわれが臨床的に有意義なマーカーは腫瘍マーカーとしてのAFPとPIVKAIIであり，こ

れらはある程度腫瘍進展度と相関性を示す場合がある．これら既存のバイオマーカーのみならず，新しいマーカーの発展が不可欠になってくるものと思われる．

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＜Editorial＞

わが国における肝移植の現状

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索引用語：　脳死肝移植　　生体肝移植　　脳死ドナー　　生体ドナー

はじめに

わが国では平成22年1月17日に法改正の一弾として家族間への脳死ドナー提供が可能になったのち，同年7月17日には家族の同意による脳死ドナーによる脳死肝移植が可能になったことで，格段に脳死ドナーが増加するようになり，合わせて脳死肝移植の実施数が増加してきた．

今まで，年間10数例に満たなかった脳死肝移植が半年間で30例にまで増加したことは今後の肝移植医療の発展に大いなる影響があるものと推測される．筆者は日本脳死肝移植適応評価委員会の委員長として平成20年の12月から脳死肝移植施設からの脳死肝移植の適応評価を行ってきた．その立場で本号に韓国の肝移植事情を報告した金論文（編集部註：號末の特別寄稿参照）に対して，わが国の肝移植事情と合わせて所見を述べさせていただく．

脳死肝移植の増加推移

わが国では1997年10月の臓器移植法案施行後，しばらくは，脳死肝移植は実践されず，1999年3月に初めて成人間の脳死肝移植が施行された．その後，年間10例前後の実施数で，具体的には2010年末まで95件の脳死肝移植が施行されている（Fig. 1）（2011年1月3日，1月15日に2件の脳死肝移植があり，1月31日現在97件である）．最大で2008年に見られた13例に比して，2010年の32例は諸外国に比べると少ないかも知れないが，その進捗率は肝移植医療に長年携わっている者としては劇的であると思わざるを得ない．

この劇的な変化は，紆余曲折をへて実施された臓器移植法案の改正が成された2010年7月17日以降に認められるようになってきた．

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とくに2010年における心停止下提供と脳死下提供の推移は極めて興味深い．心停止下の臓器提供は年間平均80例であり，その年次推移を見ると一年間を通して少ない年で59例，多い年で102例の心停止後の臓器提供があった．

一方，脳死下の臓器提供数は最大で年間13例であったが，2010年になると29例にまで増加した．当初から臓器移植法改正後の脳死下提供数は心停止下提供の数と同じぐらいであろうと推測されていた．2010年でみると，心停止下提供は法改正前で45例，法改正後36例の81例と過去の心停止下提供の年間平均とそれほど変動はない．そして，脳死下提供数は2010年では法改正前に3例であったが，法改正後は29例と圧倒的にその数が増加した．

このことは，単に脳死下提供の増加が今までの心停止下提供数をそのまま受け継ぐのでなく，それらを上回る脳死下臓器提供数であることが判明しつつある．すなわち，家族の同意により脳死下提供が許されるようになってから，少なくとも臓器提供の関心の高まりが数字に反映されているものと推測される（Fig. 2）．このことは2010年の単年度を見ても理解され，心停止下提供は法改正後も改正前と同程度もしくはそれ以上の提供数として認識される．すなわち法改正前は月平均7例であったが，法改正後では6.4例と大きな変化はないことを物語っている．

臓器移植法改正後の脳死ドナーと肝移植実施状況（Table 1）（2011年1月19日現在）

個人情報の守秘の観点から法改正後の脳死下提供と脳死肝移植の具体的な項目は述べることは困難であるがTable 1にその概略を示した．

31例の脳死ドナー提供があり，28例の脳死ドナーが肝移植に適応された．その中で2例の脳死ドナーから二人のレシピエントへの提供となった分割肝による肝移植が実施された．したがって，脳死肝移植を受けた

