

Comparing the accuracy of three diagnostic criteria and a refined pathological scoring system in drug-induced liver injury: From suspected to definite

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Abstract

Background Drug-induced liver injury(DILI) is difficult in diagnose, criteria used now are mostly based on history review. We tried to evaluate the value of these criteria and histopathology features in DILI to perform a method diagnosing DILI more definitely.

Methods We enrolled 458 consecutive hospitalized DILI patients from 1st January 2012 to 31st December 2018, using Roussel-Uclaf Causality Assessment Method(RUCAM), Maria&Victorino scale (M&V) and Digestive Disease Week-Japan criterion(DDW-J) to perform the evaluation. A refined pathological scale was calculated and combined with those criteria using logistic regression analysis. Area under receiver operating characteristics(AUROC) were used to estimate diagnostic accuracy.

Results The AUROC of the three clinical diagnostic criteria were 0.730 (95%CI:0.667-0.793), 0.793(95%CI:0.740-0.847) and 0.764(95%CI:0.702-0.826) respectively. The AUROC of the refined pathological scale combined with the three criteria were 0.843(95%CI:0.747-0.914), 0.907(95%CI:0.822-0.960) and 0.881(95%CI:0.790-0.942) respectively. In hepatocellular type, the AUROCs were 0.894(95%CI:0.787-0.959), 0.960(95%CI:0.857-0.994) and 0.940(95%CI:0.847-0.985); In cholestatic type, the AUROCs were 0.750(95%CI:0.466-0.931), 0.500(95%CI:0.239-0.761) and 0.500(95%CI:0.239-0.761); In mixed type, the AUROCs were 0.786(95%CI: 0.524-0.943), 0.869(95%CI:0.619-0.981) and 0.762(95%CI: 0.498 -0.930).

Conclusion Combined with pathological scale can significantly improve the accuracy of clinical diagnostic criteria, no matter in alone or combined condition, M&V might be more accurate in diagnosing DILI from suspected patients.

Introduction

Drug-induced liver injury (DILI) is a serious, worldwide health problem. In the United States and Europe, it is the most common reason for acute liver failure, even though it accounts for <1% of acute liver injury cases(1–3). Studies showed that DILI occurs with an annual incidence of approximately 13.9 per 100,000 inhabitants in France compared to 19.1 per 100,000 in Iceland(4, 5). In China, a retrospective study of 22,030 DILI patients showed that only 50.65% of them were cured, but 1.60% died(6). Additionally, DILI is a potentially severe adverse drug reactions that is a major concern for healthcare systems and the pharmaceutical industry, with a cost of £1 billion in the United Kingdom and \$4 billion in the United States(7).

Despite its potentially severe outcomes and drug post-marketing restrictions, diagnosing DILI is still a major challenge, and remains a diagnosis of exclusion. Based on patient data and the typical 'signatures' associated with certain drugs, expert opinion recommends using causality scores to help diagnose, but due to the lack of a reliable method, no objective scales that assesses the causality of a given drug in DILI patients, beyond expert opinion, has been developed(8). On the other hand, histopathology plays an irreplaceable role in providing direct and objective information about the characteristics of liver injury, for example, defining injury patterns(9). Popper. et al. were the first who divided DILI into six patterns: zonal necrosis, simple cholestasis, hepatitis with/without cholestasis, acute haptatis-like with/without massive necrosis, reactive hepatitis and steatosis(10). However, a prospective study showed that liver biopsy was performed in only approximately 50% of patients(11).

Thus, we compared the diagnostic accuracy of three kinds of clinical diagnostic criteria: the RUCAM, Maria & Victorino scale (M&V) and Digestive Disease Week-Japan scale (DDW-J) to assess DILI patients, and analysed their

sensitivity and specificity in diagnosing DILI, and then, for patients with liver biopsy, we explored the value of histopathological characteristics and the role of a pathological scale in diagnosing DILI combined with the clinical criteria.

