OPEN

Real-Time Tissue Elastography to Evaluate Hepatic Hypoxic-Ischemic Injury Caused by Brain Death

Guoying Zhang, MD Ying Tang, MD Huimin Yu, MD Weina Kong, MD Yun Chen, MD Yang Liu, BSN and Jingwen Zhao, MD

Abstract: This study aimed to explore the potential of real-time tissue elastography (RTE) in evaluating hepatic hypoxic-ischemic injury caused by brain death. We performed RTE and biopsy for 50 donated liver. Hematoxylin-eosin staining was used to observe hepatocyte acidophilic change. Liver grafts were divided into 2 groups, one nonacidophilic change (n = 7) and the other with acidophilic change (n = 43). Correlation and difference analysis were performed for hematoxylin-eosin staining results and RTE parameters. The result indicated that 4 of the 11 RTE parameters, namely, the area of low strain within the region of interest (%AREA), contrast (CONT), inverse difference moment (IDM), and correlation (CORR) were related to hepatocytes acidophilic change ($r = 0.284$, $P = 0.046$; $r = 0.349$, $P = 0.013$; $r = -0.444$, $P = 0.001$; $r = -0.381$, $P = 0.00$). Whereas %AREA and CONT of the nonacidophilic change group were lower than that of the acidophilic change group ($P < 0.05$), IDM and CORR in nonacidophilic change group were higher than that of the acidophilic change group ($P < 0.05$); the remaining parameters were not statistically different between 2 groups ($P > 0.05$). Analysis of receiver operating characteristic curve indicated that the area under the curve of % AREA, CONT, IDM, and CORR were 0.75, 0.79, 0.81, and 0.77, respectively. Based on this, we concluded that the quantitative analysis parameters of RTE could preliminary assess hepatic hypoxic-ischemic injury caused by brain death.

Key Words: ultrasonography, real-time tissue elastography, brain death, donated liver, hypoxic-ischemic injury, hepatocytes acidophilic change

(*Ultrasound Quarterly* 2021;37: 138–143)

Donor from brain death (DBD) is the predominant source of donated organ for liver transplantation in China[1] and accounts for more than 80% of organ transplantation.[2] However, brain death is a complicated dynamic pathophysiological process directly affecting the morphology and function of the organ.[3,4] Experimental and clinical studies have indicated that, compared with living donor liver transplantation, livers from DBD exhibit a more severe ischemia-reperfusion injury and a higher chance of acute rejection, leading to a large risk of initial liver dysfunction and primary graft nonfunction.[5,6] Therefore, brain death is often considered an independent risk factor for graft injury.

Studies have suggested increased hepatocytes apoptosis as the major cause of liver injury in DBD.[7] In addition, it also plays an important role in the nonfunction of the graft after transplantation.[7] Hepatocyte acidophilic change is a common form of apoptosis induced by viral hepatitis. However, it can also occur when hepatocytes are damaged because of severe ischemia and hypoxia in the state of brain death,[8] where the degeneration of cytoplasm enhances its acidophilic staining and causes dehydration and concentration of the cytoplasm, as well as shrinking of hepatocyte, thereby affecting single or multiple hepatocytes that are scattered around the hepatic lobules. At present, the accurate evaluation of hepatocyte acidophilic change heavily relies on biopsy, the major disadvantage of which is its invasiveness. The use of conventional imaging techniques (including 2-dimensional ultrasound, computed tomography, and magnetic resonance imaging) for its evaluation has not been reported yet, besides the fact that the latter two cannot be performed at bedside. Therefore, it is necessary to find an alternative noninvasive and objective evaluation method for the same.

Real-time tissue elastography (RTE) is a functional ultrasound elastography technique, developed based on the concept that all biological tissues will experience a certain degree of deformation or displacement, when facing an external force, because of elasticity or hardness. Real-time tissue elastography collects the echo signals from the tissue before and after application of the external force and converts them into real-time color images to demonstrate tissue hardness.[9–11] Furthermore, the new-generation RTE is equipped with a quantitative analysis software. It can not only directly observe tissue elasticity distribution via color coding but also extract and analyze the characteristics of tissue images,[12] allowing the acquisition of quantitative analysis parameters to describe tissue elasticity information in three aspects (deformation, color histogram, and texture).[13] Compared with conventional ultrasound techniques, RTE can provide diagnostic information independent of patient anatomy and perfusion.[14,15] Its use in the quantitative evaluation of donor kidney injury in an animal model with brain death has already been verified in a previous study.[16]

Received for publication December 21, 2019; accepted February 19, 2020.
Department of Ultrasound, Tianjin First Central Hospital, No. 24 Fukang Road, Nankai District, Tianjin, 300192, China.
The authors declare no conflict of interest.
This study was funded by the program of Tianjin Major Science and Technology Foundation (18ZXZNSY00250).
Address correspondence to: Ying Tang, MD, Department of Ultrasound, Tianjin First Central Hospital, Tianjin, China (e-mail: drtang2002@aliyun.com).