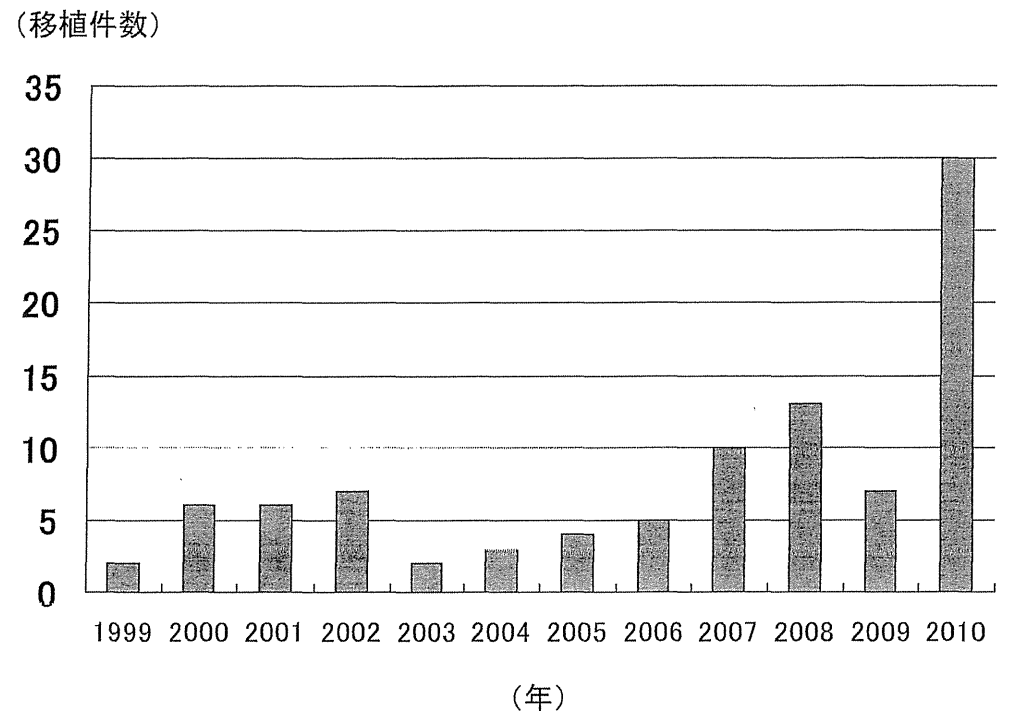

Fig. 1　脳死肝移植件数（1999年から2010年）95例の年次別推移

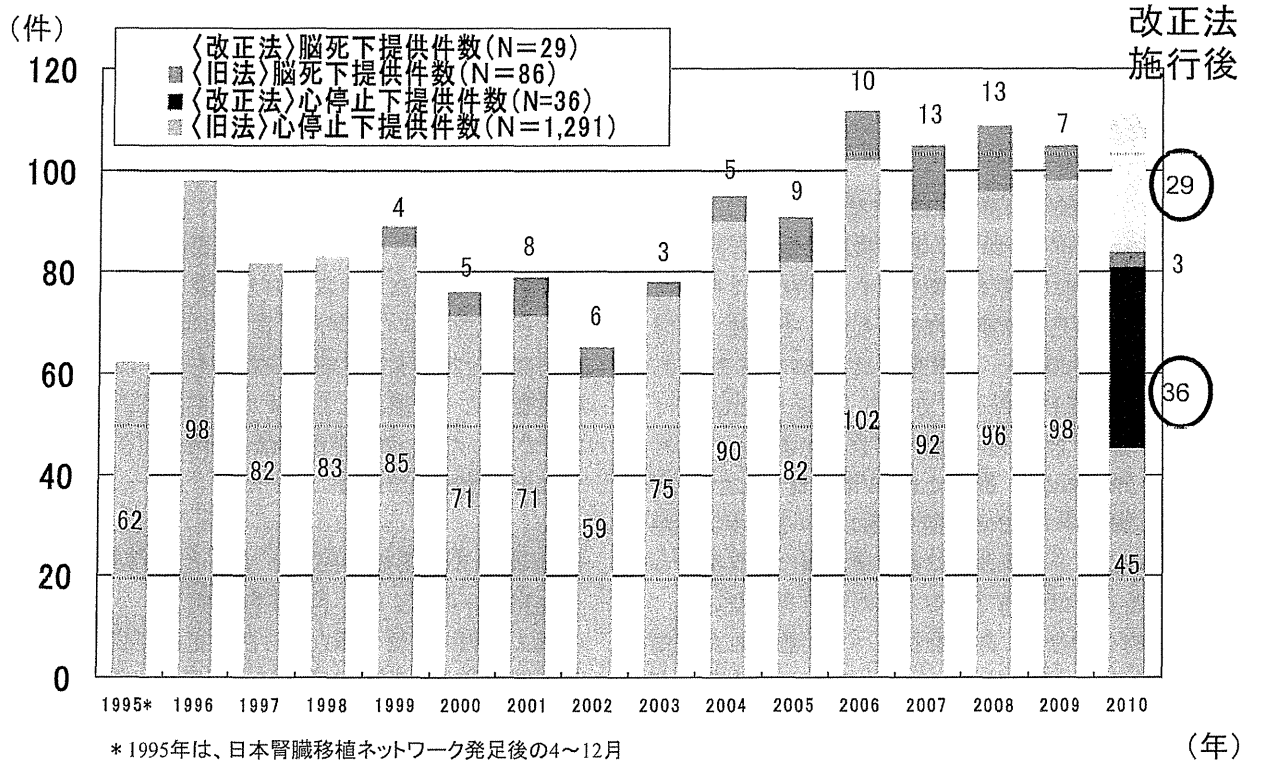

Fig. 2　臓器提供件数の年次別推移

Table 1　法改正後の脳死ドナーと肝移植の実施状況

提供日	移植施設	レシピエント	病名	提供施設	ドナーカード
2010/8/10	関東地区大学病院	60歳代女性	C型ウイルス性肝硬変	関東甲信越	無
2010/8/19	近畿地区大学病院	40歳代男性	ウィルソン病	近畿	無
2010/8/22	近畿地区大学病院	60歳代男性	B型ウイルス性肝硬変	東海	無
2010/8/27	北海道地区大学病院	30歳代女性	二次性胆汁性肝硬変	愛媛県	有
2010/8/29	関東地区研究センター	10歳未満女児	劇症肝炎	関東甲信越	無
2010/8/29	近畿地区大学病院	50歳代男性	C型ウイルス性肝硬変	関東甲信越	無
2010/9/2	中部地区大学病院	60歳代男性	C型ウイルス性肝硬変	九州	無
2010/9/4	中部地区大学病院	50歳代女性	非B非C型肝硬変	東北	無
2010/9/7	北海道地区大学病院	60歳代女性	C型ウイルス性肝硬変	関東甲信越	無
2010/9/12	関東地区大学病院	50歳代女性	C型ウイルス性肝硬変	北海道	無
2010/9/18	近畿地区大学病院	10歳代女性	原発性硬化性胆肝炎, 生体肝移植後グラフト不全	近畿	無
2010/9/18	中四国地区大学病院	40歳代男性	B型ウイルス性肝硬変	近畿	無
2010/9/25	肝提供なし			九州	無
2010/9/27	近畿地区大学病院	50歳代男性	C型ウイルス性肝硬変	北海道	無
2010/9/30	近畿地区大学病院	60歳代女性	C型ウイルス性肝硬変	北海道	無
2010/9/30	近畿地区大学病院	20歳代女性	胆道閉鎖症，肝再移植後肝不全	宮城県	無
2010/10/3	中四国地区大学病院	40歳代男性	B型ウイルス性肝硬変	関東	無
2010/10/13	近畿地区大学病院	30歳代男性	胆道閉鎖症	西日本	無
2010/11/3	中四国地区大学病院	50歳代男性	C型ウイルス性肝硬変	九州	無
2010/11/21	関東地区大学病院	40歳代男性	C型ウイルス性肝硬変	中部	無
2010/11/26	肝提供なし			中四国	無
2010/11/26	関東地区研究センター	10歳代男性	Oxalosis	北海道	無
2010/12/2	関東大学病院	50歳代男性	劇症肝炎	関東	無
2010/12/4	肝提供なし			九州	無
2010/12/10	中四国地区大学病院	30歳代男性	劇症肝炎	近畿	無
2010/12/13	中四国地区大学病院	50歳代女性	B型ウイルス性肝硬変	九州	無
2010/12/17	中部地区大学病院	50歳代女性	C型ウイルス性肝硬変	北海道	無
2010/12/18	中部地区大学病院	30歳代男性	非B非C型肝硬変	中部	無
2010/12/18	近畿地区大学病院	40歳代女性	原発性胆汁性肝硬変	関東	無
2010/12/25	近畿地区大学病院	10歳代女性	胆道閉鎖症・ 生体肝移植後肝不全腎不全	中部	無
2010/12/29	北海道地区大学病院	40歳代女性	原発性胆汁性肝硬変	近畿	無
2011/1/2	中四国地区大学病院	60歳代男性	B型ウイルスによる急性肝不全	北陸	無
2011/1/14	北海道地区大学病院	50歳代女性	B型ウイルス性肝硬変	北海道	

レシピエントは30例になる．

その脳死ドナー31例を検討すると，1例にのみドナーが意思表示カードを持参していたが，残りの30例は意思表示カードを持参せず，その脳死肝移植は家族の同意に基づくドナー提供であった．

脳死肝移植の成績；保存時間と総阻血時間との関係

肝臓ドナー提供施設の地域と脳死肝移植を施行した地域を見ると，北海道で提供があったドナー肝臓が近畿地方，中四国地方へと輸送され脳死肝移植が行われているという実態が判明している．脳死肝移植は脳死腎臓移植のような地域内での移植という機能は作動せず，現在は医学的緊急性が高く，血液型が合致した順番にレシピエントが選択される方法である．したがって，上述のようなドナー肝臓を遠隔地に運ぶという事態に及んでいる．このことは極めて不合理で，かつ脳死肝移植の成績にも影響を及ぼす極めて重要な検討課題である．

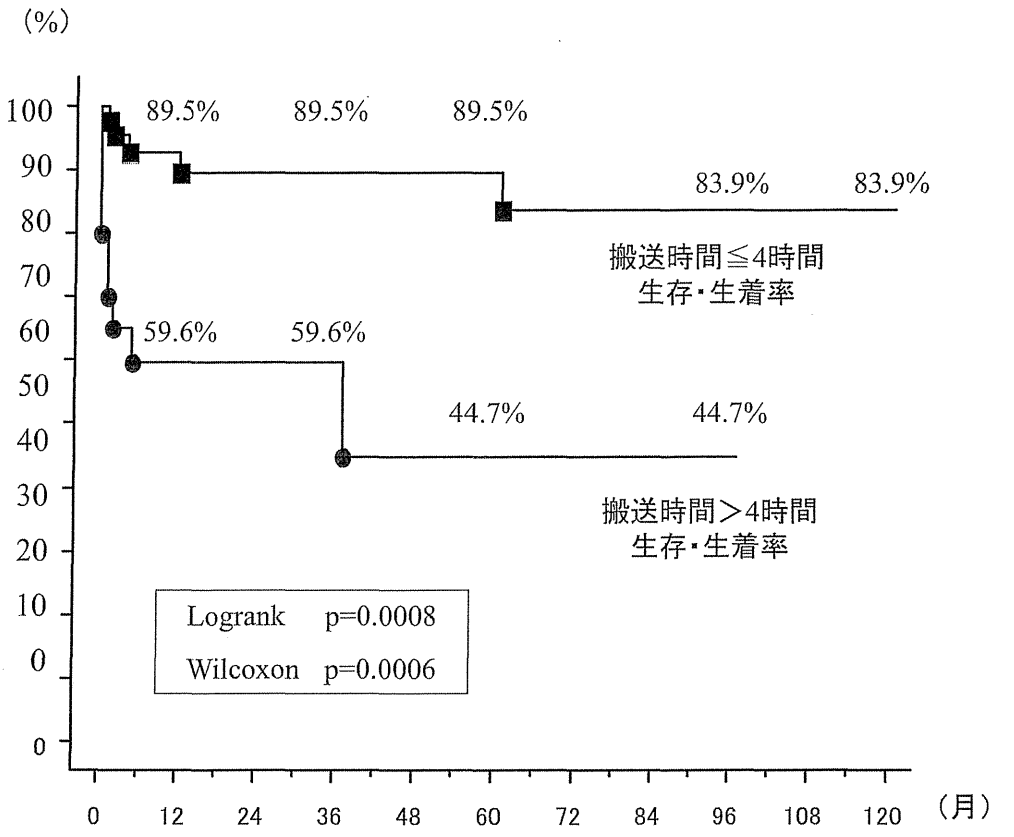

Fig. 3　脳死肝移植における搬送時間とその生存，生着率
搬送時間4時間以内と以上で優位に生存，生着率が異なる．

日本臓器移植ネットワークから，法改正前の脳死肝移植における脳死ドナーの保存時間と総阻血時間と生存率を検討した報告が成されている．過去の脳死肝移植症例の長期生存率あるいはグラフト生存率はグラフトの保存時間と総阻血時間が短い方が明らかに延長している（Fig. 3，4）．すなわち，狭いわが国とは言いながらも北海道で提供されたドナー肝臓を長い時間をかけて運搬するということは理屈が合わない．

