Hepatic fibrosis and cirrhosis in the Fontan circulation: a detailed morphological study

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ABSTRACT

Aims: To describe the histological features of the liver in patients with a Fontan circulation.

Methods: Specimens from liver biopsies carried out as part of preoperative assessment prior to extracardiac cavopulmonary conversion of an older style Fontan were examined and scored semi-quantitatively for pertinent histological features. To support the use of the scoring, biopsy specimens were also ranked by eye for severity to allow correlation with assigned scores.

Results: Liver biopsy specimens from 18 patients with a Fontan circulation were assessed. All specimens showed sinusoidal fibrosis. In 17 cases there was at least fibrous spur formation, with 14 showing bridging fibrosis and 2 showing frank cirrhosis. In 17 cases at least some of the dense or sinusoidal fibrosis was orcein positive, although a larger proportion of the dense fibrous bands were orcein positive compared with the sinusoidal component. All specimens showed marked sinusoidal dilatation, and 14 showed bile ductular proliferation; 1 showed minimal iron deposition, and 1 showed mild lobular lymphocytic inflammation. There was no cholestasis or evidence of hepatocellular damage. Similar appearances were observed in 2 patients with severe tricuspid regurgitation.

Discussion: The histological features of the liver in patients with a Fontan circulation are similar to those described in cardiac sclerosis. Sinusoidal dilatation and sinusoidal fibrosis are marked in the Fontan series. The presence of a significant amount of orcein negative sinusoidal fibrosis suggests there may be a remediable component, although the dense fibrous bands are predominantly orcein positive, suggesting chronicity and permanence. No inflammation or hepatocellular damage is evident, suggesting that fibrosis may be mediated by a non-inflammatory mechanism.

The Fontan operation was first described in 1971 for the treatment of tricuspid atresia,[1] and has subsequently been used for other cardiac abnormalities with a single functional ventricle. In the classical Fontan procedure a direct connection between the right atrium and the pulmonary trunk is created, bypassing the right ventricle. The left ventricle supports the circulation and blood is drawn into the lungs passively. Total cavopulmonary anastomosis was the successor to the classical Fontan as it evolved over time. These procedures lead to central venous hypertension, depressed dynamic cardiac output, impaired ventricular arterial coupling and late ventricular dysfunction. These characteristics, together with the longstanding hypoxia preceding the Fontan, are all recognised risk factors for hepatic injury. In the immediate postoperative period, right-sided pressures greater than 20 mm Hg are associated with morbidity and mortality. However, long-term survivors have chronically raised systemic venous pressures associated with deranged liver function, particularly increased prothrombin times and cholestasis.[2–5] The potential long-term impact on hepatic architecture and function is very poorly described. Four cases of cirrhosis identified at postmortem examination in patients having undergone the Fontan procedure 4–18 years before death are described,[6] but description of the hepatic architecture in surviving Fontan patients is limited to an isolated case report.[7]

We have recently described a series of 12 patients who had extensive clinical, radiological and histological assessment by means of liver biopsy prior to extracardiac cavopulmonary conversion of an older style Fontan.[8] The histological features of the liver were broadly similar to those seen in patients with right heart failure, and a significant relationship between the duration of the Fontan circulation and the extent of fibrosis/cirrhosis was described. Here we describe in detail the complete morphological features of an extended series of liver biopsies from 18 patients who have undergone assessment for Fontan conversion, with semi-quantitation using scoring systems specifically adapted for these features. Where possible the assigned scores have been correlated with independent ranking of the same features to support the semi-quantitation. Furthermore, liver biopsies from two patients with severe tricuspid regurgitation were also assessed using the same systems to allow comparison with a related aetiology.