Method

Patients

Consecutive DILI inpatients at Tianjin Second People's Hospital from 1 January 2012 to 31 December 2018 were enrolled. The standard of definite DILI and suspected DILI were based on the diagnosis and treatment guideline published in 2015, by The Drug Induced Liver Disease Study Group of Chinese Medical Association (which were published in English in 2017(12)) and determined again in a multidisciplinary consultation held by a panel of hepatologists, pharmacologists, clinical toxicologists and pathologists. The Study protocol was approved by the Ethics Committee of Tianjin Second People's Hospital and conformed to the Declaration of Helsinki. All patients signed an informed consent form before enrolment in this study.

Biochemical data

Serum samples were collected on the first day of hospital admission. The laboratory data included serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB), fasting plasma glucose (Glu), triglyceride (TG), and total cholesterol (CHO) levels, measured by a Hitachi 7600-110 automatic analyzer (Hitachi Co., Tokyo, Japan). Serum HBsAg, HBeAg and HBV-DNA were measured by a Roche COBAS e411 (Roche Co., Basel, Switzerland). R values were defined as the ALT/upper limit of normal (ULN) ratio divided by the ALP/ULN ratio according to the Council for International Organizations of Medical Sciences (CIOMS) criteria(13), and DILI was classified as hepatocellular, cholestatic or mixed types based on its R-value.

Diagnostic criterion scales

Three diagnostic criterion scales: the RUCAM, M&V and DDW-J, were used in this study. Each patient was scored with the three different diagnostic rating scales by three physicians. RUCAM(14) has five degrees: score = 0, relationship "excluded"; 1-2: "unlikely"; 3-5: "possible"; 6-8: "probable"; and >8: "highly probable". M&V(15) has five degrees: score <6, "excluded"; 6-9: "unlikely"; 10-13: "possible"; 14-17: "probable"; and ≥ 18 : "definite". DDW-J(16, 17) has three degrees: ≤ 2 : "possible"; 3-4: "probable"; and ≥ 5 "highly probable".

Liver biopsy and refined DILI-PSS

Patients who underwent a percutaneous ultrasound-guided liver biopsy using a MaxCore disposable automatic biopsy needle (C. R. Bard, Inc., Murray Hill, USA) were included. Each specimen was fixed in formalin, embedded in paraffin and stained with hematoxylin-eosin (H&E), Special staining included Masson's trichrome, Gomori collagen and Perls blue. Immunohistochemical staining included keratin 19, HBsAg, HBeAg, preS1 antigen and CD68. The refined DILI-Pathological Scoring System (rDILI-PSS) in our study was based on Hu's studies in China(18, 19) which include: hepatocellular steatosis (macrovesicular steatosis counts for 1 point, microvesicular steatosis counts for 2

and mixed steatosis counts for 3), hepatocellular cholestasis (1 point), apoptosis (1 point), eosinophil infiltration (2 point), vascular inflammation (1 point), iron deposition (1 point) and pigmented macrophages (1 point, in the original DILI-PSS, this was intraepithelial granuloma). H&E and specific staining reagents were purchased from Bogoo (Bogoo., Shanghai, China). Immunohistochemical antigens were purchased from Abcam (Abcam Co., Cambridge, UK). Blinded to the clinical data, two experienced hepatic pathologists independently reviewed the histologic findings. Consensus was reached in cases of disagreement.

Statistical analysis

Continuous variables were compared using the Mann-Whitney U test for two nonnormal datasets and the Kruskal-Wallis H test for more than two nonnormal datasets. A chi-square test was used to compare categorical data between groups. Diagnostic performances of RUCAM, M&V, DDW-J and new parameters which were combined with refined DILI-PSS using logistic regression analysis were evaluated by computing receiver operating characteristics (ROC) curves, the area under the ROC (AUROC) and its 95% confidence intervals (CI). The optimal diagnostic cut-off for each scale was found by the maximum Youden Index. For each cut-off, a corresponding positive predictive value (PPV), a negative predictive value (NPV), and positive and negative likelihood ratios (LR+ and LR-) were also calculated. Logistics regression analysis was used to fit new parameters. A *P* value of <0.05 was considered indicative of significance. Statistical analyses were performed using SPSS 21.0 (SPSS, Chicago, USA) and MedClac 15. (MedCalc Software, M&Vkerke, Belgium).