脳死肝移植が増加に伴いこのような不備が少しずつ現実のものに成りつつある．移植医療で先陣を切って素晴らしい医療構築を整えている腎移植に見習い，少なくとも地域内で脳死ドナーが活用できるシステムを早急に構築しなければならないと考える．

韓国の移植事情と日本の肝移植事情の比較

本号では金守良氏は脳死肝移植も生体肝移植もわが国と韓国ではおおよそその成績は同じであり，異なるのは生体肝移植，脳死肝移植の実施数であるとしている．その実施数の差が何によるかを韓国の外科や内科の教授の意見を踏まえて私見を述べている．そこで，氏はわが国と韓国の脳死ドナー提供の数的差異の要因として三つの項目を挙げている．一つは，韓国では子の親に対する孝を原理とする儒教的美風であり，このことが容易に生体肝移植のドナーになり得る風土を形成しているとしている．次いで，全人口の三分の一を占めるカトリック教徒も一部否定的な宗派の存在もあるが要因として示している．そして三つ目に社会的風潮を高揚させたプロボクサーと枢機卿の脳死ドナー提供も重要な要因として示している．

生体肝移植に関して考えてみよう．韓国では肝移植適応疾患の80％以上がB型肝炎ウイルス由来の肝不全，肝細胞癌である．この場合，レシピエントの多くはHBVによる家族内集積により生体ドナーとしての両親，兄弟，姉妹は不適格となる．一方，医学的進歩からHBVの母親から出産に対してHBVのワクチンが早期に実施され，現実的にはレシピエントの子どもたちはHBV

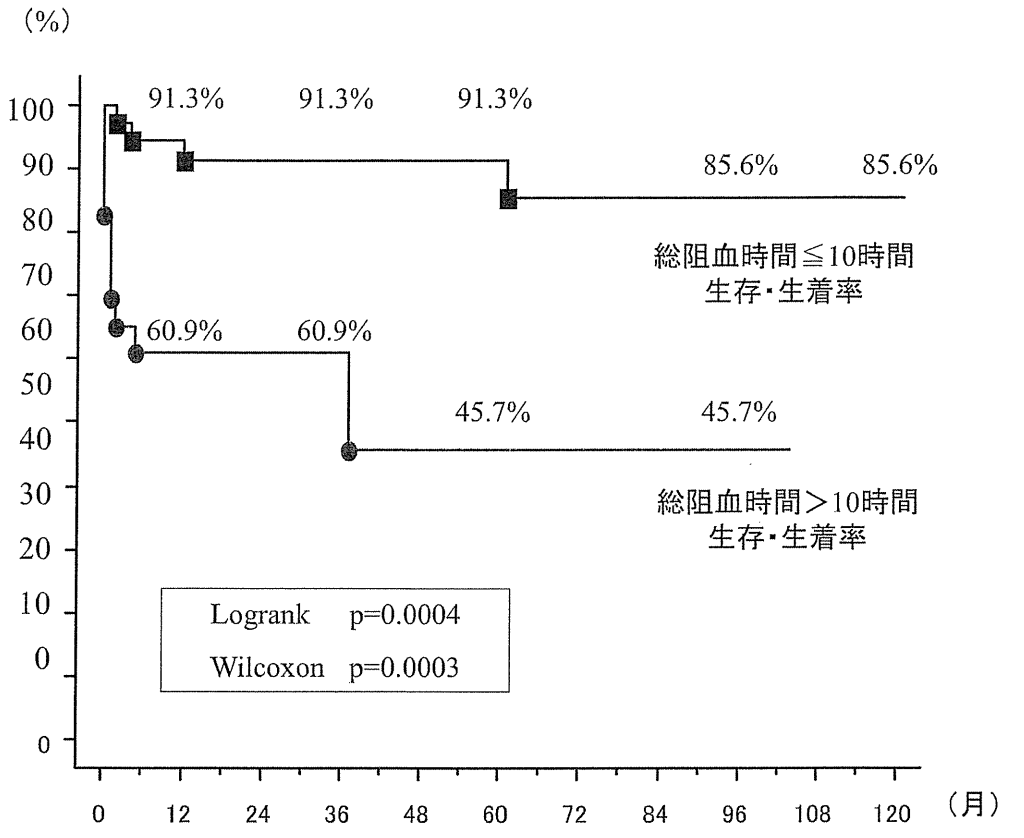

Fig. 4 脳死肝移植における総阻血時間とその生存，生着率
総阻血時間 10 時間以内と以上で優位に生存，生着率が異なる．

が陰性である．そこに，儒教的風土が子供からの生体肝移植ドナーが比較的容易に成されると考える．もちろんこんな簡単な推論通りとは思わず，もう少し深い事情があるかもしれないが筆者は金論文を読んでこのように考える．このことにより生体肝移植のドナーが必然的に若年傾向となり，その移植成績は良好となる訳である．一方，わが国は肝移植の適応疾患の多くがC型肝炎ウイルス由来の肝不全，肝細胞癌であるために，ドナー候補者の年齢が高くなり，心理的，社会的側面から容易に家族内のドナー候補者に制限があると述べられているが，この意見にはある程度同意できるところがある．

しかし，肝移植に関する韓国から世界への発信として，子女の多くが儒教的価値観から容易にドナーになることが韓国的特性と述べているが，果たしてこの普遍性を世界に発信して受諾されるのであろうか．親が病気になると，子女が生体ドナーになるべしという風潮は避けるべきであり，またその精神が蔓延して生体肝移植が推進されるのも少し違和感を受ける．ましてや，韓国で盛んに行われている Dual Donor に関して氏はドナーの安全を守るために合理的な術式と述べている点に少し不安を覚える．さらに生体ドナーの減少が危惧されているという認識は理解されないであろう．移植医療として筆者は基本的に生体肝移植が必要悪という考えであるが故にこの点は些か納得できない面がある．さらに，それら生体ドナーとなった多くの子女に対して国として社会として敬い，特別な待遇，恩恵を被ることができるような儒教的風潮が韓国社会において機能しているかどうかも，すこし懐疑的でもある．

一方，脳死肝移植の増加は素直に韓国の実施される医療に敬意を表する．成績もさることながら，その増加はカトリック教徒の数と有名人の脳死 donation だけによるものではないであろう．脳死肝移植を社会の一つのルールとして認めている韓国国民の移植医療に対する理解と成熟度を示しているものと理解している．

さいごに，韓国の肝移植医療の事情が明らかになり，

わが国でも法改正後に脳死肝移植症例が少しずつではあるが増加傾向にあるこの時期にタイムリーな論文を寄稿された金氏に敬意を表するとともに，さらなる健全な移植医療の推進を望むものである．

文　献

1）市田隆文．肝移植医療の最前線．内科　2010；99（1）：349―357

Present state of liver transplantation in Japan

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Key words: deceased liver transplantation　living donor liver transplantation　deseased donor living donor

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Advanced histology and impaired liver regeneration are associated with disease severity in acute-onset autoimmune hepatitis

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Advanced histology and impaired liver regeneration are associated with disease severity in acute-onset autoimmune hepatitis

Aims: Some cases of acute-onset autoimmune hepatitis (AIH) develop into severe or fulminant forms showing massive/submassive hepatic necrosis, and have a poor prognosis. The pathological features of acute-onset AIH remain uncertain. Ductular (intermediate) hepatocytes after massive/submassive necrosis may serve as hepatic progenitor cells, and could be seen as cytokeratin 7 (CK7)-positive hepatocytes in immunohistochemistry. Therefore, the aim was to examine histological features to obtain a better evaluation of acute-onset AIH.

Methods: The histological features of 27 clinically acute-onset AIH patients were examined by immunohistochemistry using CK7.

Results: On staining for CK7, intermediate hepatocytes were less commonly present ($P < 0.001$) and ductular reactions were more commonly present ($P < 0.001$) in severe/fulminant patients than in non-severe ones. In severe and fulminant patients, intermediate hepatocytes and intralobular progenitor cells were more commonly present ($P < 0.005$ and $P < 0.05$, respectively) and ductular reactions were less commonly present ($P = 0.007$) in recovered patients than in dead ones. Severe patients had more clinically and histologically advanced disease.