METHODS

Patients

All patients were referred to the Wessex Adult Congenital Heart Unit, Southampton, between 1 September 2003 and 1 March 2007 for extracardiac cavopulmonary conversion of an older style Fontan.[9] Patients were identified from the Wessex Congenital Heart Disease Database. The unit had established a clinical protocol of evaluation in September 2003 to assess suitability and potential risk associated with Fontan conversion. All patients gave informed consent for the individual procedures. This protocol was adopted after our group had experienced recurrent perioperative hepatic and hepatorenal complications, as well as reduced catecholamine sensitivity similar to patients with chronic liver disease. In addition to clinical, haemodynamic and radiological assessment, a liver biopsy was performed. Liver biopsy specimens from 2 patients with severe tricuspid regurgitation (TR) were also examined.

Table 1 Scoring criteria used to assess histological features in the livers of Fontan patients

Histological feature	Score	Comment
Gross architectural distortion (modified from METAVIR system[10])	0	No definite fibrosis
	1	Minimal fibrosis (no septa or rare thin septum)
	2	Mild fibrosis (occasional thin septa)
	3	Moderate fibrosis (moderate thin septa; up to incomplete cirrhosis)
	4	Cirrhosis definite or probable
Sinusoidal fibrosis (modified from published system[11])	0	No sinusoidal fibrosis
	1	Sinusoidal fibrosis present in <1/3 sinusoids
	2	Sinusoidal fibrosis present in 1/3–2/3 sinusoids
	3	Sinusoidal fibrosis affecting >2/3 sinusoids
Orcein positivity (gross architecture or sinusoidal fibrosis, possibly reflecting matrix maturity[12])	0	None of the fibrotic matrix is orcein positive
	1	<1/3 fibrotic matrix is orcein positive
	2	1/3–2/3 of fibrotic matrix is orcein positive
	3	>2/3 fibrotic matrix is orcein positive
Sinusoidal dilatation (previously published system[13])	0	No sinusoidal dilatation
	1	<1/3 sinusoids dilated
	2	1/3–2/3 sinusoids dilated
	3	>2/3 sinusoids dilated
Bile ductular reaction (previously published system[14])	0	No ductular reaction
	1	Mild ductular reaction
	2	Moderate ductular reaction
	3	Severe ductular reaction
Cholestasis	0	No cholestasis
	1	Cholestasis present
Iron deposition	0	No iron deposition
	1	Stainable iron present
Hepatocellular damage	0	No evidence of hepatocyte damage
	1	Hepatocyte ballooning, necrosis or apoptosis
Inflammation	0	No lobular inflammation
	1	Lobular inflammation

Liver biopsy

A transjugular or transfemoral liver biopsy was performed by conventional means at the time of cardiac catheterisation. Biopsy specimens were processed routinely and stained using H&E, Gordon and Sweet's reticulin, orcein, periodic acid Schiff after diastase digestion and Perl's Prussian blue. Histological analysis was performed by two histopathologists (TK and HM-S), who were blinded to all clinical data. The histological specimens were graded semi-quantitatively for a number of features identified in the previous work (table 1).

On a separate occasion to the scoring, the biopsy specimens were reviewed while blinded to all clinical information and assigned scores; they were ranked 1–20 (1 being the least severe) for gross architectural fibrosis, sinusoidal fibrosis and sinusoidal dilatation.

Statistics

For gross architectural distortion, sinusoidal dilatation and sinusoidal fibrosis, the assigned semi-quantitative scores and the assigned severity rank were correlated by Spearman rank order correlation. The assigned ranks for gross architecture and sinusoidal fibrosis were also correlated by Spearman rank order comparison.

RESULTS

Summary of scoring

Liver biopsy specimens from 18 patients with a Fontan circulation were assessed; 2 biopsy specimens from patients with severe TR but without a Fontan circulation were also available for assessment. Table 2 provides a summary of the assigned scores for the histological features of the liver in patients with a Fontan circulation, with the equivalent scores for the patients with TR in parentheses.