DOI: 10.1097/RUQ.0000000000000497

TABLE 1. Basic Characteristics of Brain-Dead Donors

Year	43.30 ± 12.21
Sex (female/male)	n = 5/n = 45
Causes of brain death	
Cerebral hemorrhage	n = 29
Brain trauma	n = 16
Cerebral infarction	n = 2
Hypoxic-ischemic encephalopathy	n = 1
Other brain disease	n = 2

In this study, we used RTE technique to quantitatively evaluate donor liver injury due to acidophilic change in the state of hypoxic-ischemic caused by brain death, so as to investigate its application value in the assessment of hepatic histological injury in the state of brain death.

MATERIALS AND METHODS

Patients

Fifty livers from brain-dead donors were performed from May 2017 to January 2018, and their clinical and pathological data were collected. There were 45 males and 5 females, whose average ± SD age was 43.30 ± 12.21 years, ranging from 17 to 66 years. The cause of brain death included cerebral hemorrhage (n = 29), brain trauma (n = 16), cerebral infarction (n = 2), hypoxic-ischemic encephalopathy (n = 1), and other brain disease (n = 2) (Table 1). For the inclusion criterion, only brain-dead donor grafts were included. Seriously infected patients who cannot conduct RTE were excluded. The criterion of brain death was adopted in reference to "Criteria for brain death" (Adult) (Revision) (brain death criteria drafting group, Ministry of Health, China) in 2009[17] and "Criteria and technical specifications for brain death (Adult version)" (brain death criteria drafting group, China) in 2013.[18] Family of the donor had signed the relevant informed consent form with Red Cross workers as witness. Information of the donor was reported to China organ transplant response system. The donor was examined for vital signs in the intensive care unit, and physiological conditions were monitored by the relevant imageology and laboratory examination. This study was approved by the ethics committee of Tianjin First Central Hospital.

Real-Time Tissue Elastography

1. Equipment: HITACHI Noblus portable color Doppler ultrasound diagnostic instrument (Hitachi Noblus, Tokyo, Japan) was used, and it was equipped with a C5–1 convex probe (1–5 MHz) for RTE examination.
2. Image acquisition: Ultrasonic examination was done after the confirmation of brain-dead state and before organ procurement. The instrument was adjusted to have the clearest image under 2-dimensional condition, opening liver elastography mode. A real-time dual-amplitude RTE image was manifested for measurement. The region of interest (ROI) of the RTE image for analysis was defined, and the position of the ROI was under the surface of the liver at least 1 cm and away from large vessels or shadows from ribs. The external driving force was originated from cardiac impulse.[15] At the RTE image, there was a curve at the bottom of the image, which represented the degree of the stress; *peak* was in the state of tissue compression, and *trough* was in the state of tissue relaxation. The values at the trough were obtained for analysis. We totally recorded the value of 5 RTE images obtained from 5 different cardiac cycle for each patient, and the average of these 5 image parameters was used in analysis. Eleven quantitative parameters were totally obtained for each patient, including average relative strain value in the ROI (MEAN), standard deviation of relative strain value within the ROI (SD), area ratio of low-strain region (%AREA), complexity (COMP), kurtosis (KURT), skewness (SKEW), contrast (CONT), entropy (ENT), inverse difference moment (IDM), the angular second moment (ASM), and correlation (CORR).[19–21] Depending on the differences in characterizing RTE image, the obtained 11 quantitative parameters for the image were divided into 3 types: parameters to describe color histogram (MEAN, SD, KURT, and SKEW), those for deformation (%AREA and COMP), and those for image texture (include CONT, ENT, IDM, CORR, and ASM).

