Effectiveness of contrast-enhanced ultrasound and serum liver enzyme measurement in detection and classification of blunt liver trauma

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Abstract
Objective: This study was performed to identify the correlation between contrast-enhanced ultrasound (CEUS) and contrast-enhanced multidetector computed tomography (CE-MDCT) as well as the correlation between serum liver enzyme concentrations and CE-MDCT in classification of the severity of blunt hepatic trauma using CE-MDCT as a reference standard.

Materials and methods: A blunt liver trauma model was created using 20 rabbits, and CE-MDCT, CEUS, and serum liver enzyme assays were performed. A radiologist and an ultrasound physician independently evaluated the degree of liver trauma. The diagnostic performance of CEUS and serum liver enzyme measurements was compared with that of CE-MDCT using Spearman’s correlation analysis and Pearson’s correlation analysis, respectively.

Results: Spearman’s rank correlation coefficient between the CEUS-based classification and CE-MDCT was 0.888. The aspartate aminotransferase and lactate dehydrogenase concentrations and the aspartate aminotransferase/alanine aminotransferase ratio were positively correlated with the grade of liver injury; Pearson’s correlation coefficients were 0.664, 0.704, and 0.503, respectively. The gamma-glutamyltransferase concentration had a significantly negative correlation with the grade of liver injury (r = −0.467).

Conclusions: CEUS and serum liver enzyme measurement exhibited high consistency with CE-MDCT for both detection and grading of intraparenchymal lesions in blunt liver trauma. These techniques may permit more accurate diagnosis of liver trauma.

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Introduction

The liver is the solid intra-abdominal organ that is most frequently injured by blunt trauma to the abdomen because of its abundant blood supply and superficial location.1 The main cause of liver trauma-related death is uncontrolled haemorrhage, which reportedly has high mortality rates ranging from 5% to 54%.2,3 Computed tomography (CT) is typically used to enable a reliable diagnosis of liver injury within a short time after admission to the emergency room and has become the gold standard for assessing patients with trauma.3 Contrast-enhanced multidetector CT (CE-MDCT) is essential to diagnose active bleeding or blunt injury to a solid organ.4 CT-based classifications of liver injuries allow for the selection of patients who can be managed conservatively.5 However, CT is also expensive and results in excess radiation exposure when repeated imaging is required during treatment or follow-up.

The protocol in many emergency centres requires that rapid performance of abdominal ultrasound (US), termed focused assessment with sonography for trauma (FAST), is performed before the CT scan.6 Conventional US is a rapid, repeatable, noninvasive, inexpensive, and real-time sonographic examination technique with high sensitivity for the detection of free peritoneal fluid but with fairly low sensitivity for the detection of traumatic lesions in solid abdominal organs.6,7 Contrast-enhanced US (CEUS) was recently reported to have higher accuracy and sensitivity than conventional US for the diagnosis of liver trauma, improving the identification and grading of liver trauma with a sensitivity of >90%.5,8–10

Various liver trauma classifications have been established to allow surgeons to adopt the most appropriate therapy to individual patients. The Moore score is the most widely accepted scoring system; it was revised in 1994 by the American Association for Surgery of Trauma.11 The Moore score, which is based on anatomical criteria, defines six grades of injury and is considered the gold standard for description of liver injuries. This scoring system is based on radiologic, autopsy, and surgical findings and is now widely used for abdominal parenchymal trauma before the performance of invasive diagnostic and operative measures.

Several research teams have attempted to use liver enzymes to detect hepatic injury. If an association between serum markers and hepatic injuries is present, early identification of hepatic injuries could be performed. This is important because patients with liver trauma require further repeated monitoring during subsequent management or follow-up at institutions with limited resources.

The aim of this study was to evaluate the accuracy of CEUS and measurement of selected liver enzymes in the detection and classification of hepatic trauma in animals with different grades of hepatic trauma using CE-MDCT (considered the gold standard) as the reference standard.

Materials and methods

Ethical statement

The Institutional Animal Care and Use Committee of the Third Military Medical University reviewed and approved all experimental procedures according to the Guide for the Care and Use of Laboratory Animals (permit no. SYXK 2012-0010).
All surgeries were performed under sodium pentobarbital anaesthesia, and all efforts were made to reduce the number of animals and minimize their suffering.