Histopathological features

Gross architectural distortion

The majority of biopsy specimens showed markedly distorted architecture with the formation of dense fibrotic bands (fig 1). The least affected specimens showed a pattern of fibrous septa formation arising from central veins. In those specimens with more advanced fibrosis there was bridging of vascular structures. In only 2 cases was there sufficient tissue to confidently assign a score of 4, cirrhosis, although the biopsies were all performed with a narrow gauge needle at the time of cardiac catheterisation, meaning that an underestimation of the severity of fibrosis graded 3 may have occurred. In 16/18 cases, more than two thirds of the fibrotic matrix within the fibrotic

Table 2 Summary of histological features of the liver in patients with a Fontan circuit (or tricuspid regurgitation)

Sinusoidal fibrosis	Score	0	1		2	3
	Frequency (n)	0	9		7 (1)	2 (1)
Sinusoidal fibrosis maturity	Score	0	1		2	3
	Frequency (n)	1	6		5 (1)	6 (1)
Gross architectural distortion	Score	0	1	2	3	4
	Frequency (n)	1	1	9	5 (2)	2
Gross architectural maturity	Score	0	1		2	3
	Frequency (n)	1	0		1	16 (2)
Sinusoidal dilatation	Score	0	1		2	3
	Frequency (n)	0	5		8	5 (2)
Ductular reaction	Score	0	1		2	3
	Frequency (n)	4	6		8 (2)	0
Iron deposition	Score	0			1	
	Frequency (n)	17 (2)			1	
Hepatocellular damage	Score	0			1	
	Frequency (n)	18 (2)			0	
Sinusoidal inflammation	Score	0			1	
	Frequency (n)	17 (2)			1	
Cholestasis	Score	0			1	
	Frequency (n)	18 (2)			0	

septa and bands stained positively with orcein (fig 2). The 2 cases of TR also showed marked fibrous septa formation.

Sinusoidal fibrosis

All the Fontan biopsy specimens, and both specimens from patients with TR, showed some degree of sinusoidal fibrosis. This fine neomatrix is deposited within the space of Disse in a pericellular manner (fig 1). It is typically seen in liver biopsy specimens showing steatohepatitis. In all but one case, some of the sinusoidal neomatrix showed some orcein positivity (fig 2). The distribution of the sinusoidal fibrosis component within the biopsy specimens was difficult to establish consistently due to the limited nature of the biopsy material.

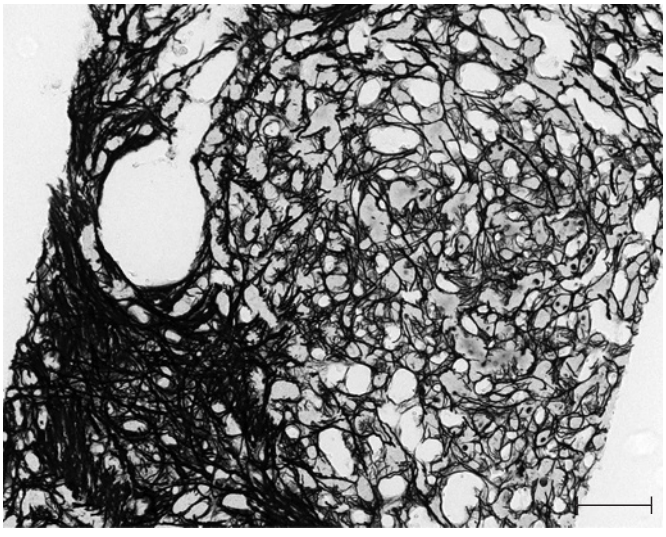

Figure 1 Liver biopsy specimen from a patient with the Fontan circulation, showing a dense fibrous septum with extensive sinusoidal fibrosis in adjacent parenchyma, reticulin stain. Scale bar 100 μm.

Sinusoidal dilatation

The most marked feature of the biopsy specimens from Fontan patients was the degree of sinusoidal dilatation/ectasia (fig 3). All specimens showed some dilatation, with equal numbers having up to one third, up to two thirds, and more than two thirds of the sinusoids affected. This dilatation appeared to extend from the perivenular region (zone 3) in a portal direction, such that a score of 1 represented dilatation of zone 3 sinusoids, and a score of 2 represented dilatation of both zones 3 and 2. Where present the degree of sinusoidal expansion was marked. Marked sinusoidal dilatation was also evident in the patients with severe TR.