Histopathological Examination

In the process of donor procurement, tissue samples were taken for pathological examination. Hematoxylin-eosin (HE) staining was performed to evaluate acidophilic change in hepatocytes. Acidophilic change was identified as enhanced eosinophilic staining in cytoplasm, the volume of hepatocytes decreased, the surface of it shrunken, and the water in the cytoplasm decreases and concentrates.[22] If the structure of hepatocytes was normal and have no change in HE staining, it would be defined as nonacidophilic change. Forty-three cases had acidophilic change, whereas 7 cases did not.

Statistical Analysis

SPSS version 23.0 (IBM, Armonk, NY) was used for statistical analysis. First, K-S normality test was used for data normality test. Those normally distributed variables were expressed by mean value ± SD, and group comparisons were undertaken using 2 independent samples *t* test. Variables not consistent with normal distribution were expressed as median (25% quantile, 75% quantile), and differences were tested using rank sum test.

TABLE 2. Correlation Between RTE Parameters and Donor Liver Hepatocyte Acidophilic Change

	Histogram				Deformation		Texture			
	Mean	SD	KURT	SKEW	%Area	COMP	CONT	ENT	IDM	CORR
r	−0.222	0.241	0.003	0.163	0.284	0.169	0.349	0.146	−0.444	−0.381
P	0.121	0.091	0.982	0.259	0.046	0.241	0.013	0.313	0.001	0.006

TABLE 3. Comparison of RTE Parameters Between 2 Groups (Mean Value ± SD) / M (Q25, Q75)

		Nonacidophilic Change (7 Cases)	Acidophilic Change (43 Cases)	*t/Z*	*P*
Histogram	MEAN	128.23 ± 8.9	123.07 ± 7.88	1.57	0.121
	SD	38.22 ± 7.9	43.7 ± 7.84	−1.72	0.09
	KURT	2.79 (2.54–2.99)	2.79 (2.63–2.93)	0.000	1.000
	SKEW	−0.04 ± 0.19	0.05 ± 0.20	−1.142	0.259
Deformation	%AREA	3.18 ± 2.48	6.98 ± 4.76	−2.051	0.046
	COMP	15.23 (7.33–16.04)	2.79 (2.54–2.99)	−1.356	0.184
Texture	CONT	141.27 ± 56.6	236.55 ± 94.43	−2.581	0.013
	ENT	3.47 ± 0.14	3.54 ± 0.16	−1.020	0.313
	IDM	0.11 (0.10–0.14)	0.09 (0.08–1.10)	−2.859	0.003
	CORR	0.95 (0.94–0.96)	0.94 (0.93–0.95)	−2.646	0.010
	ASM	0.00 ± 0.00	0.00 ± 0.00	0.00	>0.05

Results of ultrasonic elasticity parameters correlating with HE staining was analyzed using Spearman correlation analysis. Receiver operating characteristic curve was established, and the cutoff value for each parameter was determined.

RESULTS

Correlation Between RTE Parameters and Donated Liver Acidophilic Change

According to the Spearman rank correlation analysis, the RTE image parameter of %AREA, CONT, IDM, and CORR were related to hepatocyte acidophilic change of donated liver, positively correlated with %AREA and CONT (r = 0.284, P = 0.046; r = 0.349, P = 0.013), and negatively correlated with IDM and CORR (r = −0.444, P = 0.001; r = −0.381, P = 0.006) (Table 2).

Comparison of RTE Parameters Between the Two Groups of Donated Liver, With or Without Hepatocyte Acidophilic Change

The values of %AREA, CONT, IDM, and CORR were different between the 2 groups, with and without acidophilic change groups, whereas the remaining parameters failed to show significant difference between the 2 groups. Specifically, the values of %AREA and CONT were higher in acidophilic change group (%AREA, 6.98 ± 4.76 vs 3.18 ± 2.48, P = 0.046; CONT, 236.55 ± 94.43 vs 141.27 ± 56.6, P = 0.013), and those of IDM and CORR were higher in without acidophilic change group (IDM, 0.11 (0.10, 0.14) vs 0.09 (0.08, 1.10), P = 0.003; CORR, 0.95 (0.94, 0.96) vs 0.94 (0.93, 0.95), P = 0.01) (Table 3; Figs. 1, 2).

ROC Analysis of the 4 Correlated Parameters, % AREA, CONT, IDM, and CORR

The meaningful quantitative parameters of RTE for evaluating hepatocyte acidophilic change of donated liver, including %AREA, CONT, IDM, and CORR, were analyzed by ROC curve. Each parameter showed moderate evaluation performance. Especially, the %AREA and CONT had relative high positive predictive value (Table 4, Fig. 3).

