Concurrent increase in mitosis and apoptosis: a histological pattern of hepatic arterial flow abnormalities in post-transplant liver biopsies

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Liver biopsies are critical in managing patients after liver transplantation. One key histological pattern in transplant liver pathology is spotty hepatocyte necrosis without significant lobular inflammation, which is typical of recurrent hepatitis C. Over the past years, we have observed several liver biopsies with a pattern of injury that mimicked the histological findings of early recurrent hepatitis C. This pattern consisted of increased lobular hepatocyte spotty necrosis without significant inflammation, but with the additional finding of numerous concurrent mitotic figures. To better understand this unique pattern of injury, we studied a group of 8 liver biopsies with this pattern and a control group of 22 biopsies with typical recurrent hepatitis C. Hepatocyte apoptosis and mitosis were quantified by counting 10 high-power fields (HPFs). The mean interval between transplantation and biopsy was 62 days in both groups. There was no significant difference between the study and the control groups in portal and lobular inflammation. In contrast, there was more hepatic apoptosis (acidophil bodies) in the study cases than the controls (average of 10.3 vs 2.8 apoptotic bodies/10HPF; $P=0.0004$). Likewise, there were more mitoses in the study cases than the controls (average 6.3 vs 0.1 /10HPF; $P<0.0001$). Interestingly, examination of the medical records for the cases with increased apoptosis and mitoses found a very strong association with hepatic arterial problems including thrombosis ($N=3$), stenosis (1), flow abnormalities consistent with stenosis (3), and arteritis associated with acute rejection (1). In summary, our findings indicate that the histological pattern of concurrent increases in both hepatocyte mitosis and apoptosis in a post-transplant liver biopsy without significant lobular inflammation is strongly associated with hepatic arterial insufficiency and should prompt evaluation of the hepatic artery.

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Liver biopsies are important in managing patients who have undergone liver transplantation. In patients transplanted for chronic viral hepatitis, liver biopsies are commonly performed in the first 6 months after transplantation to evaluate mild elevations in the liver enzymes and help differentiate recurrent viral hepatitis from acute cellular rejection. In this setting, lobular spotty necrosis in the absence of significant inflammation is a key histological pattern that suggests early recurrent viral

hepatitis.^{1,2} Preservation injury in some cases may also be in the differential, but in most cases, the time of the biopsy since transplantation makes preservation injury unlikely. Likewise, preservation injury can usually be excluded based on available liver function tests, as they will typically reveal continuous abnormalities after the transplantation,³ in contrast to the abrupt onset of increased liver function tests that is more typical of recurrent viral hepatitis and acute cellular rejection. Thus, the histological pattern of lobular spotty necrosis in the absence of significant inflammation remains a key element in the diagnosis of early recurrent viral hepatitis. However, over the years we have noticed occasional cases that closely mimicked this pattern, but had an additional distinctive feature of numerous mitotic figures. Also of note, a number of these cases were in allografts transplanted for underlying liver diseases that did not include chronic viral

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Table 1 Characteristics of the study group with increased apoptosis and mitoses

Case	Age	Sex	Reason for liver transplant	Rejection	Imaging study	Days post-transplantation	Apoptosis (/10 HPF)	Mitosis (/10 HPF)	Outcome
1	55	F	Autoimmune hepatitis	No	HA stenosis	46	12	2	Normal LFT after 4 years
2	47	M	Chronic hepatitis C	No	HAT	8	24	9	Graft failure 3 weeks after transplantation
3	59	M	Chronic hepatitis C	No	HAT	12	7	7	Normal LFT after thrombus removal
4	66	M	Chronic hepatitis C	Mild	HAT	150	4	7	Graft failure 6 months after transplantation
5	15	M	Autoimmune hepatitis	Mild	Suggestive of HA stenosis	71	2	18	Normal LFT after 5 years
6	60	M	Chronic hepatitis C	No	Suggestive of HA stenosis	10	19	3	Graft failure 2 months after transplantation
7	24	M	Polycystic liver disease	Mild	No HAT or stenosis	150	8	2	Features of chronic rejection at 9 months post-transplantation
8	62	M	Chronic hepatitis C	No	Suggestive of HA stenosis	47	6	2	Normal LFT after 4 months
Average	49	1F, 7M				62	10.3 (± 2.70)	6.3 (± 1.94)	

Abbreviations: F, female; HA, hepatic artery; HAT, hepatic artery thrombosis; HPF, high-power field; LFT, liver function tests; M, male.

hepatitis. Furthermore, several of the biopsies were temporally associated with hepatic artery thrombosis or stenosis. This suggested to us that the constellation of findings of (1) increased lobular spotty necrosis, (2) increased mitotic figures, and (3) little or no inflammation may represent a histological pattern of early acute hepatic artery insufficiency. To further characterize the significance of this distinctive histological pattern, we retrospectively studied the associated clinical and pathological findings. As recurrent viral hepatitis is the main alternative diagnosis, we used cases of early recurrent hepatitis C cases as controls.