The development of scoring systems for histological features within liver biopsy specimens has been most formally achieved in hepatitis C virus infection. The development of the METAVIR system required examination of a large number of biopsy specimens by a large number of observers. In this context, 20 specimens is a small number, so a pragmatic adaptation of previously published scoring systems was used based on the obvious histological features identified. In order to support the use of some of these scoring systems, the specimens were crudely ranked for the degree of architectural distortion, sinusoidal fibrosis and sinusoidal dilatation, so that supportive correlation with scores could be made. The ranking of the specimens for these features strongly correlated with the assigned score (table 3).

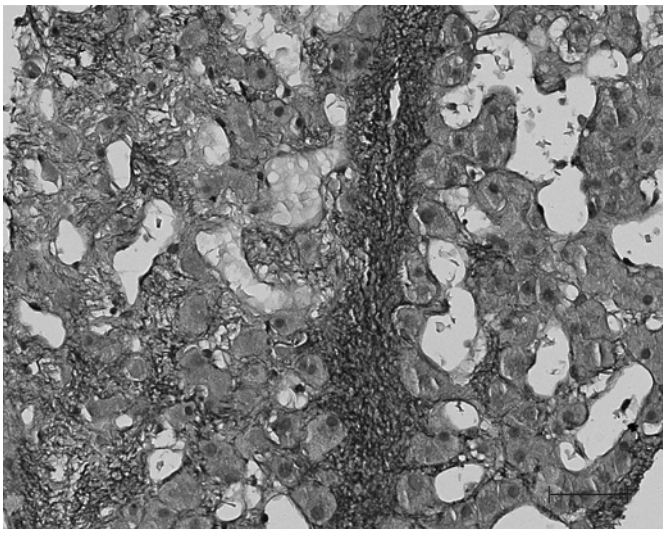

Figure 2 Liver biopsy specimen from a patient with the Fontan circulation, showing a marked orcein positive central dense fibrous septum with adjacent orcein positive sinusoidal fibrosis, orcein stain. Scale bar 50 μm.

Furthermore there was a significant positive correlation between the assigned rank of a biopsy for sinusoidal fibrosis and gross architectural distortion (correlation coefficient = 0.656, p = 0.002). There was no correlation between sinusoidal dilatation rank or score and either gross architectural or sinusoidal fibrosis rank or score.

Ductular reaction

Sixteen Fontan biopsies showed some degree of bile ductular proliferation (fig 4), in all cases exclusively in portal areas. Both biopsy specimens from patients with TR showed similar changes in portal areas.

Other features

None of the biopsy specimens showed any evidence of ongoing or recent hepatocellular damage. Specifically there were no ballooned hepatocytes or apoptotic bodies, and no ceroid-laden macrophages were evident. One specimen from a patient with a Fontan circulation showed a mild degree of iron deposition, and another showed a mild degree of lobular mononuclear inflammation.

DISCUSSION

We have previously described the correlation of right heart pressure and duration of Fontan circuit with liver fibrosis. The pathology of the liver in these cases showed similarities with that of cardiac cirrhosis, confirming the profibrogenic effect of raised venous pressure within the liver. In this study we have described in detail the histopathology of the liver in an extended group of patients with the Fontan circuit.