Conclusions: Immunohistochemical evalution using CK7 might be a useful tool for evaluating liver regeneration, and intermediate hepatocytes and progenitor cells might play an important role in liver regeneration after massive and submassive necrosis in acute-onset AIH.

Keywords: autoimmune hepatitis, cytokeratin 7, fulminant hepatitis, liver regeneration, severe hepatitis

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; ANA, antinuclear antibody; ASMA, anti-smooth muscle antibody; CK19, cytokeratin 19; CK7, cytokeratin 7; CMV, cytomegalovirus; DR, ductular reaction; EBV, Epstein–Barr virus; HBc, hepatitis B core; HCV, hepatitis C virus; HSV, herpes simplex virus; IM, intermediate hepatocyte; iPC, intralobular progenitor cell; PC, progenitor cell; PT, prothrombin time; T-Bil, total bilirubin; UNV, upper normal value

Introduction

Autoimmune hepatitis (AIH) is an unresolving inflammation of the liver of unknown cause, and is characterized by the presence of hypergammaglobulinaemia, autoantibodies, and interface hepatitis and plasma cell infiltration on histological examination.[1,2] A prospective study has indicated that as many as 40% of patients with untreated severe disease die within 6 months of diagnosis.[3] Cirrhosis develops in at least

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40% of survivors.[4] An acute onset of illness is common,[5–8] and fulminant presentation is possible.[9]

There are two types of acute-onset AIH: one is AIH with acute presentation of the disease, and the other is AIH with acute exacerbation of underlying chronic disease. Some cases of the former type develop into a severe or fulminant form showing massive/submassive hepatic necrosis, and there is a risk of mistiming the start of immunosuppressive therapy, because of the difficulty in diagnosis resulting from atypical clinicopathological features; the patients are sometimes resistant to immunosuppressive therapy and have a poor prognosis.[10–12]

Histological liver cell regeneration after massive/submassive necrosis resulting from severe and fulminant AIH has not been examined in detail. In a case report of a patient with fulminant hepatitis B who underwent auxiliary partial orthotopic liver transplantation, examination of sequential liver biopsy specimens showed that ductular hepatocytes [intermediate hepatocytes (IMs)] after massive necrosis were transformed into mature hepatocytes.[13] Mature bile ducts are specifically stained by anti-cytokeratin 7 (CK7) and anti-cytokeratin 19 (CK19),[14] and IMs, which are considered to originate from activated progenitor cells (PCs), express CK7 and, to a lesser extent, CK19.[15] We have noticed that IMs are frequently found in the liver biopsy specimens of patients recovering from acute-onset AIH, but not in those of dead patients. Therefore, we used CK7 immunohistochemistry, not CK19 staining, in this study.

The term 'ductular reaction' (DR) in a wide sense has been applied to the overall phenomenon of PC activation and differentiation in the regenerative and reparative response, including the progenitor cells (single CK7-positive small cells with an oval nucleus and small rim of cytoplasm), DR in a narrow sense (bile ductules with poorly defined lumina located at the portal parenchymal interface, arranged in anastomosing cords, and lined with small CK7-positive cells with little cytoplasm) and IMs (cells intermediate in size and immunohistochemical profile between PCs and hepatocytes). In our immunohistochemical findings, DR in a narrow sense is used for one of the CK7-positive cell types, as described above.

Recently, we examined the clinicopathological features of acute-onset AIH patients presenting with acute hepatitis, and found that histological examination of the liver was useful for early diagnosis and that centrilobular necrosis was characteristic of this disease.[16] In the current study, we examined the association between the clinical severity of the disease and histological features, including the state of liver regeneration, using immunohistochemistry with CK7 for better evaluation of the nature of acute-onset AIH.

Materials and methods

PATIENTS

A diagnosis of AIH was made on the basis of the presence of antinuclear antibody (ANA) and/or anti-smooth muscle antibody (ASMA), according to the criteria defined by the International Autoimmune Hepatitis Group,[17] and of liver biopsy findings compatible with AIH, consisting of interface hepatitis, centrilobular necrosis, and plasma cell infiltration. Eligibility criteria for clinically 'acute-onset' AIH were as follows, in addition to the AIH criteria described above: (i) acute-onset liver injury; (ii) no history of liver injury; and (iii) no signs of chronicity on the basis of physical examination, laboratory data, and abdominal ultrasound findings.

Eligibility criteria for severe and fulminant AIH, in addition to the criteria described above, were as follows: (i) prothrombin time (PT) activity <50% of control or total bilirubin level more than 20 mg/dl during the disease course were defined as severe hepatitis; and (ii) patients with a prothrombin time <40% of control and hepatic encephalopathy were defined as having fulminant hepatitis. Informed consent was obtained from all patients or appropriate family members. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committee.

Twenty-seven patients were enrolled in the study: 14 non-severe acute-onset AIH, seven severe AIH, and six fulminant AIH.

CLINICAL, BIOCHEMICAL AND IMMUNOSEROLOGICAL ANALYSIS

Data obtained from patients were as follows: sex; age at diagnosis; time of onset, for severe disease and fulminant disease; complications; serum levels of alanine aminotransferase (ALT), total bilirubin (T-Bil), alkaline phosphatase, PT, IgG, IgM, ANA, ASMA, liver kidney microsomal antibody-1 and antimitochondrial antibody; types of therapy; and response to therapy. Patients were also questioned for any history of recent exposure to drugs and chemical agents, as well as heavy alcohol consumption (>50 g/day for >5 years). In acute-onset AIH, early symptoms, including fever, general malaise, fatigue, nausea, vomiting and right upper quadrant discomfort, are frequently observed, so

we defined the beginning of these symptoms as clinical onset.

VIROLOGICAL ANALYSIS

Patients were examined for viral markers such as anti-hepatitis A virus IgM, anti-hepatitis B core IgM, hepatitis B surface antigen, anti-hepatitis C virus (HCV) antibody, HCV RNA, anti-Epstein–Barr virus IgM, anti-herpes simplex virus IgM and anti-cytomegalovirus IgM. None of the patients had clinical or laboratory evidence of acquired immune deficiency syndrome.

HISTOLOGICAL EXAMINATION

Histological examination was performed before the administration of corticosteroids, in the convalescent phase, or after death. There were 22 needle biopsies, one explanted liver, and four post-mortem specimen. Three specialists reviewed the histopathological changes by evaluating the degrees of portal and lobular changes and plasma cell infiltration by haematoxylin and eosin staining with or without Victoria blue-staining. Staging and grading were evaluated according to the classification of Desmet *et al.*[18]

IMMUNOHISTOCHEMISTRY

Formalin-fixed, paraffin-embedded liver biopsy specimens were used for immunohistochemical studies, with a standard avidin–streptavidin complex technique.[19] Anti-CK7 (dilution 1:200; Dako, Glostrup, Denmark) was used for staining bile duct, ductular epithelium and hepatic PCs.

Single CK7-positive small cells with an oval nucleus and small rim of cytoplasm were regarded as PCs. A DR consisted of bile ductules with poorly defined lumina located at the portal–parenchymal interface, arranged in anastomosing cords, and lined with small CK7-positive cells with little cytoplasm. IMs were defined as cells intermediate in size and with an immunohistochemical profile between that of PCs and hepatocytes.[20]

The term DR has been applied to the overall phenomenon of PC activation and differentiation in the regenerative and reparative response in a wide sense, including the PCs, DRs and IMs described above. In our immunohistochemical findings, DR is used for one of the CK7-positive cell types in a narrow sense.

Semiquantitative assessment of CK7-positive cells was performed concerning periportal DRs, periportal IMs, and intralobular PCs (iPCs): 0 (point 0) represents absent or rare single PCs or DRs around occasional portal tracts; 1+ (point 1) represents rare single or focal DRs or focal clustering of positively stained PCs around most portal tracts; 2+ (point 2) represents continuous DRs or clusters of positively stained PCs occupying <50% of portal tracts; 3+ (point 3) represents continuous DRs or clusters of positively stained PCs occupying >50% of portal tracts. The number of iPCs was assessed by counting these cells in three non-overlapping fields showing parenchyma, using higher-power examination (×400), and the average of these scores was taken.[20,21]

STATISTICAL ANALYSIS

Differences in proportions among the groups were compared by Fisher's exact probability test, Student's *t*-test and Welch's *t*-test ($P < 0.05$ was considered to be significant).