Results

Patients' demographic and clinical characteristics

A total of 458 DILI patients at Tianjin Second People's Hospital were enrolled during the study period. The CONSORT diagram is shown in Table 1. The majority of the DILI patients had the hepatocellular type, accounting for 290 (63.32%) patients, 71 patients had the cholestatic type (15.5%), and 97 patients had the mixed type (21.18%). More female patients than male patients were affected by all three types of DILI injuries, 188 (64.83%) had the hepatocellular type, 52 (73.24%) had the cholestatic type, and 68 (70.10%) had the mixed type. The patient ages (mean \pm SD) for the three types were 47.47 \pm 13.87, 51.82 \pm 12.77 and 50.71 \pm 12.03, respectively. The median (range) values of ALT, AST, GGT, ALP, TB, DB, Glu, TG and CHO are shown in Table 2. Except for Glu and TG, other biochemical data were statistically significant (*P*<0.05) among the different DILI types, using the Kruskal-Wallis H test, followed by a step-down pairwise comparison test. The results are shown in Figure 1.

Causative drugs involved in DILI patients

In this study, Chinese herbal medicines were the most commonly used drugs in 240 (52.41%) patients. Multiple herbal medicine use was the most common cause in 158 (34.50%) patients, and the top three medicines used individually were Polygonum multiflorum [46 (10.04%)], Alismae rhizome [7 (1.53%)] and Radix bupleuri [5 (1.09%)]. The second highest major category was chemotherapeutics used in 40 (8.73%) patients, followed by non-steroidal anti-inflammatory drugs (NSAIDs) used in 37 (8.08%) patients, antibiotics used in 29 (6.38%) patients and healthcare products used in 25 (5.46%) patients. Detailed results are shown in Table 3.

The diagnostic value of three clinical criteria

Among all 458 DILI patients, 340 were ultimately diagnosed with definite DILI, 118 were suspected DILI. For the three diagnosis scales of DILI, the scores (mean \pm SD) of RUCAM, M&V and DDW-J were 8.04 ± 1.66 , 11.59 ± 2.63 and 8.24 ± 1.2 , respectively. RUCAM confirmed DILI diagnosis with an AUROC of 0.730 (95% CI: 0.667–0.793), $Z = 7.147$, $P < 0.001$, the optimal cut-off was 8, and the Youden Index were 0.3558 for “>8”, 0.3446 for “ ≥ 8 ”. M&V confirmed DILI diagnosis with an AUROC of 0.793 (95% CI: 0.740–0.847), $Z = 10.753$, $P < 0.001$, the optimal cut-off was 11, and the Youden Index were 0.4084 for “>11”, 0.3907 for “ ≥ 11 ”. DDW-J confirmed DILI diagnosis with an AUROC of 0.764 (95% CI: 0.702–0.826), $Z = 8.303$, $P < 0.001$, the optimal cut-off was 8, and the Youden Index were 0.3558 for “>8”, 0.4185 for “ ≥ 8 ”. The ROC curves are shown in Figure 2. The AUROCs, sensitivities, specificities, PPVs, NPVs and LR+, LR- values are shown in Table 4.

A sub-analysis of ROC and AUROC was performed according to the clinical injury type. In the hepatocellular type, the AUROCs of RUCAM, M&V and DDW-J were 0.688 (95% CI: 0.617–0.753), $Z = 4.207$, $P < 0.001$, 0.741 (95% CI: 0.673–0.802), $Z = 6.297$, $P < 0.001$ and 0.759 (95% CI: 0.692–0.818), $Z = 6.552$, $P < 0.001$, respectively. In the cholestatic type, the AUROCs of RUCAM, M&V and DDW-J were 0.701 (95% CI: 0.534 –0.837), $Z = 2.030$, $P = 0.042$, 0.807 (95% CI: 0.649 –0.915), $Z = 4.283$, $P < 0.001$ and 0.656 (95% CI: 0.487–0.800), $Z = 1.606$, $P = 0.108$ respectively. In the mixed type, the AUROCs of RUCAM, M&V and DDW-J were 0.765 (95% CI: 0.637 –0.865), $Z = 4.173$, $P < 0.001$, 0.886 (95% CI: 0.777 –0.953), $Z = 8.528$, $P < 0.001$ and 0.794 (95% CI: 0.670–0.888), $Z = 4.444$, $P < 0.001$, respectively.