Table 3 Correlation of assigned scores with assigned rank

Histological feature	Correlation coefficient (rank vs score)	p Value
Gross architectural distortion	0.858	<0.0001
Sinusoidal fibrosis	0.877	<0.0001
Sinusoidal dilatation	0.938	<0.0001

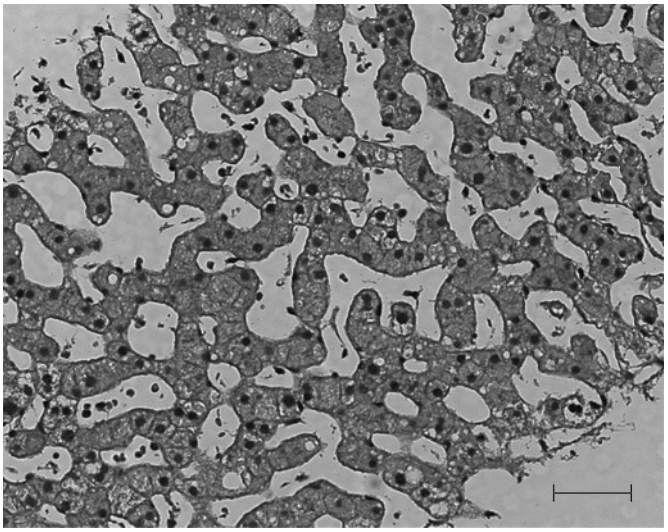

Figure 3 Liver biopsy specimen from a patient with the Fontan circulation, showing severe grade 3 sinusoidal dilatation, H&E stain. Scale bar 50 μm.

The typical features of liver fibrosis in patients with cardiac failure consist of a spectrum of changes that depend on the chronicity of cardiac failure. In those patients with acute cardiac failure, there is centrilobular inflammation and necrosis, with associated increases in serum transaminases. Sinusoidal dilatation was associated with higher right atrial pressure.[15] With chronic cardiac failure there is a pattern of centrilobular fibrosis with the formation of fibrotic spurs,[16] although cirrhosis is said to be uncommon.

The livers of patients with a Fontan circuit are exposed to high venous pressure, with the hepatic vein pressure in the range 9–30 mm Hg and the hepatic wedge pressure in the range 10–30 mm Hg.[8] Associated with this there is also frequently deep intra-hepatic reflux due to right atrial contraction, particularly in patients with an atrio-pulmonary connection. The appearance of the liver in these patients shows significant similarities with that described in cardiac failure, but is more

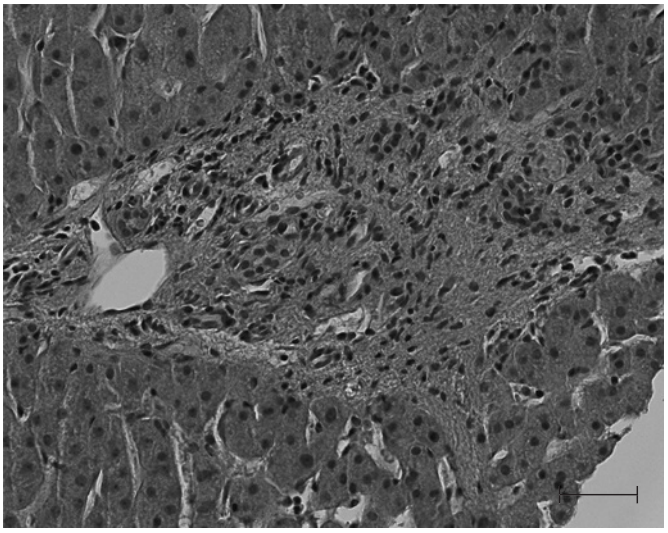

Figure 4 Liver biopsy specimen from a patient with the Fontan circulation, showing bile ductular reaction within a fibrotic portal tract, H&E stain. Scale bar 50 μm.