**Preparation of liver trauma model**

Twenty male New Zealand white rabbits weighing 2.20 ± 0.29 kg (Animal Center of Daping Hospital) were housed in a temperature-controlled room on a 12-/12-h light/dark cycle with access to food and water ad libitum until 12 h before the experiment. The rabbits were fasted overnight with free access to water. They were then anaesthetized by intravenous injection of 2.5% sodium pentobarbital at a dose of 30 mg/kg, and an intravenous catheter was introduced into the proximal auricular vein to allow for infusion of the contrast agent. The rabbits were immobilized on the operating platform in the supine position. The hair on the abdomen was shaved and the skin sterilized with 10% povidone iodine. A midline laparotomy was carried out to access the liver. Liver trauma was randomly induced in different liver lobes with a forceps. The liver trauma was inflicted by an experimenter who did not participate in the CT and US examinations or in the imaging analysis. During the experiment, fluid was administered through an intravenous line in the ear vein to maintain the animals’ blood pressure.

**CE-MDCT examination**

Immediately after induction of liver trauma, the rabbits were fixed in the supine position on the table and CT scans were performed with a conventional 64-channel multidetector CT system (LightSpeed VCT; GE Healthcare, Milwaukee, WI, USA). The CT parameters were as follows: rotation time, 0.5 s; 80 kV; 150 mA; pitch, 0.516:1; slice thickness, 0.625 mm; slice gap, 0 mm; display field of view, 20 cm; and matrix, 512 × 512. Contrast medium (ioversol injection; Jiangsu Hengrui Medicine Co., Ltd., Jiangsu, China) was administered via the auricular vein by an injector (Missouri XD2001; Ulrich Medical GmbH & Co. KG, Germany) as a bolus injection of 740 mg I/kg. CECT was performed 20 s after injection of the contrast medium from the upper border of the heart to the level of the umbilicus to include the whole liver. CT images were consensually analysed by one certificated radiologist with 10 years of experience in abdominal CT imaging; this radiologist was not involved in the production of the animal models.

**CEUS examination**

All US examinations were performed immediately after the CT examinations with a diagnostic US system (LOGIQ E9; GE Healthcare, Wauwatosa, WI, USA) with a 6- to 15-MHz transducer. The US imaging was performed by a radiologist with 8 years of experience in abdominal US. After the conventional US, the mode was changed to a contrast pulse sequence at a mechanical index of 0.08 to perform the CEUS. A second-generation US contrast medium (SonoVue; Bracco, Milan, Italy) was administered through a catheter with a mean diameter of 2.5 μm in the auricular vein at a dose of 0.2 mL/kg diluted in 5 mL saline; this was followed by a 1-ml saline flush to ensure that no residual contrast remained in the catheter. The CEUS was performed immediately after contrast medium injection and lasted for 5 min. US was performed in the high mechanical index mode between two injections for another 5 min to destroy residual bubbles within the liver.

**Image analysis**

Blunt liver trauma is usually graded using the organ injury severity scale of the American Association for the Surgery of Trauma. Because the rabbit liver is smaller than the human liver and differs in lobar
anatomy, we used a modified grading system (Table 1).\textsuperscript{12} Based on previous studies of CE-MDCT imaging findings, the liver was considered injured when its enhancement differed from that of the adjacent parenchyma; i.e., the liver was classified as hyperenhancing, isoenhancing, or hypoenhancing.\textsuperscript{7} The injured areas were also classified by comparison with the adjacent parenchyma on CEUS; i.e., hypervascular, isovascular, or hypovascular.\textsuperscript{7,13} The radiologist and US physician, neither of whom participated in the animal experiment, independently evaluated the presence or absence of liver trauma and the grade of trauma, if present.

| Grade | Description of injuries                                      |
|-------|-------------------------------------------------------------|
| 0     | No trauma                                                   |
| I     | Minor trauma involving <10% of one half of the involved liver (right lobe or left lobe), either laceration or hematoma |
| II    | Moderate liver trauma involving >10% but <50% of one half of the liver |
| III   | Severe liver trauma involving >50% of one half of the liver  |

AAST, American Association for Surgery of Trauma

Results

Gross pathologic observations

Gross pathologic examination findings confirmed that all 20 rabbits had sustained blunt hepatic trauma. All liver lesions were located in the left and middle hepatic lobes. Minor lacerations (Figure 1a), capsular tears and intraparenchymal lesions (Figure 2a), and complete rupture (Figure 3a) were found in all cases.