Materials and methods

Patient and Material

All biopsies were between the time interval of 2001 to 2011 and had been performed within 6 months of liver transplantation. The study group was post-transplant liver biopsy specimens with the pattern of bland lobular spotty necrosis (ie, spotty necrosis with little or no lobular inflammation) and increased mitoses. For the control group, allograft liver biopsies were studied from individuals transplanted for hepatitis C that had been diagnosed as consistent with recurrent hepatitis C with no evidence for acute cellular rejection. All biopsies in both the study and control groups had been performed for elevated liver enzymes and had been obtained within 6 months of liver transplantation. Clinical and radiological findings were reviewed.

Histological Assessment

For each biopsy, the amount of portal and lobular inflammation was scored as per the Ishak scoring

system.⁴ No biopsies had significant fibrosis because of the short time since transplantation. Biopsies were also evaluated for acute cellular rejection as per the Banff criteria^{5,6} and for any other pathological findings.

Routine H&E stains were examined for the presence of apoptosis (acidophil bodies) and the amount of apoptosis was quantified as the average number of apoptotic bodies per 10 high-power fields (HPFs). The presence of mitosis was likewise quantified as the average number of mitoses per 10 HPF. For both mitotic and apoptotic counts, fields were arbitrarily chosen for counting. Statistical analysis was performed using unpaired *t*-tests and Fisher's exact test. Significance was defined as $P < 0.05$.

Results

Patient Demographics

Eight cases with the pattern of a concurrent increase in apoptosis and mitosis but without significant inflammation were collected (Table 1). These study cases included seven men and one woman with average age of 49 years at the time of liver biopsy (range, 15–66 years). The underlying causes of native liver disease were chronic hepatitis C ($n = 5$), autoimmune hepatitis ($n = 2$), and polycystic liver disease ($n = 1$). The average interval between transplantation and biopsy was 61 days (range, 8–150 days).

The recurrent hepatitis C control group (Table 2) included 18 men and 4 women with an average age of 52 years (range, 39–67 years). The average interval between transplantation and biopsy was 62 days (range, 8–157 days).

The liver function tests in the study group often showed both hepatitic and cholestatic patterns, whereas the patients with recurrent hepatitis C

Table 2 Characteristics of the control group with recurrent hepatitis C

Case	Age	Sex	Days post-trans-plantation	Apoptosis (/10 HPF)	Mitosis (/10 HPF)
1	48	M	49	3	0
2	67	M	48	3	0
3	54	M	15	0	0
4	42	M	64	3	0
5	44	F	60	0	0
6	53	M	51	6	1
7	53	M	8	2	1
8	54	M	31	6	1
9	66	M	120	1	0
10	49	M	51	2	0
11	59	M	110	1	0
12	39	M	141	0	0
13	62	F	157	5	0
14	54	M	78	2	0
15	51	M	61	3	0
16	45	M	93	0	0
17	50	F	21	12	0
18	44	M	39	3	0
19	53	M	37	1	0
20	46	M	35	2	0
21	53	M	72	3	0
22	49	F	27	2	0
Average	52	4F, 18M	62	2.8 (± 0.6)	0.1 (± 0.07)

Abbreviations: F, female; HPF, high-power field; M, male.

mainly showed hepatitic but not cholestatic patterns of injury.

Histological Evaluations

All of the biopsies in the study group showed prominent hepatic apoptosis with little or no lobular inflammation (Figures 1a and b). They all had increased mitotic figures. Three cases in the study group also showed features of mild acute cellular rejection (Table 1). In the hepatitis C control group, all biopsies showed a mild increase in apoptosis with no significant lobular or portal chronic inflammation. No additional pathological conditions were noted in the control group, as the cases had been selected to have only recurrent hepatitis C.

After formal scoring of the liver biopsies, there was no significant difference between the two groups in the presence and severity of portal and lobular inflammation ($P=NS$). In contrast, the amount of hepatic apoptosis differed significantly between the study cases and control cases. The study cases with increased lobular spotty necrosis plus increased mitoses had an average ($\pm$ s.d.) of 10.3 ± 2.7 apoptotic bodies/10 HPF, whereas the control group with recurrent hepatitis C had an average of 2.8 ± 0.6 apoptotic bodies/10 HPF ($P=0.0004$, t -test). Likewise, the average number of mitoses differed significantly between the two groups. The study group showed an average of 6.3 ± 1.9 mitotic figures/10 HPF, whereas the control group showed an average of only 0.1 ± 0.1 mitotic

figures/10 HPF ($P<0.0001$, Student's t -test). Tissue was available on four of the study cases for Ki-67 immunostaining and all showed marked nuclear labeling of hepatocytes (Figure 1c), as anticipated given the increased mitotic counts. However, the small number of study cases with available tissue for immunostaining precluded formal analysis and comparison of Ki-67 immunostaining to the recurrent HCV controls.