Results

CLINICAL AND BIOCHEMICAL FEATURES

Of 27 patients, four were men and 23 were women. Mean age at the time of diagnosis was 51.2 ± 15.4 years. The mean ALT level was 556 ± 435 IU/l, the mean T-Bil level was 10.0 ± 10.0 mg/dl, and the mean PT activity was 66% ± 29%.

The mean IgG level was 2160 ± 1139 mg/dl. The IgG level was normal [<1.0 × upper normal value (UNV)] in nine of 27 patients (33%), 1.0–1.5 × UNV in 14 patients (52%), 1.5–2.0 × UNV in one patient (4%), and >2.0 × UNV in three patients (11%). ANA was positive (≥1:40) in 24 of 27 patients (89%), <1:40 in three patients (11%), 1:40 in four patients (15%), 1:80 in nine patients (33%), and >1:80 in 11 patients (41%). ASMA was positive (≥1:40) in nine of 24 patients (38%).

The clinical and biochemical features of patients according to disease severity at admission are provided in Table 1. The mean T-Bil level was higher in severe than in non-severe patients ($P = 0.007$), in fulminant than in non-severe patients ($P < 0.001$) and in fulminant than in severe patients ($P = 0.03$). Mean age, sex, mean ALT level, mean IgG level, ANA positivity and AIH scores were not different among the three groups.

Regarding the duration from initial symptoms to admission to our unit, the differences were statistically significant between non-severe and fulminant patients ($P < 0.001$) and between severe and fulminant patients ($P = 0.03$). Regarding the duration from initial symptoms to histological examination, the difference was not statistically significant.

Table 1. Comparison of findings at presentation of acute-onset autoimmune hepatitis (AIH) patients according to disease severity

	Non-severe	Severe	Fulminant
n	14	7	6
Age*	55 ± 12	42 ± 17	53 ± 18
Sex (male/female)†	1/13	0/7	3/3
ALT (IU/l)‡	598 ± 333	441 ± 391	593 ± 699
T-Bil (mg/dl)§	2.6 ± 3.2	13.2 ± 7.1	23.5 ± 7.2
PT (%)¶	90 ± 14	49 ± 3	29 ± 8
IgG (mg/dl)**	1797 ± 456	2692 ± 1923	2385 ± 970
ANA (≥ ×40)††	11/13	8/8	6/6
AIH score, histology-negative‡‡	11.1 ± 2.8	13.7 ± 3.0	12.7 ± 2.2
AIH score, histology-positive§§	14.2 ± 3.4	17.4 ± 3.6	16.2 ± 2.4
Days from onset to admission¶¶	36.1 ± 27.1	38.0 ± 10.7	102.0 ± 48.4
Days from onset to histological examination***	48.2 ± 28.9	75.3 ± 28.9	126.5 ± 104.2
Death†††	0	0	5

ALT, Alanine aminotransferase; ANA, antinuclear antibody; PT, prothrombin time; T-Bil, total bilirubin.
Values are mean (standard deviation) or number.
*No significant difference among the three groups.
†No significant difference among the three groups.
‡No significant difference among the three groups.
§Significant difference between non-severe ($P = 0.007$), severe, non-severe and fulminant ($P < 0.001$), and severe and fulminant ($P = 0.03$).
¶Significant difference between non-severe and severe ($P < 0.001$), non-severe and fulminant ($P < 0.001$), and severe and fulminant ($P = 0.001$).
**No significant difference among the three groups.
††No significant difference among the three groups.
‡‡No significant difference among the three groups.
§§No significant difference among the three groups.
¶¶Significant difference between non-severe and fulminant ($P < 0.001$), and severe and fulminant ($P = 0.03$).
***No significant difference among the three groups.
†††Significant difference between non-severe and fulminant ($P < 0.001$), and severe and fulminant ($P = 0.005$).

No patients were positive for the viral markers described above. Suspected hepatotoxic drugs were excluded in this study, using the drug-induced liver injury diagnostic scale by Maria and Victorino.[22]

TREATMENT RESPONSE AND OUTCOME

An initial dose of 40–60 mg of prednisolone or 1000 mg of methylprednisolone daily was administered in nine of 14 non-severe patients, in all of seven severe patients, and in five of six fulminant patients. All of the nine non-severe and seven severe patients responded well and recovered. Of the six fulminant patients, one responded and recovered, four did not respond and died, and one received a liver transplant and died.

Five (36%) of the non-severe patients recovered with ursodeoxycholic acid and intravenous glycyrrhizin

(Stronger Neominophagen C) at 100 ml daily, an aqueous extract of liquorice root that is reported to have anti-inflammatory activity and has been used for the treatment of chronic viral hepatitis in Japan. One fulminant patient who was already suffering from terminal stage liver failure on admission was not treated with corticosteroids and died while waiting for liver transplantation.

As a result, all non-severe and severe patients recovered, and five of six fulminant patients died, suggesting an unresponsive tendency in fulminant patients.

HISTOLOGICAL FEATURES

The pathological characteristics of the patients are summarized in Tables 2 and 3. Centrizonal necrosis and plasma cell accumulation in portal and centrilobular areas were characteristic for acute-onset AIH. Thirteen (93%) of 14 non-severe patients showed severe activity, with four (29%) showing severe acute hepatitis, and nine (64%) showing severe activity with fibrosis stage 2–3. Only one showed mild focal centrizonal necrosis with fibrosis stage 1. Of 13 severe and fulminant patients, six (46%) showed massive necrosis, one (8%) submassive necrosis, four (31%) severe acute hepatitis, and two (15%) chronic hepatitis with fibrosis stage 2–3 and moderate to severe activity. Twenty-five (93%) of the 27 patients showed severe activity with or without plasma cell accumulation in portal and centrilobular areas, and all showed centrilobular necrosis. Plasma cell infiltration was found in 25 (93%) of 27 patients.

Histological heterogeneity was seen especially in severe and fulminant AIH, and it was clearly seen in multiple specimens from post-mortem examination: there was a mixture of massive hepatic necrosis and regenerative islands.

IMMUNOHISTOCHEMICAL STAINING FOR CK7

DRs showed a strong homogeneous cytoplasmic and membranous CK7 staining pattern at the portal–parenchymal interface. PCs showed a strong homogeneous cytoplasmic and membranous staining pattern located distal to the DRs and occasionally in the sinusoids. IMs showed a variable cytoplasmic and membranous staining pattern in the periportal areas, occasionally with extension into the lobular areas (Figure 1).

The findings from CK7 immunohistochemistry of non-severe patients are described in Table 2. The mean

Table 2. Cytokeratin 7 immunostaining findings in 16 non-severe patients

Patient	Histology	Ductular reaction	Intermediate hepatocyte	Intralobular progenitor cell
1	CH (F3, severe)	2	3	0
2	CH (F3, severe)	2	3	5
3	AH (severe)	1	2	2
4	CH (F2, severe)	1	3	5
5	CH (F2, severe)	1	2	1
6	CH (F3, severe)	1	2	2
7	CH (F2, severe)	1	2	2
8	AH on CH (F1, mild)	1	1	1
9	AH (severe)	1	1	0
10	CH (F3, severe)	1	1	0
11	CH (F2, severe)	1	3	2
12	CH (F2, severe)	1	3	2
13	AH (severe)	1	2	4
14	AH (severe)	2	3	4

AH, Acute hepatitis; CH, chronic hepatitis; F, fulminant.

Table 3. *Cytokeratin 7 immunostaining findings in 14 severe and fulminant patients*

Patient	Outcome	Histology	Ductular reaction	Intermediate hepatocyte	Intralobular progenitor cell
S1	Recovery	AH (severe)	3	2	5
S2	Recovery	CH (F3, severe)	2	1	3
S3	Recovery	AH (severe)	2	2	1
S4	Recovery	Massive necrosis	2	1	0
S5	Recovery	AH (severe)	2	1	0
S6	Recovery	Massive necrosis	0	0	0
S7	Recovery	AH (severe)	2	1	1
F1	Recovery	CH (F2, moderate)	2	2	2
F2	Death	Massive necrosis	3	0	0
F3	Death	Massive necrosis	3	0	0
F4	Death	Submassive necrosis	3	0	0
F5	Death	Massive necrosis	3	0	0
F6	Death	Massive necrosis	3	0	0

AH, Acute hepatitis; CH, chronic hepatitis; F, fulminant; S, severe.

scores for DRs, IMs and iPCs were 1.21 ± 0.43, 2.21 ± 0.80 and 2.14 ± 1.75, respectively. The mean scores in non-severe chronic hepatitis were 1.22 ± 0.44, 2.44 ± 0.73 and 2.11 ± 1.83, respectively. The mean scores in non-severe acute hepatitis were 1.20 ± 0.45, 1.80 ± 0.84 and 2.20 ± 1.79, respectively. The differences between chronic hepatitis and acute hepatitis were not significant.