Histological findings and its diagnostic value combined with clinical criteria

We used immunohistochemistry HBsAg(-), HBcAg(-), preS1(-) to histologically confirming patients without hepatitis B virus infection and occult infection, CD68(+) were used to explain the pigmented macrophages. Among 458 DILI patients, 149 refused and 7 because of physical condition couldn't perform liver biopsy. Finally, 302 DILI patients' liver biopsies were included (Figure 1), 248 were diagnosed as definite DILI and 54 were suspected DILI. Although there were numerous histological manifestations in DILI (9, 20–22), we used rDILI-PSS to evaluate: steatosis in 204 cases (67.5%), $\chi^2 = 4.487$, $P = 0.106$; cholestasis in 151 cases (50%), $\chi^2 = 3.886$, $P = 0.143$; cell apoptosis in 139 cases (46%), $\chi^2 = 0.840$, $P = 0.657$; eosinophil granulocyte infiltration in 131 cases (43.4%), $\chi^2 = 0.30$, $P = 0.985$; central and/or portal phlebitis in 103 cases (34.1%), $\chi^2 = 25.948$, $P < 0.001$; iron deposition in 90 cases (29.8%), $\chi^2 = 5.737$, $P = 0.057$; and pigmented macrophages in 92 cases (30.5%). $\chi^2 = 6.616$, $P = 0.037$. Table 5 shows the results of the characteristics of histological findings according to injury type. The mean \pm SD of the refined DILI-PSS score was 3.26 ± 1.34 . The new parameters: (pre1, pre2 and pre3) were DILI-PSS combined with RUCAM, M&V and DDW-J, respectively. The logistic regression formulas were expressed as pre1 = PSS+0.374*RUCAM, pre2 = PSS+0.338*M&V, and pre3 = PSS+0.578* DDW-J. The AUROCs of pre1, pre2 and pre3 were 0.843 (95% CI: 0.747–0.914), $Z = 7.653$, $P < 0.001$, with a sensitivity of 77.94%, specificity of 85.71%; 0.907 (95% CI: 0.822–0.960), $Z = 10.467$, $P < 0.001$, with a sensitivity of 77.94%, specificity of 92.86%; and 0.881 (95% CI: 0.790–0.942), $Z = 9.352$, $P < 0.001$, with a sensitivity of 77.94%, specificity of 85.71%, respectively. The ROC curves are shown in Figure 2. and the diagnostic performance of pre1, pre2 and pre 3 are also shown in Table 4.

In the hepatocellular type, the AUROCs of pre1, pre2 and pre3 were 0.894 (95% CI: 0.787–0.959), $Z = 9.086$, $P < 0.001$, 0.960 (95% CI: 0.857–0.994), $Z = 19.015$, $P < 0.001$ and 0.940 (95% CI: 0.847–0.985), $Z = 14.544$, $P < 0.001$, respectively; in the cholestatic type, the AUROCs were 0.750 (95% CI: 0.466 –0.931), $Z = 2.000$, $P = 0.045$, 0.500 (95% CI: 0.239 –0.761), $Z = 0.000$, $P = 1.000$, and 0.500 (95% CI: 0.239 –0.761), $Z = 0.000$, $P = 1.000$, respectively; in the mixed type, the AUROCs were 0.786 (95% CI: 0.524–0.943), $Z = 2.146$, $P = 0.032$, 0.869 (95% CI: 0.619–0.981), $Z = 3.058$, $P = 0.002$ and 0.762 (95% CI: 0.498 –0.930), $Z = 2.052$, $P = 0.040$, respectively.