Clinical Correlates

Examination of the medical records found a very strong association with hepatic arterial problems in the study group, with 7/8 cases having arterial flow abnormalities. In three of the eight cases, acute hepatic artery thromboses were identified, all within 7 days of the liver biopsy. Another case showed hepatic artery stenosis on imaging studies. Three additional cases showed changes in imaging studies of the graft vasculature, with new onset increased hepatic artery velocity on Doppler ultrasound that was suggestive of stenosis. In the last case, no arterial flow changes were noted on imaging studies. However, the biopsy showed features of mild acute cellular rejection, in addition to the increased apoptosis and lobular spotty necrosis, and a follow-up biopsy was performed because of worsening enzymes showed moderate acute cellular rejection with arteritis (Figure 1d).

In all, 16 of the 22 individuals in the control group had imaging studies performed for visualization of the vasculature, of which 9 were performed within 30 days of biopsy. Of note, only one case showed an elevated arterial velocity consistent with hepatic artery stenosis and this was on an imaging study performed 15 days after the liver biopsy. The frequency of hepatic arterial flow abnormalities in the study group was then compared with the frequency in the subset of the control group that had imaging studies within 30 days of the biopsy ($N=9$): arterial flow abnormalities strongly correlated with histological pattern of bland lobular spotty necrosis and increased mitoses ($P<0.0001$, Fisher's exact test).

Discussion

Allograft liver biopsies are an important part of patient care, but they can be challenging to interpret in some cases. One of the classic patterns in allograft liver pathology is that of bland lobular necrosis, which suggests recurrent viral hepatitis in the right clinical context.² In this study, we describe a pattern that can closely mimic recurrent hepatitis C, but overall has more hepatic apoptosis and also has increased mitotic activity. This pattern in our case series was strongly associated with hepatic arterial problems, most of which were hepatic artery thromboses or stenosis. Our findings suggest that

Figure 1 Histological findings associated with hepatic arterial flow abnormalities. **(a)** Original magnification $\times 100$. The hepatic lobules in a case of hepatic arterial thrombosis show little or no inflammation, but shows increased hepatocyte apoptosis (white arrows) and mitotic figures (black arrows). **(b)** Original magnification $\times 260$. An example from a second case with new onset hepatic arterial stenosis shows increased lobular apoptosis (white arrows) and mitotic figures (black arrows). **(c)** Original magnification $\times 100$. A Ki-67 immunostain shows marked increased nuclear labeling of hepatocytes. Same case as shown in panel **b**. **(d)** Original magnification $\times 160$. One case with the pattern of increased hepatic apoptosis and mitoses showed acute cellular rejection with focal arteritis.

the application of simple morphological findings can identify a pattern of injury that strongly suggests hepatic artery thrombosis/stenosis. Recognition of this pattern can be of importance in patient care and should prompt evaluation of the allograft vasculature.

The association between lobular apoptosis and hepatic artery thrombosis is further supported by previous studies that used the TUNEL assay to demonstrate that liver biopsies with hepatic artery thromboses had significantly increased apoptosis.^{7,8} The increased apoptosis in cases of hepatic artery thrombosis is most likely caused by ischemia, but the explanation for why some cases have increased mitoses while others do not is not clear. It may be related to the time interval from arterial flow problems to the time of the biopsy, the severity of the arterial flow changes, or from other variables such as donor age. We did not identify any strong zonation pattern to the apoptosis or mitoses, though

others have identified either a zone 1 or zone 3 pattern using the TUNEL assay.^{7,8}

In one of our cases, the histological pattern of spotty necrosis and increased mitoses was associated with arteritis on a subsequent liver biopsy. Although the available information cannot directly establish causality, the arteritis does fit into the overall findings linking this pattern with arterial problems. Interestingly, one previous study also found increased apoptosis in a biopsy with foam cell arteritis.⁸

As an important caveat, it has not been our experience that all cases of early hepatic arterial thromboses will have this pattern. In fact, we have noted anecdotal cases of hepatic artery thrombosis diagnosed by imaging studies that had only increased apoptosis, but not increased mitoses, on the corresponding liver biopsy. Furthermore, it is worth noting that this study is focused on the specific differential for the finding of increased lobular

spotty necrosis with increased mitoses and was not designed to study all of the changes associated with either hepatic artery thromboses or that of recurrent hepatitis C. For example, the later changes (weeks and months) that can be seen in a biopsy after a hepatic artery thrombosis have been previously described and include a range of findings from coagulative necrosis, to zone 3 hepatocyte drop out, to bile duct injury with a ductular reaction.^{9,10} Likewise, the later changes of recurrent hepatitis C are not the focus of this study, but include varying degrees of lobular and portal chronic inflammation and fibrosis that resemble chronic hepatitis C in the non-transplanted population.^{1,11} None of these later findings were present in either the study or the control group in this study.

In summary, the finding of a concurrent increase in apoptosis and mitosis in post-transplant liver biopsies was strongly associated with hepatic arterial flow abnormalities in this cohort of cases. When this pattern is observed in allograft liver biopsy specimens, our findings indicate it should prompt further workup of the hepatic artery.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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