CE-MDCT findings

CE-MDCT showed 26 hepatic lesions in 20 animals (Figures 1b, c through 3b, c). The acute hepatic traumatic lesions appeared

Table 1. Modified liver injury scale according to AAST classification.\textsuperscript{11}

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Measurement of serum liver enzyme concentrations

Blood samples were collected from the heart after all examinations to measure changes in the serum liver enzyme concentrations and thus analyse changes in hepatic function. The blood samples were centrifuged at 3500 rpm for 10 min at 4°C. The supernatant was collected and stored at −80°C, then sent to the clinical laboratory to assay the aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), and lactate dehydrogenase (LDH) concentrations using a serum multiple biochemical analyser. The AST/ALT ratio was calculated from these measurements.

Statistical analysis

Data were analysed using the SPSS statistical package (SPSS 13.0 for Windows; SPSS, Chicago, IL, USA) and are presented as mean ± standard deviation. Spearman’s rank correlation was used to compare the CEUS and CE-MDCT findings. Kappa consistency checking was done. Pearson correlation analysis was performed to compare the CE-MDCT findings with the serum liver enzyme concentrations. Differences in the AST, ALT, ALP, GGT, and LDH concentrations and the AST/ALT ratio before and after trauma were compared with a two-tailed paired t test. Differences in the AST, ALT, and LDH concentrations and the AST/ALT ratio within different grades of severity were compared by one-way analysis of variance and Dunnett’s multiple-comparison test when the overall difference was significant. A difference with a P value of < 0.05 was considered statistically significant.
with no enhancement in the three phases. According to the modified liver injury scale, we observed six grade I, seven grade II, and seven grade III hepatic injuries.

**CEUS findings**

After CE-MDCT, all animals underwent CEUS examination. According to the CEUS examination results, minor (grade I), moderate (grade II), and severe (grade III) liver trauma was found in six, six, and seven rabbits, respectively. No trauma was found in one rabbit. Because of poor or absent blood reperfusion, minimal or no US contrast agent was able to reach the traumatic area, and the blunt traumatic liver lesions appeared as hypovascular defects in the arterial phase, portal phase, and late phase of CEUS (Figures 1d, 2d, and 3d). The margins of the hepatic injuries were clear compared with the adjacent normal parenchyma on CEUS.

**Comparison of CEUS with CE-MDCT**

Using CE-MDCT, the radiologist detected six, seven, and seven cases of grade I, II, and III liver trauma, respectively, among all animals. Using CEUS, the US physician diagnosed one, six, six, and seven cases of grade 0, I, II, and III liver trauma, respectively. A total of 16.7% (1/6) of the grade I traumas were missed on CEUS, 14.3% (1/7) of the grade II traumas were diagnosed as
grade I and III liver traumas, and 14.3% (1/7) of the grade III traumas were diagnosed as grade II liver traumas. The grading results of blunt liver trauma diagnosed by CE-MDCT and CEUS are shown in Table 2. Spearman’s rank correlation coefficient between CEUS and CE-MDCT was 0.888 (P < 0.0001), and the kappa value was 0.71. The two examinations had a high level of concordance (P < 0.0001).