Take-home messages

- Patients with a long-standing Fontan circuit develop liver fibrosis without obvious inflammation.
- Sinusoidal fibrosis and sinusoidal dilatation are marked.
- Some fibrosis remains orcein negative, suggesting a reversible component.

severe. The degree of sinusoidal dilatation is much more extreme, even in the absence of acute haemodynamic disturbances. The pattern of liver fibrosis is broadly the same as that described as cardiac sclerosis, with a predominantly perivenular distribution of the initial changes, including spur formation. However, the extent of fibrosis and the degree of fibrous septum formation is more severe than generally described for long-standing cardiac failure. Although only two patients were scored as having definite cirrhosis, another 16 had some degree of septum formation; 3 cases that were previously scored as METAVIR fibrosis score "4"[8] were only scored "3" on rescoring in this study. The narrow nature of the biopsy specimens also suggests that a number of patients scoring 3 for gross architectural distortion may actually have cirrhosis, only evident if more tissue were available,[17–19] and also may reflect the inter-observer variability of scoring systems, including that of METAVIR,[20 21] which is more evident in the scoring of suboptimal specimens. The extent of pericellular sinusoidal fibrosis is also not described in cardiac sclerosis. This represents neomatrix deposition within the space of Disse, and is typically seen in steatohepatitis. The livers of patients with severe TR showed appearances as marked as those with a Fontan circuit, consistent with the similarly marked right atrial pressure in these patients.

The maturity of the fibrotic tissue, as assessed by orcein positivity, also reflects the chronicity of the injury. Orcein binds to elastin and cross-linked collagen fibrils. In an experimental model of liver fibrosis, prolonged 12 week injury that produces a micronodular cirrhosis is associated with orcein positive staining of fibrous septa.[12] In contrast, a 6-week period of injury that leads to reversible fibrosis shows no orcein positive matrix. In addition to representing maturity of fibrotic neomatrix, orcein-positivity may also represent the point of irreversibility. In the same animal model, after cessation of injury at 6 weeks, complete architectural resolution is observed. However, after a period of 12 weeks resolution is only partial, leading to a macronodular appearance, with the broad orcein positive fibrous bands remaining. The observation that, in most cases described here, some neomatrix in the sinusoids remained orcein negative suggests that there is a reversible component, and that once Fontan revision has been successfully undertaken, some remodelling may be possible.

The pathogenesis of liver fibrosis in patients with the Fontan circuit is unknown. The majority of fibrogenic injurious stimuli act by initiating inflammation within the liver, leading to the transdifferentiation of hepatic stellate cells into hepatic myofibroblasts (MFBs). Hepatic MFBs are the primary fibrogenic cells, producing fibrotic neomatrix in an environment that favours deposition rather than matrix breakdown. In the descriptions of liver disease in patients with cardiac failure, inflammation and hepatocellular necrosis have been observed. However, in this series of 18 patients, only a small amount of parenchymal lymphocytic inflammation was seen in a single biopsy specimen. It may be that episodes of inflammation are occurring in the livers of Fontan patients either not represented in the biopsy material or not occurring at the time of the biopsy. However, it may also be that liver fibrosis in patients with the Fontan circulation develops independently of inflammation. This effect may be mediated purely by mechanical stretch of the sinusoids and resident cells, a consequence of not only higher venous pressures but also the repetitive deep intra-hepatic reflux associated with atrial systole, since this has been shown to produce profibrogenic effects in hepatic stellate cells[22 23] in vitro, and/or the result of hypoxia on parenchymal and non-parenchymal liver cells. The similar findings in patients with TR support a generic non-inflammatory pressure related mechanism.

Competing interests: None declared.