Discussion

The three diagnostic criteria were RUCAM, designed in 1993 by Danan G, et al.(14), M&V, also called the clinical diagnostic scale (CDS) scoring system, and improved by Maria and Victorino in 1997(15), and DDW-J, put forward by Japanese scholars at the Digestive Disease Week (DDW) meeting in 2003(16). However, studies have shown that diagnosis scales may not be the best way to diagnose DILI. For example, in the case of patients diagnosed with DILI when a low score is obtained or opposite and different results are obtained using different scales(23, 24). Although it may be agreed that M&V and DDW-J were based on original RUCAM, they were invented to better diagnosis DILI. The M&V scoring system is different from RUCAM in terms of time limit and score setting, and extra-hepatic clinical manifestations are added as diagnostic criteria, however, the diagnostic accuracy for patients with chronic liver injury after a long-term incubation period and drug withdrawal is poor(25). DDW-J concerned the genes encoding drug-metabolizing enzymes in different ethnics groups, and was probably proposed for Asian populations, but further clinical research is still needed(26). Our study showed that the M&V was better in confirming DILI in suspected patients. Occasionally, the reviewer's opinions begrudgingly abided by the final assessment, and thus, the reviewer decision was different from that produced by the grading process, as in cases of score of 3 or 4 in the RUCAM categories, where the likelihood of DILI was balanced around a 50% likelihood(27). Although the DDW-J score was proposed by Japanese scholars for Asian populations, virtually no drug lymphocyte stimulation test (DLST) is performed during our actual clinical diagnosis and treatment process. Also, questions remained that: on which grade of these scales, can we say a it is DILI.

Some emerging biomarkers, such as microRNAs(28, 29), high mobility group box 1 (HMGB1) and caspase-cleaved keratin18 (ccK18)(30, 31), have been identified in the assessment of DILI. Coupled with traditional liver enzyme tests, these new biomarkers are still questionable. ALT and AST are also present in skeletal muscle and elevated in patients in polymyositis or during extreme exercise(32), and ALP is also present in bone tissue and increased by osteoblast activity; TBIL is elevated after the processing of erythrocytes and subsequent degradation of haemoglobin or alteration of bilirubin transporters(33). Thus, the physiological processes underlying changes in these markers may be unrelated to damage to the liver(34). Therefore, no biomarkers are currently suitable for diagnosing DILI.

As histopathological examination can detect damage directly, diagnosis can be assisted by eliminating (or confirming) conflicting causes of liver injury and by conducting a biopsy associated with DILI patterns(35). In our study, hepatological pathologists (Liu and Shi) carefully reviewed 302 slides without knowing the clinical diagnosis, and used a descriptive method for the assessment of the typical histological features. The seven histological characteristics in our refined pathological scoring system were based on DILI-PSS(18, 19) by Hu, such as steatosis, cholestasis, apoptosis and vascular inflammation are similar to features reported in other published studies focused on the histopathological characteristics of DILI. We found that vascular inflammation and pigmented macrophages in DILI patients were significantly different among the three clinical types ($p < 0.05$), which may prove the correlation between clinical classification and pathological classification. Moreover, to explore the

specific pigmented macrophages in our histopathological findings, we further studied immunohistochemical expression of CD68 (Figure 3). CD68 is known as a specific marker expressed in various kinds of macrophages, in the liver, where it is mainly expressed in Kupffer cells(36), Kupffer cells are a type of nonessential cells that originated from the yolk sac and were first identified as macrophages by Naito in 1990(37), these cells play an important role in the natural immune response and can effectively phagocytize pathogens or other toxic particulate matter through the portal vein or arterial circulation(38–40). The pigmented macrophages observed by H&E staining may be related to the type and timing of drugs taken and may progress to granuloma performance to help diagnose DILI in an early stage, which is why we propose the concept of deposition rather than granuloma in the refined DILI-PSS. Fortunately, our study showed that the refined DILI-PSS can be helpful for improving diagnostic accuracy: combined with clinical diagnostic criteria, the diagnosis efficiency of the new parameters increased with AUROCs of 0.843 (95% CI:0.747–0.914); 0.907 (95% CI:0.822–0.960) and 0.881 (95% CI: 0.790–0.942). These values were better than those of RUCAM alone with an AUROC of 0.730 (95% CI: 0.667–0.793), M&V alone with an AUROC of 0.793 (95% CI: 0.740–0.847), and DDW-J alone with an AUROC of 0.764 (95% CI: 0.702–0.826).