**Relationship between serum liver enzyme concentrations and severity of liver trauma**

According to the CE-MDCT grading system, six (30%) rabbits had grade I injury, seven (35%) had grade II injury, and seven (35%) had grade III injury. All animals had significant differences in the AST, ALT, and LDH concentrations and AST/ALT ratio before and after liver trauma (all P < 0.05), but there was no significant difference in ALP or GGT (Figure 4). When the animals were divided according to the grade of liver injury, we found that the AST, ALT, and LDH concentrations and the AST/ALT ratio gradually increased with the grade of liver trauma, and there were significant differences compared with before liver trauma (Figure 5). In addition, the AST and LDH concentrations and the AST/ALT ratio were positively correlated with

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**Figure 2.** Grade II blunt liver trauma. (a) Gross pathologic image of blunt hepatic trauma. (b, c) Axial and sagittal contrast-enhanced multidetector computed tomography showed low-density unenhanced injuries (black arrows) in the liver parenchyma. (d) Contrast-enhanced ultrasound showed a complete hypovascular laceration of the liver parenchyma with an additional capsular hematoma (black arrows).
the grade of liver injury, and the Pearson correlation coefficients were 0.664 ($P = 0.0014$), 0.704 ($P = 0.0005$), and 0.503 ($P = 0.024$), respectively. The GGT level was negatively correlated with the grade of liver injury ($r = -0.467$, $P = 0.038$) (Table 3).

Figure 3. Grade III blunt liver trauma. (a) Gross pathologic image of a complete rupture of the liver lobe. (b, c) Axial and sagittal contrast-enhanced multidetector computed tomography showed a large, low-density, unenhanced lobe (black arrows) in the liver parenchyma. (d) Contrast-enhanced ultrasound showed an irregular hypovascular area in the liver parenchyma (black arrows).

Table 2. Results of different scales for classification of blunt liver trauma by CE-MDCT and CEUS

| CEUS | 0 | I | II | III | Overestimated | Underestimated |
|------|---|---|----|-----|---------------|----------------|
| 0    | 1 |   |    |     | 0             | 1              |
| I    | 5 | 1 |    |     | 0             | 1              |
| II   | 5 | 1 |    |     | 0             | 1              |
| III  | 1 | 6 |    |     | 0             |                |

CE-MDCT, contrast-enhanced multidetector computed tomography; CEUS, contrast-enhanced ultrasound
Discussion

The liver is the most frequently injured intra-abdominal organ following blunt abdominal trauma. Complex liver injuries are a challenging problem. Rapid and accurate diagnosis of the haemorrhage and intraparenchymal lesions is crucial in patients with blunt hepatic trauma because such injuries warrant immediate treatment. Physicians often use radiographic imaging and sonographic imaging to aid in the initial evaluation of trauma patients involved in high-energy accidents or with loss of consciousness. For patients with stable vital signs, total-body CT examination is a useful technique for rapid detection of all possible body injuries in a single examination and allows the physician to promptly detect prognostic negative factors and thus make decisions regarding conservative or surgical management in patients with polytrauma. However, total-body CT may only be carried out if it is available; it also exposes young patients to radiation, removes

Figure 4. Changes in serum liver enzyme parameters before and after liver trauma. (a) Aspartate transaminase concentration, (b) alanine transaminase concentration, (c) aspartate transaminase/alanine transaminase ratio, (d) lactate dehydrogenase concentration, (e) alkaline phosphatase concentration, and (f) gamma-glutamyltransferase concentration were measured before and after liver trauma. *P < 0.05 vs. before (two-tailed t test).
patients from direct clinical care, and may lead to inappropriate delays in patient care. In hemodynamically unstable patients, the FAST examination is another useful tool for performing the initial survey in emergency centres without interrupting resuscitation manoeuvres, and is frequently used to detect free intraperitoneal fluid with a sensitivity rate of 63% to 99%. However, its major limitation is the technical expertise of the operator. Furthermore, FAST has poor sensitivity for the direct detection of parenchymal lesions on conventional US images, and when used as the sole diagnostic tool, it may miss approximately one-third

Figure 5. Various serum liver enzyme concentrations in different grades of liver trauma. (a) Aspartate transaminase concentration, (b) alanine transaminase concentration, (c) aspartate transaminase/alanine transaminase ratio, and (d) lactate dehydrogenase concentration were measured after liver trauma. *P < 0.05 vs. before (one-way analysis of variance and Dunnett’s multiple-comparisons test).