The findings from CK7 immunohistochemistry of severe and fulminant patients are shown in Table 3. The mean scores for DRs, IMs and iPCs were 2.31 ± 0.85, 0.77 ± 0.83 and 0.92 ± 1.55, respectively. The mean scores in severe patients were 1.86 ± 0.90, 1.14 ± 0.69 and 1.43 ± 1.90, respectively. The mean scores in fulminant patients were 2.83 ± 0.41, 0.33 ± 0.82 and 0.33 ± 0.82, respectively.

The score for IMs was higher in non-severe than in severe and fulminant patients, with statistical significance ($P < 0.001$). That for DRs was higher in severe and fulminant than in non-severe patients, with statistical significance ($P < 0.001$). The mean scores for DRs, IMs and iPCs in recovered patients with severe and fulminant hepatitis were 1.88 ± 0.83, 1.25 ± 0.71 and 1.50 ± 1.77, respectively. The mean scores in dead patients were 3 ± 0, 0 ± 0 and 0 ± 0, respectively. The mean scores for IMs and iPCs were significantly higher ($P < 0.005$ and $P < 0.05$, respectively) and that for DRs was significantly lower ($P = 0.007$) in recovered patients than in dead patients.

Immunohistochemistry for CK7 in a non-severe patient, a severe patient and a fulminant patient are shown in Figures 2–5. Marked periportal PCs, periportal IMs and intralobular IMs and a few periportal DRs were found in a non-severe, recovered patient (Figures 2 and 3). Marked periportal DRs were found, but periportal IMs, iPCs and intralobular IMs were not found in a fulminant, dead patient (Figure 5). In a severe, recovered patient, staining showed a pattern between that of the non-severe one and that of the fulminant, dead one (Figure 4).

AIH SCORING SYSTEM

The provisional scoring system (AIH score) proposed by the International Autoimmune Hepatitis Group[17] was used to score all patients (Table 1).

The AIH scores ranged from 7 to 14 (11.1 ± 2.8), 11 to 18 (13.7 ± 3.0) and 10 to 16 (12.7 ± 2.2) before treatment without histological scoring in non-severe, severe and fulminant patients, respectively. The difference among the three groups was not statistically

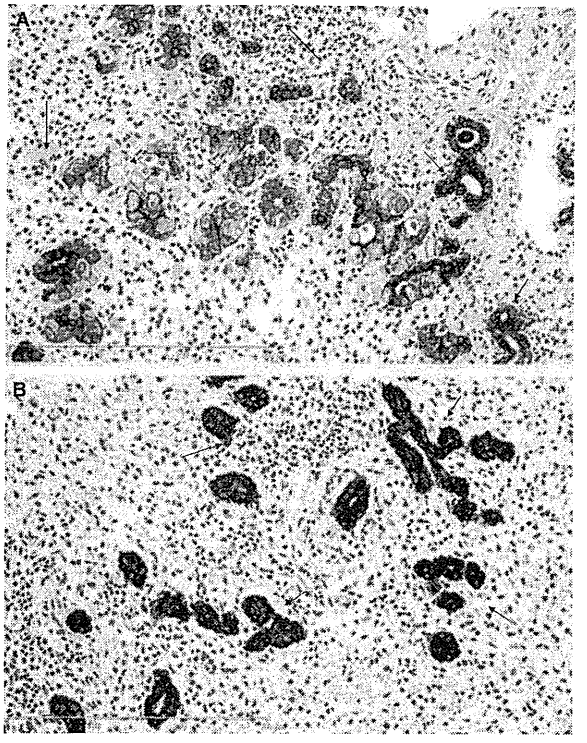

Figure 1. Immunohistochemistryl for cytokeratin 7 (CK7) shows ductular reactions (short arrow), progenitor cells (long arrow) and intermediate hepatocytes (arrowhead). Semiquantitative assessment of CK7-positive cells was performed with regard to periportal ductular reactions (pDRs), periportal progenitor cells (pPCs), periportal intermediate hepatocytes (pIMs), intralobular progenitor cells (iPCs) and intralobular intermediate hepatocytes (iIMs).

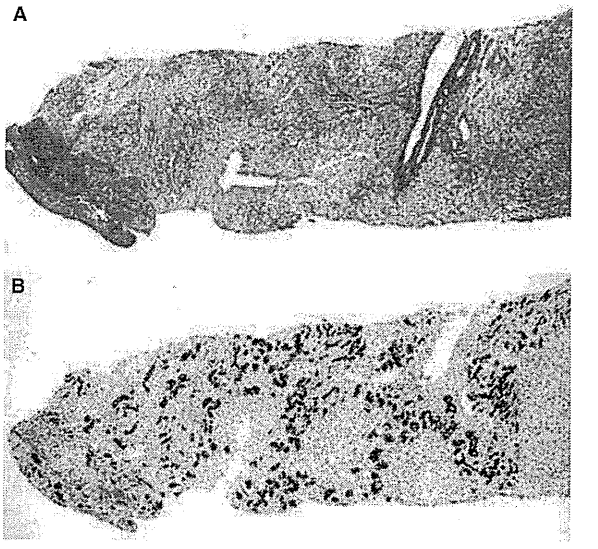

Figure 2. Histological findings in a non-severe patient (patient 1). A, Haematoxylin and eosin and Victoria blue staining shows interface hepatitis and plasma cell infiltrations in centrilobular necrotic regions. B, Immunohistochemistry stain for cytokeratin 7 (CK7) shows positive cells in periportal and intralobular regions.

significant. Without histology, the score was non-diagnostic in four (29%), probable in 10 (71%) and definite in none among non-severe patients, and it was non-diagnostic in none, probable in 10 (77%) and definite in three (23%) among severe and fulminant patients.

The AIH scores ranged from 9 to 18 (14.2 ± 3.4), 15 to 23 (17.4 ± 3.5) and 13 to 20 (16.2 ± 2.4) before treatment with histological scoring in non-severe, severe and fulminant patients, respectively. The difference was not statistically significant among the three groups. With histology, the score was non-diagnostic in two (14%), probable in seven (50%) and definite in five (36%) among non-severe patients, and it was non-diagnostic in none, probable in six (46%) and definite in seven (54%) among severe and fulminant patients. Although two patients with non-severe hepatitis showed an AIH score that was less than the probable AIH score, we diagnosed them as having AIH because they were positive for ANA or ASMA, and their liver biopsy findings were compatible with AIH, consisting of interface hepatitis, centrilobular necrosis and plasma cell infiltration, which are characteristic of AIH, although not specific.

Discussion

The diagnosis of acute-onset AIH is difficult, because patients do not always show typical clinicopathological features of AIH. Although some of them progress to severe and fulminant hepatitis with poor prognosis, their specific clinicopathological features still have to be clarified.

In the present study, mean ANA positivity and IgG level were higher in severe and fulminant than in non-severe patients, although the differences were not statistically significant. The AIH score was higher, without statistical significance, and the duration from onset to admission was longer in severe than in non-severe patients, with significance. These findings mean that severe patients have more clinically and histologically advanced disease than non-severe patients, partially depending on the time durations until their diagnoses.