REFERENCES

1. **Fontan F,** Baudet E. Surgical repair of tricuspid atresia. *Thorax* 1971;**26**:240–8.
2. **Cromme-Dijkhuis AH,** Hess J, Höhlen K, *et al*. Specific sequelae after Fontan operation at mid- and long-term follow-up. Arrhythmia, liver dysfunction, and coagulation disorders. *J Thorac Cardiovasc Surg* 1993;**106**:1126–32.
3. **Kaulitz R,** Luhmer I, Bergmann F, *et al*. Sequelae after modified Fontan operation: postoperative haemodynamic data and organ function. *Heart* 1997;**78**:154–9.
4. **van Nieuwenhuizen RC,** Peters M, Lubbers LJ, *et al*. Abnormalities in liver function and coagulation profile following the Fontan procedure. *Heart* 1999;**82**:40–6.
5. **Narkewicz MR,** Sondheimer HM, Ziegler JW, *et al*. Hepatic dysfunction following the Fontan procedure. *J Pediatr Gastroenterol Nutr* 2003;**36**:352–7.
6. **Ghaferi AA,** Hutchins GM. Progression of liver pathology in patients undergoing the Fontan procedure: chronic passive congestion, cardiac cirrhosis, hepatic adenoma, and hepatocellular carcinoma. *J Thorac Cardiovasc Surg* 2005;**129**:1347–51.
7. **Lemmer JH,** Coran AG, Behrendt DM, *et al*. Liver fibrosis (cardiac cirrhosis) five years after modified Fontan operation for tricuspid atresia. *J Thorac Cardiovasc Surg* 1983;**86**:757–60.
8. **Kiesewetter CH,** Sheron N, Vettukattill JJ, *et al*. Hepatic changes in the failing Fontan circulation. *Heart* 2007;**93**:579–84.
9. **Sheikh AM,** Tang ATM, Roman K, *et al*. The failing Fontan circulation: successful conversion of atriopulmonary connections. *J Thorac Cardiovasc Surg* 2004;**128**:60–6.
10. **Bedossa P,** Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996;**24**:289–93.
11. **Issa R,** Zhou X, Trim N, *et al*. Mutation in collagen-1 that confers resistance to the action of collagenase results in failure of recovery from CCl4-induced liver fibrosis, persistence of activated hepatic stellate cells, and diminished hepatocyte regeneration. *FASEB J* 2003;**17**:47–9.
12. **Issa R,** Zhou X, Constandinou CM, *et al*. Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking. *Gastroenterology* 2004;**126**:1795–808.
13. **Ates B,** Ilker Dogru M, Gul M, *et al*. Protective role of caffeic acid phenethyl ester in the liver of rats exposed to cold stress. *Fundam Clin Pharmacol* 2006;**20**:283–9.
14. **Ara C,** Kirimlioglu H, Karabulut AB, *et al*. Protective effect of resveratrol against oxidative stress in cholestasis. *J Surg Res* 2005;**127**:112–7.
15. **Myers RP,** Cerini R, Sayegh R, *et al*. Cardiac hepatopathy: clinical, hemodynamic, and histologic characteristics and correlations. *Hepatology* 2003;**37**:393–400.
16. **Lefkowitch JH,** Mendez L. Morphologic features of hepatic injury in cardiac disease and shock. *J Hepatol* 1986;**2**:313–27.
17. **Siddique I,** El-Naga HA, Madda JP, *et al*. Sampling variability on percutaneous liver biopsy in patients with chronic hepatitis C virus infection. *Scand J Gastroenterol* 2003;**38**:427–32.
18. **Skripenova S,** Trainer TD, Krawitt EL, *et al*. Variability of grade and stage in simultaneous paired liver biopsies in patients with hepatitis C. *J Clin Pathol* 2007;**60**:321–4.
19. **Schiano TD,** Azeem S, Bodian CA, *et al*. Importance of specimen size in accurate needle liver biopsy evaluation of patients with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2005;**3**:930–5.
20. **Rozario R,** Ramakrishna B. Histopathological study of chronic hepatitis B and C: a comparison of two scoring systems. *J Hepatol* 2003;**38**:223–9.
21. **Goldin RD,** Goldin JG, Burt AD, *et al*. Intra-observer and inter-observer variation in the histopathological assessment of chronic viral hepatitis. *J Hepatol* 1996;**25**:649–54.
22. **Goto T,** Mikami KI, Miura K, *et al*. Mechanical stretch induces matrix metalloproteinase 1 production in human hepatic stellate cells. *Pathophysiology* 2004;**11**:153–8.
23. **Sakata R,** Ueno T, Nakamura T, *et al*. Mechanical stretch induces TGF-beta synthesis in hepatic stellate cells. *Eur J Clin Invest* 2004;**34**:129–36.

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