DISCUSSION

In the state of brain death, liver damage is mainly caused by ischemia, hypoxia, and release of inflammatory factors in

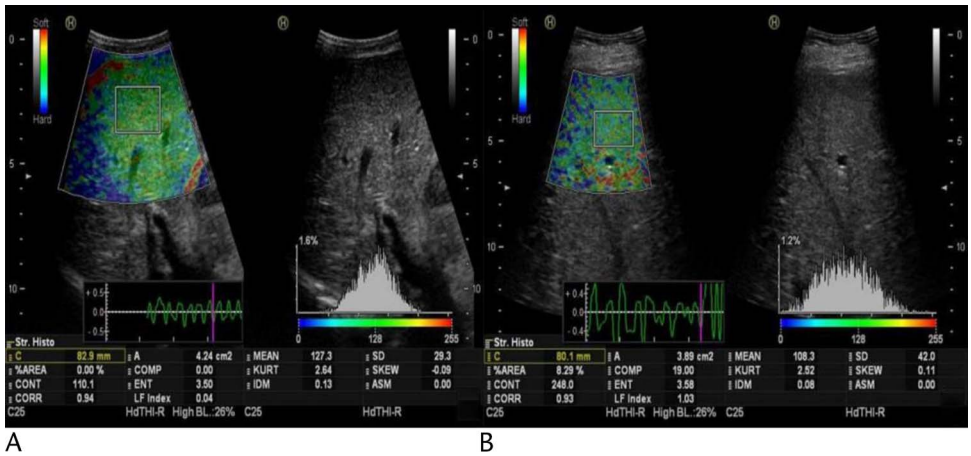

FIGURE 1. Real-time tissue elastography of donated liver from brain death donor. A, The RTE image of donated liver without hepatocyte acidophilic change by HE staining, gray scale image cannot find some abnormality. Furthermore, on RTE map of the ROI, it was mainly a plain green area. B, The RTE image of donated liver with hepatocyte acidophilic change by HE staining, gray scale image also cannot find some abnormality. However, on RTE map of the ROI, it was revealed that the blue area gradually increased.

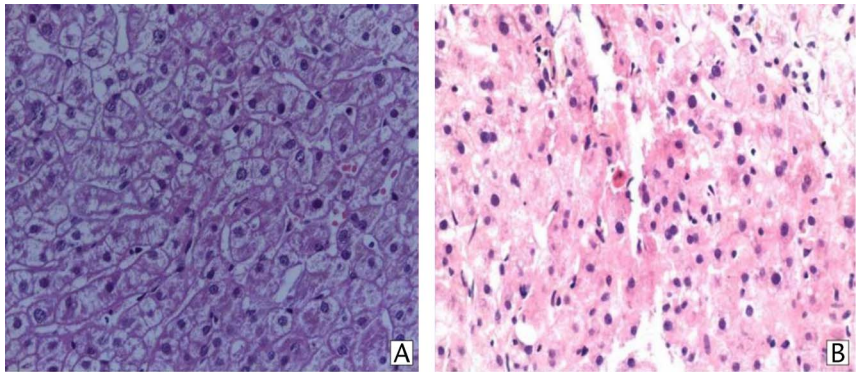

FIGURE 2. Hematoxylin-eosin staining of donated liver from brain death donor. A, The HE staining of donated liver without hepatocyte acidophilic change (by 40 × 10 times). B, The HE staining of donated liver with hepatocyte acidophilic change (by 40 × 10 times).

hepatocytes, which may often cause irreversible damage to hepatocytes. Acidophilic change of hepatocyte is a pathological change in the early stage of liver injury after brain death, which can accurately reflect on the degree of hepatocyte damage.[23,24] After brain death, the level of catecholamine surges initially, causing increased vascular resistance of body. Later, the level of it declines below the standard, leading to cardiovascular collapse due to hypovolemia.[25] In facing of deteriorating hemodynamics, a compromised abdominal organ perfusion and reduced oxygenation become evident.[25,26] Under the influence of hypoperfusion and hypotension, microcirculation changes are beginning in the liver, stimulating hepatocyte inflammatory reaction and immune activation. This seems to be the pathological basis of hepatocytes acidophilic change, indicating early stage of liver function damage and liver quality decline.[23] Therefore, it is clear that a quick identification of hepatocyte acidophilic change is crucial. Liver biopsy has traditionally been used for evaluating graft histological injury. However, its usefulness is limited by its invasiveness, being time-consuming, and associated sampling error.[27] Applications of routine imaging (including 2-dimensional ultrasound, computed tomography, and magnetic resonance imaging) have not been reported in this field, and the latter two are inapplicable at the bedside. Therefore, an optimal, noninvasive, objective, and simple method is urgently required for assessing hepatocyte acidophilic change.