There are no morphological features that are pathognomonic of AIH. Moreover, there are only a few reports on the histological features of acute-onset AIH, such as centrilobular necrosis being associated with an

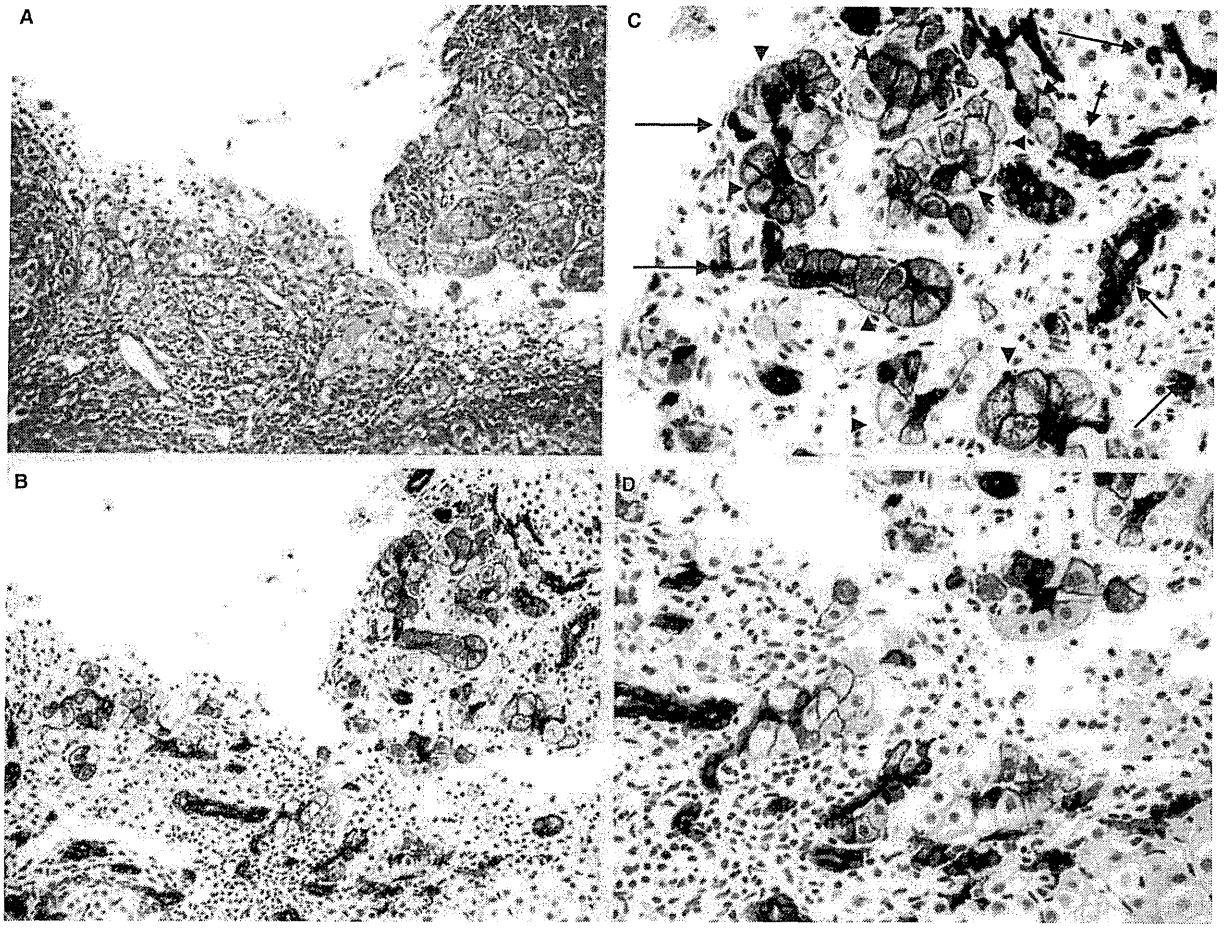

Figure 3. Higher-power examinations of patient 1. A, Haematoxylin and eosin and Victoria blue staining. B–D, Immunohistochemistry for cytokeratin 7 (CK7) shows ductular reactions (short arrow), progenitor cells (long arrow) and intermediate hepatocytes (arrowhead) in periportal regions, and intermediate hepatocytes and few progenitor cells in intralobular regions.

acute clinical presentation and possibly reflecting an early lesion preceding portal involvement.[8,23–25] In the current study, histological examination showed that, among non-severe patients, nine of 14 (64%) patients had chronic disease, whereas, among severe and fulminant patients, only two of 13 (15%) patients had chronic disease ($P = 0.013$). AIH with acute presentation might be a more severe form than AIH with acute exacerbation of underlying chronic disease. Therefore, early histological diagnosis and treatment of acute-onset AIH is indispensable for a better outcome.

In our study, one of the pathological characteristics of acute-onset AIH was its histological heterogeneity, especially in severe AIH. Therefore, bias resulting from the specimen, types of sampling (needle biopsy, explanted liver and post-mortem) and locations of sampling exist in this study. Histological heterogeneity leads to radiological heterogeneity. Unenhanced computed tomography often shows hypoattenuated and hyperattenuated areas, with the former reflecting massive hepatic necrosis and the latter regenerative islands. Ultrasound shows similar heterogeneity. These findings could be attributable to the mixture of severe necrosis and regeneration usually seen in acute-onset AIH. We supposed that the characteristic morphological patterns of liver regeneration would exist in acute-onset AIH, and that better understanding of these would be of help in the diagnosis.

Regarding liver cell regeneration, cellular restitution after different types of liver injury involves three putative types of liver cell: mature hepatocytes, ductular PCs, and periductular bone marrow-derived stem cells.[26] In the normal liver, mature hepatocytes proliferate and reconstitute the liver mass by the

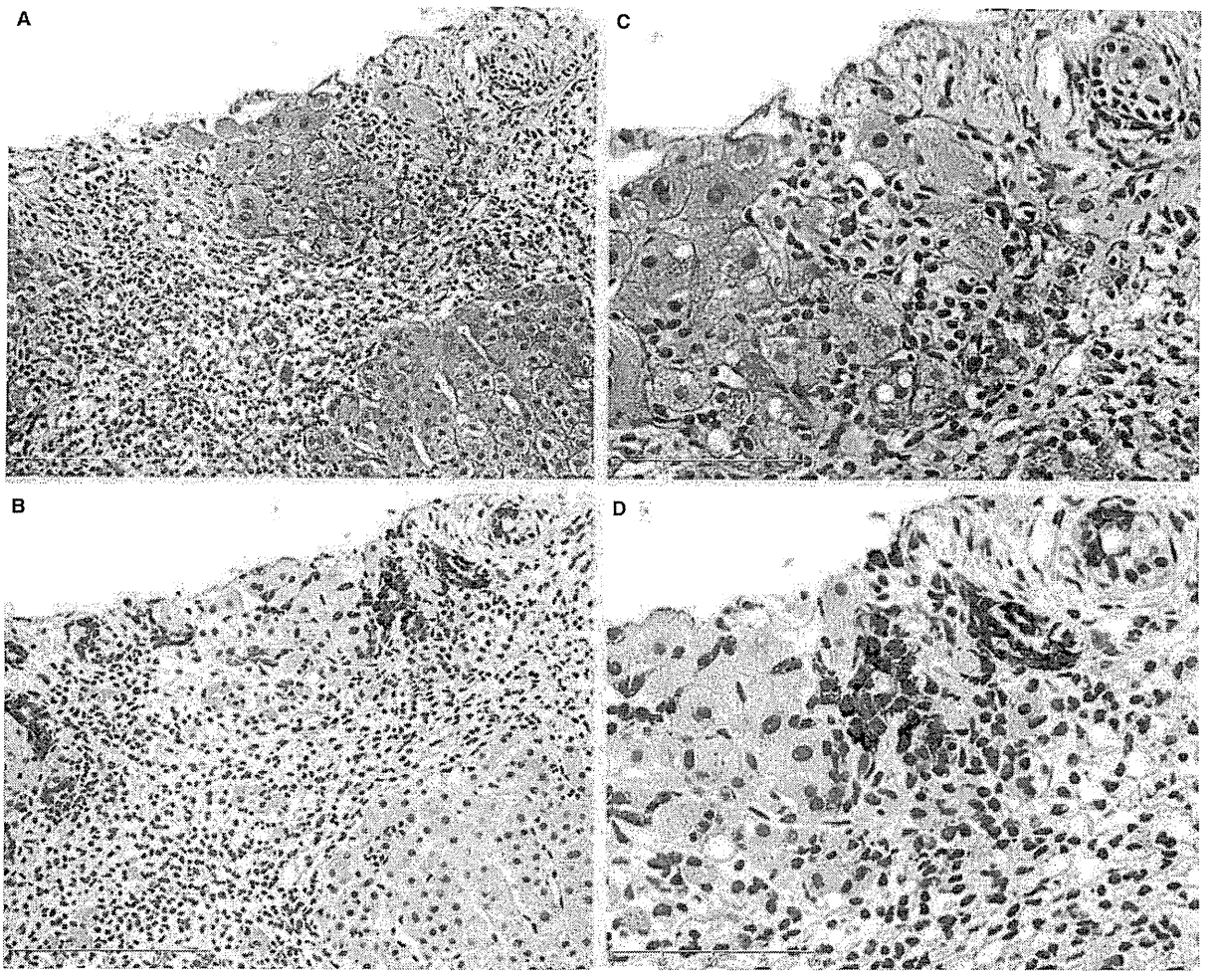

Figure 4. Histological findings in a severe patient (patient S5). **A** and **C**, Haematoxylin and eosin and Victoria blue staining shows severe acute hepatitis. **B** and **D**, Immunohistochemistry for cytokeratin 7 (CK7) shows ductular reactions and a few intermediate hepatocytes in periportal regions.