Table 3. Correlations between grade of liver injury and serum liver enzyme concentrations

| Liver enzyme | r    | P    |
|--------------|------|------|
| AST          | 0.664| 0.0014|
| ALT          | 0.189| 0.424|
| AST/ALT      | 0.503| 0.024|
| LDH          | 0.704| 0.0005|
| ALP          | 0.089| 0.708|
| GGT          | -0.467| 0.038|

r, correlation coefficient; AST, aspartate transaminase; ALT, alanine transaminase; AST/ALT, aspartate transaminase/alanine transaminase ratio; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase
(29%–34%) of abdominal injuries without hemoperitoneum.\textsuperscript{15,16} Thus, a negative FAST result cannot exclude injury.

The sensitivity of US for the detection of parenchymal lesions is < 50%.\textsuperscript{7,17,18} The application of second-generation US contrast agents, which are composed of a lipidrigid outer shell within the low-solubility gas sulfur hexafluoride,\textsuperscript{19} improve the accuracy of US in detecting parenchymal lesions after blunt abdominal trauma. The detection rate of CEUS for intraparenchymal hepatic trauma is significantly higher than that of conventional US (80%–100% vs. 57%–60%, respectively).\textsuperscript{12} On conventional US, minor trauma may be missed because of mixing and masking of the signal by adjacent normal liver parenchyma. On CEUS, lesion areas show minimal or no enhancement compared with adjacent normal liver parenchyma, which is strongly enhanced. The increase in contrast leads to greater sensitivity for detecting liver trauma. In addition, vascular perfusion of the traumatic area can be easily evaluated to diagnose vascular rupture after contrast enhancement. Notably, CEUS reduces the incidence of severe contrast agent–related adverse reactions and can be used for follow-up without problems associated with either ionizing radiation or the nephrotoxic effects of the CT contrast agent.\textsuperscript{8}

A previous study revealed that CEUS and CECT had 93.3% agreement on the grade of hepatic injury and that CEUS grading showed a high level of concordance with CECT. The authors suggested that CEUS could be used to diagnose the grade of liver trauma more accurately than conventional US.\textsuperscript{10} In the present study, Spearman's rank correlation coefficient between CEUS and CE-MDCT in the classification of liver trauma was high at 0.89, and the kappa value was 0.71. These results suggest that CEUS enables a more accurate evaluation of liver trauma.

For patients with trauma secondary to low- to moderate-energy accidents, biochemical testing is an alternative to CT and FAST. Increases in the serum liver enzymes AST and ALT reportedly indicate blunt traumatic liver injury. These transaminases, which are present in high concentrations in hepatocytes, are largely released into the circulation after acute traumatic hepatocellular injury.\textsuperscript{20} One previous study revealed that the magnitude of these enzyme concentrations was correlated with the severity of liver injury,\textsuperscript{15,20} as was observed in the present study. Additionally, the AST and ALT concentrations are reportedly sensitive diagnostic markers with which to evaluate the degree of trauma caused by blunt hepatic injuries.\textsuperscript{20,21}

In the present study, the AST, ALT, and LDH concentrations and the AST/ALT ratio significantly increased after liver trauma, and these increases were correlated with the severity of liver injury. The AST and LDH concentrations and the AST/ALT ratio were positively correlated with the CE-MDCT–based classification using Pearson's correlation analysis, and Pearson's correlation coefficients were 0.664, 0.704, and 0.503, respectively. Although the ALT level was significantly different before and after liver trauma, ALT had a poor positive correlation with the CE-MDCT–based classification. Interestingly, the GGT concentration showed a significant negative correlation with the grade of liver injury.

A combination of the AST, ALT, and LDH concentrations and the AST/ALT ratio may be useful for predicting the presence and severity of hepatic injuries. We also found that the ALT concentration in rabbits with severe liver injury (grade III) was lower than that in rabbits with moderate liver injury (grade II). Therefore, even for patients whose liver enzyme concentrations are lower and liver injury cannot be completely ruled out, measurement of liver
enzymes may still provide clues concerning liver injury, particularly in patients with high-grade liver injury.