Real-time tissue elastography was a strain elastography based on the elasticity or hardness properties of biological tissue, which is a quantitative method for measuring the tissue deformation generated by cardiac impulse.[13] Tissue deformation recognized by ultrasound equipment is converted into a real-time color image. It can quantitatively evaluate tissue features by analyzing the strain histogram and texture of the image.[12] and can provide independent diagnostic information of anatomy and perfusion of the target organ.[14,15] The provided 11 quantitative elastic parameters are divided into 3 categories according to the different description feature of image, namely, the strain histogram, deformation, and image texture.

In this study, DBD liver grafts were evaluated by both RTE and traditional HE staining method to verify the ability of RTE to predict hepatocyte acidophilic change. Results showed that the parameters describing deformation and texture of the image were correlated with the occurrence of hepatocyte acidophilic change, including %AREA, CONT, IDM, and CORR ($r = 0.284$, $P = 0.046$; $r = 0.349$, $P = 0.013$; $r = -0.444$, $P = 0.001$; $r = -0.381$, $P = 0.006$); the remaining parameters have no correlation. The results also showed that, among the 11 parameters, the values of %AREA, CONT, IDM, and CORR showed significant difference between the 2 groups ($P = 0.046$, $P = 0.013$, $P = 0.003$, $P = 0.01$). Specifically, the values of %AREA and CONT were higher in acidophilic change group, and IDM and CORR were higher in without acidophilic group. The ROC curve indicated that each parameter showed moderate evaluation performance (the area under the ROC curve of %AREA, CONT, IDM, and CORR was 0.75, 0.79, 0.81, and 0.77, respectively), and the %AREA and CONT had relative high positive predictive value.

Three of the 4 meaningful parameters describe the image texture. CONT was the contrast, which is the amount of local variations present in an image. The greater the resolution, the greater the contrast.[16] IDM was inverse difference moment, which reflects the homogeneity of image texture. The larger the value gets, the lesser image texture changes. This is inverse difference between 2 groups. CORR was correlation and is a measure of gray-tone linear dependencies in the image. If the matrix element values are evenly distributed, the value of CORR will be large.[28,29] Texture is a common concept for

TABLE 4. Results of Receiver Operating Characteristic Curve

	Cut-off Value	Area Under the Curve	Sensitivity, %	Specificity, %	PPV, %	NPV, %	LR+	LR−
%AREA	6.53	0.75	53.5	100	100	63.6	—	0.46
CONT	186.85	0.79	76.7	85.7	97.6	75.0	5.37	0.27
IDM	0.095	0.81	85.7	69.7	96.7	31.6	2.83	0.20
CORR	0.945	0.77	71.4	74.4	94.1	31.3	2.79	0.38

LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

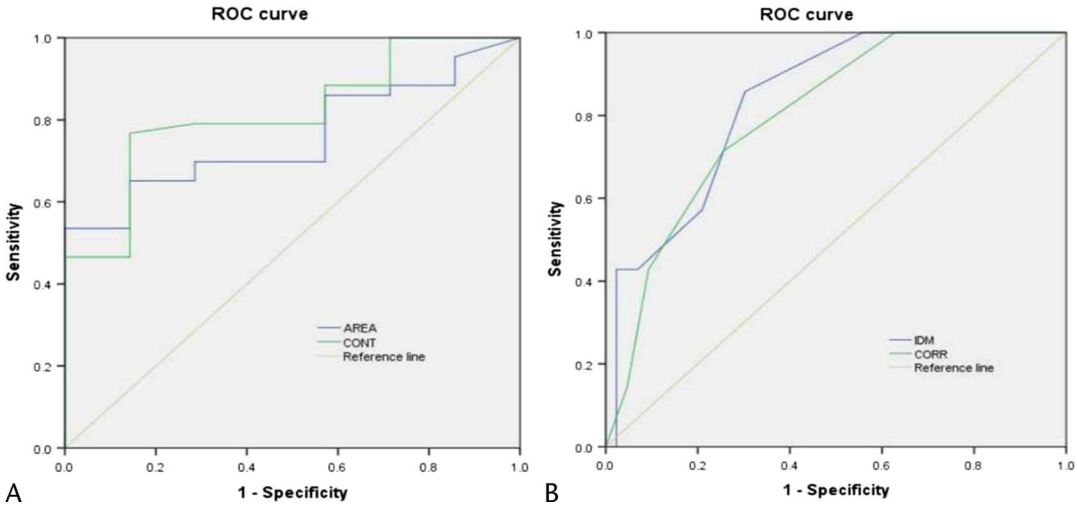

FIGURE 3. Receiver operating characteristic curves of %AREA, CONT, IDM, and CORR for evaluating hepatocyte acidophilic change of brain death donor. A, The ROC curve of %AREA and CONT. B, The ROC curve of IDM and CORR.

describing images feature, and different objects have different textures. However, compared with the computer, human eyes had obvious limitations in distinguishing texture differences in an image. Therefore, with the help of a computer, quantitative analysis of image texture can mine more subtle and rich information. Real-time tissue elastography quantitative analysis technique provides quantitative parameters for image texture analysis based on the principle of gray-tone spatial-dependence matrices,[28,29] and this study confirmed that it can quantitatively analyze liver histological damage in brain death state. The RTE image of hepatocytes acidophilic change in the brain death state is characterized by the following: (1).image definition: hepatocyte acidophilic change destroys the internal structure of hepatocytes, resulting in increased and disordered acoustic interface in the liver parenchyma, enlarging the difference of acoustic impedance. Therefore, the CONT is increased. (2) Image uniformity: the acidophilic change hepatocyte is random and uneven in the parenchyma, and the ultrasonic image texture changes from fine uniformity to rough unevenness, and the parameters IDM and CORR also decrease.

Another meaningful parameter is %AREA that describes the area ratio of low-strain region of the image. The larger the value got, the harder the tissue was. It indicates that water in the hepatocyte is lost, resulting in their shrinkage and weakened elasticity after hepatocyte acidophilic change in the state of brain death. When pressed by a force, the degree of deformation is weakened, the hardness is increased, and area of the blue region in the image is increased. However, sensitivity of it is relative lower, just a value of 53.5%. In addition, the data in this work showed that the parameters in the strain histogram have no significant difference between the 2 groups. This may relate to the small sample.

In conclusion, the RTE parameters can quantitatively evaluate the histological damages due to hepatocyte acidophilic change in the state of brain death and can be an alternative evaluation method for liver quality, especially the parameters to describe image texture. However, the number of cases enrolled in this study was small, and most of the patients (86%, 43/50) had hepatocyte acidophilic change, which could lead to a bias in the result. The 2-week postoperative follow-up of recipient who was donated from the 50 brain death donors has indicated that the liver functions of all the recipients are recovering well. Long-term follow-up and data collection are currently in progress.

ACKNOWLEDGMENTS

The authors would like to thank Ningning Niu and Jing Liu, for valuable contributions to this study.

REFERENCES

1. Niu NN, Tang Y, Guo QJ, et al. A preliminary study of real-time elastic imaging technique in evaluating the liver of brain death donor and the livers of patients after liver transplantation. *Chin J Ultrasonography*. 2016;25(10):875–878.
2. Zhang SJ, Zhai WL, Song Y, et al. How brain-dead state affects the hepatic morphology and function of Ba-ma mini pigs and its mechanism. *Zhonghua Yi Xue Za Zhi*. 2006;86(18):1244–1248.
3. Van Der Hoeven JA, Moshage H, Schuurs T, et al. Brain death induces apoptosis in donor liver of the rat. *Transplantation*. 2003;76(8):1150–1154.
4. van der Hoeven JA, Molema G, Ter Horst GJ, et al. Relationship between duration of brain death and hemodynamic (in)stability on progressive dysfunction and increased immunologic activation of donor kidneys. *Kidney Int*. 2003;64(5):1874–1182.
5. Fang HB. Experimental study of gene expression in liver tissue of rat with brain death and prevention of liver injury [master's thesis]. China: Zheng Zhou University; 2016.
6. Weiss S, Kotsch K, Francuski M, et al. Brain death activates donor organs and is associated with a worse I/R injury after liver transplantation. *Am J Transplant*. 2007;7(6):1584–1593.
7. Cao SL. The protective effects of Salubrinal on rat liver injury induced by brain death [master's thesis]. China: Zheng Zhou University; 2015.
8. Deng YL. Establishing model of uncontrolled brain death in porcine and research for mechanism of injury [master's thesis]. China: Tianjin Medical University; 2014.
9. Kalita K, Filipczak K, Bieńkiewicz M, et al. Diagnostic value of optimised real-time sonoelastography in the assessment of liver fibrosis in chronic hepatitis B and C. *Prz Gastroenterol*. 2017;12(1):28–33.
10. Teng PPC, Lo YL. A comparison study of conventional ultrasound and ultrasound strain elastography in the evaluation of myopathy. *Ultrasound Q*. 2020;36(1):32–37.
11. Turgut E, Celenk C, Tanrivermis Sayit A, et al. Efficiency of B-mode ultrasound and strain elastography in differentiating between benign and malignant cervical lymph nodes. *Ultrasound Q*. 2017;33(3):201–207.