replacement of necrotic and apoptotic hepatocytes within the lobules in the primary proliferative pathway. This pathway is easily impaired by a variety of insults, leading to the activation of a secondary proliferative pathway of hepatic PCs. These are bipotential cells that reside primarily in the periportal region and become the source of regenerating hepatocytes, as well as cholangiocytes and draining ductules. A by-product of the activation of this secondary proliferative pathway is the so-called DR. The DR is a reactive lesion at the portal tract interface, comprising small biliary ductules with an accompanying complex of stroma and inflammatory cells. Periductular stem cells respond to periportal injury when hepatocytes are unable to move into the injured area.[26]

Regarding the markers of single PCs, IMs and DRs, Eleazar *et al.*[21] reported that the pattern of CK19 staining was of a lower intensity, with fewer visualized cells, than that of CK7. Zhou *et al.*[27] also reported that CK19 was positive in transitional cells of the bile ductular lineage and bile duct cells, and negative in bipotential stem cells, transitional cells of the hepatocytic lineage and hepatocytes. Single PCs, IMs and DRs could be stained with CK7, and could be distinguished by morphological characteristics.

On the basis of our observation that IMs were frequently found in the recovering patients with acute-onset AIH, we focused on IMs as well as PCs as one of the markers of liver regeneration, and used CK7, not CK19, as a marker in this study.

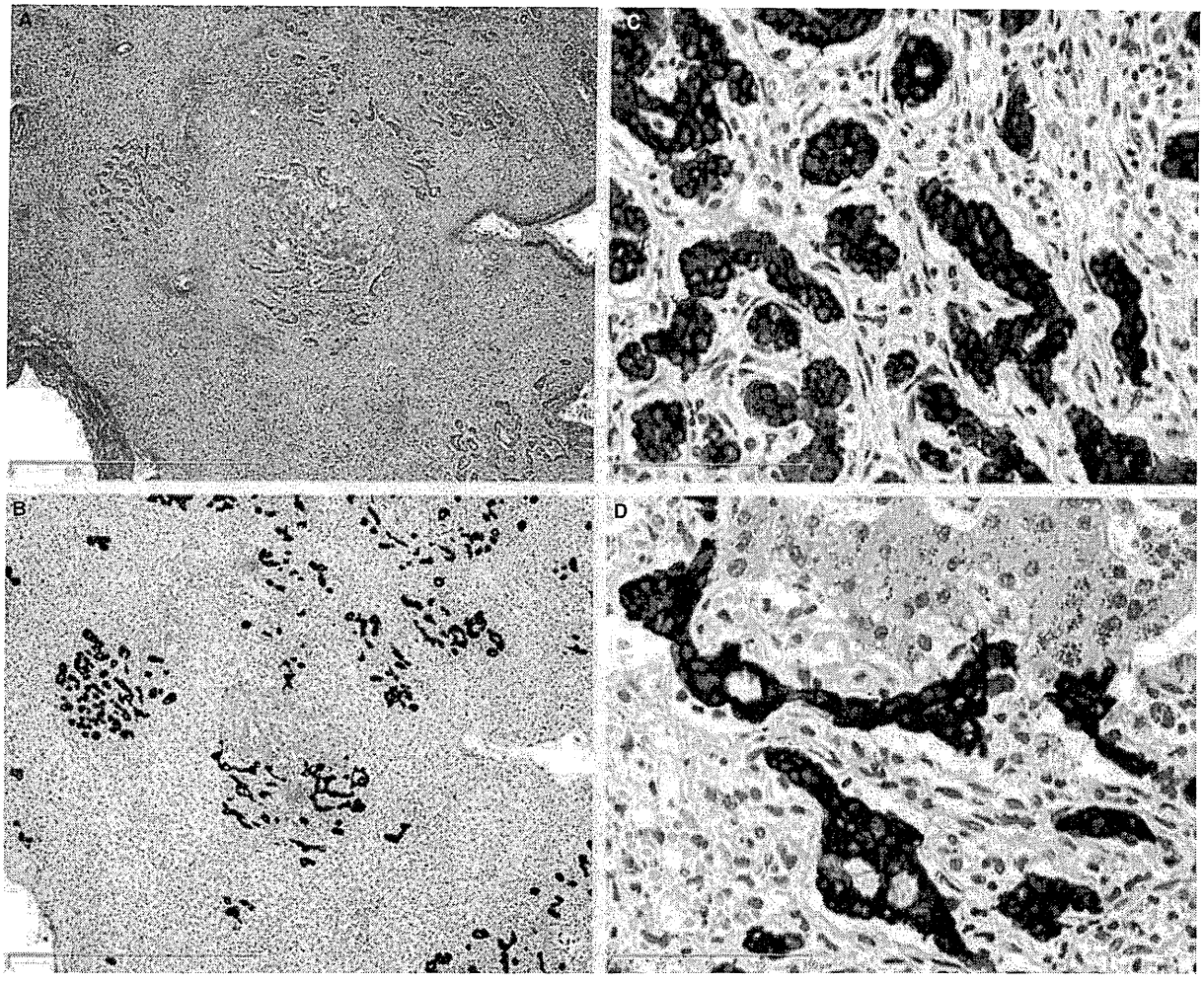

Figure 5. Histological findings in a fulminant patient (patient F5). **A**, Haematoxylin and eosin and Victoria blue staining shows massive necrosis. **B–D**, Immunohistochemistry for cytokeratin 7 (CK7) shows marked ductular reactions, few progenitor cells and no intermediate hepatocytes in periportal regions.

Immunohistochemical studies of the liver using anti-CK7 have been performed in a variety of liver diseases. Periportal DR correlates strongly with portal fibrosis in chronic hepatitis C.[28] The significant correlation between the extent of PC activation/DR and the severity and localization of inflammation in chronic hepatitis B and C suggests that inflammation is an important trigger for PC activation. PCs were strikingly scattered throughout the parenchyma and surrounded by IMs, suggesting migration of PCs away from the portal periphery into the lobular parenchyma and their differentiation towards hepatocytes.[20] DRs were present in the majority of patients with chronic hepatitis C, hepatitis B, and AIH, appeared early in the disease, and showed correlation with disease stage.[21] In non-alcoholic steatohepatitis, progressive fibrosis was associated with periportal DR.[29]

On immunohistochemistry for CK7, more IMs and iPCs and fewer DRs were found in non-severe and recovered patients, suggesting that liver regeneration was not impaired in these patients. In fulminant and dead patients in whom liver regeneration was considered to be impaired, marked DRs were found, but IMs and iPCs were not. These findings suggest that the differentiation from periportal PCs to IMs and mature hepatocytes is maintained in survivors, but it is impaired in non-survivors, resulting in the marked formation of DRs.

From the sequential histological observation of liver regeneration after massive necrosis in auxiliary partial

orthotopic liver transplantation, Fujita *et al.*[13] reported that ductular hepatocytes play the role of PCs. Fotiadu *et al.*[30] reported that PC activation was correlated with the degree of necroinflammatory activity and the stage of fibrosis in chronic hepatitis B and chronic hepatitis C, and might play a role in hepatic regeneration. Demetris *et al.*[31] reported that ductular hepatocytes seen in the livers after acute submassive necrosis may represent a transient amplifying population arising from a progenitor population located in or near the canals of Hering. Ductular hepatocytes in massive necrosis may serve as bipotential PCs.[32] DRs represent proliferation and bidirectional differentiation of facultative hepatic stem cells in a variety of acute and chronic liver diseases.[33]

In summary, immunohistochemical study using CK7 might be a useful tool for evaluating liver cell regeneration, and histological examination of the states of liver regeneration might be valuable for the diagnosis and evaluation of treatment response, although multicentre studies are also needed to clarify the features of acute-onset AIH.

Disclosures

All authors have nothing to disclose. No conflicts of interest exist.

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