In summary, hepatic trauma can be divided into different grades using the CE-MDCT imaging modality described herein. Our study showed a high correlation between the CE-MDCT–based classification of the severity of hepatic trauma, CEUS, and the serum liver enzyme concentrations. CEUS was shown to be as sensitive as CE-MDCT in the detection of traumatic liver lesions and clearly demonstrated the size of the injury and involvement of capsular tears, intraparenchymal lesions, and rupture. The AST and LDH concentrations and the AST/ALT ratio were positively correlated with the grade of liver trauma. It appears that CEUS findings and serum liver enzyme concentrations can play an important role in the emergency management of hepatic trauma with respect to demonstrating the severity of injury. These parameters allow for rapid and accurate depiction of traumatic injuries and aid clinicians in choosing the optimal therapeutic strategy such as conservative management, minimally invasive treatment, or surgery.

**Conclusion**

CEUS examination and serum liver enzyme measurement show high consistency with CE-MDCT in both the detection and grading of intraparenchymal lesions in blunt liver trauma and may permit more accurate diagnosis of liver trauma.

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**Declaration of conflicting interests**

The authors declare that there is no conflict of interest.

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**References**

1. Chien LC, Lo SS and Yeh SY. Incidence of liver trauma and relative risk factors for mortality: a population-based study. *J Chin Med Assoc* 2013; 76: 576–582.
2. Prichayudh S, Sirinawin C, Sriussadaporn S, et al. Management of liver injuries: predictors for the need of operation and damage control surgery. *Injury* 2014; 45: 1373–1377.
3. Slotta JE, Justinger C, Kollmar O, et al. Liver injury following blunt abdominal trauma: a new mechanism-driven classification. *Surg Today* 2014; 44: 241–246.
4. Yu J, Fulcher AS, Turner MA, et al. Multidetector computed tomography of blunt hepatic and splenic trauma: pearls and pitfalls. *Semin Roentgenol* 2012; 47: 352–361.
5. Lv F, Ning Y, Zhou X, et al. Effectiveness of contrast-enhanced ultrasound in the classification and emergency management of abdominal trauma. *Eur Radiol* 2014; 24: 2640–2648.
6. Miele V, Piccolo CL, Galluzzo M, et al. Contrast-enhanced ultrasound (CEUS) in blunt abdominal trauma. *Br J Radiol* 2016; 89: 20150823.
7. Clevert DA, Weckbach S, Minaifar N, et al. Contrast-enhanced ultrasound versus MS-CT in blunt abdominal trauma. *Clin Hemorheol Microcirc* 2008; 39: 155–169.
8. Manetta R, Pistoia ML, Bultrini C, et al. Ultrasound enhanced with sulphur-hexafluoride-filled microbubbles agent (SonoVue) in the follow-up of mild liver and spleen trauma. *Radiol med* 2009; 114: 771–779. [in Italian, English Abstract].
Seitz K, Strobel D, Bernatik T, et al. Contrast-enhanced ultrasound (CEUS) for the characterization of focal liver lesions - prospective comparison in clinical practice: CEUS vs. CT (DEGUM multicenter trial). Parts of this manuscript were presented at the ultrasound Dreilandertreffen 2008, Davos. *Ultraschall Med* 2009; 30: 383–389.

Tang J, Li W, Lv F, et al. Comparison of gray-scale contrast-enhanced ultrasonography with contrast-enhanced computed tomography in different grading of blunt hepatic and splenic trauma: an animal experiment. *Ultrasound Med Biol* 2009; 35: 566–575.

Moore EE, Cogbill TH, Jurkovich GJ, et al. Organ injury scaling: spleen and liver (1994 revision). *J Trauma* 1995; 38: 323–324.

You JS, Chung YE, Lee HJ, et al. Liver trauma diagnosis with contrast-enhanced ultrasound: interobserver variability between radiologist and emergency physician in an animal study. *Ann Emerg Med* 2012; 30: 1229–1234.

Yekuo L, Shasha W, Xiansheng Z, et al. Contrast-enhanced ultrasound for blunt hepatic trauma: an animal experiment. *Am J Emerg Med* 2010; 28: 828–833.

Sessa B, Trinci M, Ianniello S, et al. Blunt abdominal trauma: role of contrast-enhanced ultrasound (CEUS) in the detection and staging of abdominal traumatic lesions compared to US and CE-MDCT. *Radiol med* 2015; 120: 180–189.

Zhao et al.