12. Tatsumi C, Kudo M, Ueshima K, et al. Non-invasive evaluation of hepatic fibrosis for type C chronic hepatitis. *Intervirology*. 2010;53(1):76–81.
13. Dietrich CF, Saftoiu A, Jenssen C. Real time elastography endoscopic ultrasound (RTE-EUS), a comprehensive review. *Eur J Radiol*. 2014;83(3): 405–414.
14. Frulio N, Trillaud H. Ultrasound elastography in liver. *Diagn Interv Imaging*. 2013;94(5):515–534.
15. Fujimoto K, Kato M, Kudo M, et al. Novel image analysis method using ultrasound elastography for noninvasive evaluation of hepatic fibrosis in patients with chronic hepatitis C. *Oncology*. 2013;84(1):3–12.
16. Tang Y, Zhao J, Liu D, et al. Evaluation of early kidney damage caused by brain death using real-time ultrasound Elastography in a Bama pig model. *Ultrasound Med Biol*. 2017;43(10):2395–2401.
17. Drafting Group of Ministry of Health. Brain death criteria (Adult) (Revised version). *Chin J Cerebrovasc Dis*. 2009;6(4):220–224.
18. National Health Brain Injury Quality Control Evaluation Center. Brain death criteria and technical specifications (Adult quality control version). *Chin J Neurol*. 2013;46(9):637–639.
19. Shi Y, Wang XH, Zhang HH, et al. Quantitative analysis of real-time tissue elastography for evaluation of liver fibrosis. *Int J Clin Exp Med*. 2014;7(4): 1014–1021.
20. Yoon JH, Yoo J, Kim EK, et al. Seo JY. Real-time elastography in the evaluation of diffuse thyroid disease: a study based on elastography histogram parameters. *Ultrasound Med Biol*. 2014;40(9):2012–2019.
21. Yoon HY, Lee JH, Kim YE, et al. Clinical significance of histogram parameters on elastography in patients with papillary thyroid microcarcinomas. *Ultrasound Q*. 2017;33(3):219–224.
22. Li J, Gao J, Yan D, et al. Neutralization of chemokine CXCL14 (BRAK) expression reduces CCl4 induced liver injury and steatosis in mice. *Eur J Pharmacol*. 2011;671(1–3):120–127.
23. Ali JM, Davies SE, Brais RJ, et al. Analysis of ischemia/reperfusion injury in time-zero biopsies predicts liver allograft outcomes. *Liver Transpl*. 2015; 21(4):487–499.
24. Sereinigg M, Stiegler P, Puntschart A, et al. Establishing a brain-death donor model in pigs. *Transplant Proc*. 2012;44(7):2185–2189.
25. Liu QW, Ye QF, Ming YZ, et al. Quality of donated liver from citizen passed away. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*. 2016;41(1): 101–108.
26. Su YN, Gu WQ, Liang YR. Effect of donor in brain death on liver transplantation. *Chin J Hepatobiliary Surg*. 2017;23(5):351–354.
27. Schulman AR, Lin MV, Rutherford A, et al. A prospective blinded study of endoscopic ultrasound elastography in liver disease: towards a virtual biopsy. *Clin Endosc*. 2018;51(2):181–185.
28. Haralick RM, Shanmugan K, Dinstein I. Textural features for image classification. *IEEE Trans Syst Man Cybern*. 1973;3:610–621.
29. Wang Yi. Experimental and clinical studies on quantitative diagnosis of liver fibrosis through ultrasound [dissertation]. China: Fu Dan University; 2011.