In our study, the following limitations should be considered. First, the standards of diagnosed DILI and suspected DILI were based on China's clinical guidelines and confirmed by multidisciplinary consultation, thus, the diagnosis of patients may not be applicable to a wider population. Second, we used liver biopsies as a reference standard but not all patients underwent a liver biopsy. Only 302 patients were enrolled after exclusion (65.94% of the total 458 patients), and the uneven specific deposition in specimens may present a bias. Third, the number of cases such as non-resident DILI patients is lacking. Moreover, the refined DILI-PSS were not performed in biopsy slices from patients with other diseases or healthy volunteers, which was not available for a more scientific and rigorous evaluation.

In conclusion, our study compared the accuracy of the three diagnostic criteria in diagnosing DILI from suspected patients and validated diagnostic ability of the pathological scoring system. Either alone or combination, M&V had the highest diagnostic accuracy among the three diagnostic criteria. However, further study is still needed, especially on suspected DILI patients who are difficult to diagnose definitely.

Conclusions

1. The main age of onset of DILI patients in this study was 40–60 years old, and female patients were significantly more than male. The main drug caused DILI is Chinese herbal medicine. The most common type among the three clinical subtypes of DILI is hepatocyte injury in this study, followed by mixed subtypes, and cholestasis subtypes; 2. Pathological findings of DILI patients in this study are basically consistent with previous studies, indicating that patients with DILI have certain commonalities in pathological manifestations, and the pathological injury patterns are not completely consistent with the clinical subtypes of DILI. The improved pathological scoring system (rDILI-PSS) in this study can provide a scientific and objective assessment of liver pathological damage in patients with DILI, providing a basis for clinical diagnosis of DILI; 3. This study shows that among the three clinical diagnostic scales of DILI, M&V has the highest diagnostic performance when a patient was suspected to have DILI, and pre1, pre2, pre3 (RUCAM, M&V and DDW-J combined with rDILI-PSS respectively) all have higher diagnostic efficacy than the clinical diagnostic scale alone, and pre2 in which the M&V combined with rDILI-PSS had the best efficacy to diagnose DILI definitely.

List Of Abbreviations

DILI: Drug-induced liver injury

RUCAM: Roussel-Uclaf Causality Assessment Method

M&V: Maria & Victorino scale

DDW-J: Digestive Disease Week-Japan criterion

DILI-PSS: DILI-pathological scoring system

CIOMS: The Council for International Organizations of Medical Sciences

ROC curve: Receiver Operating Characteristic curve

AUROC: Area Under the ROC

ALT: Alanine transaminase

AST: Aspartate aminotransferase

GGT: γ -glutamyltransferase

ALP: Alkaline phosphatase

TB: Total bilirubin

DB: Direct bilirubin

Glu: blood-glucose

TG: triglyceride

CHO: total cholesterol

NSAIDs: Non-steroidal anti-inflammatory drugs

H&E: hematoxylin-eosin staining

CK19: cytokeratin-19

CCK18: Caspase-cleaved keratin 18

HMGB1: High mobility group box 1

HBV: hepatitis B virus

HBsAg: hepatitis B virus surface antigen

HBcAg: hepatitis B virus core antigen

CD68: Cluster of Differentiation

ULN: upper limits of normal

DLST: drug lymphocyte stimulation test

95% CI: 95% Confidence Interval

PPV: positive predictive value

NPV: negative predictive value

LR+: positive likelihood ratio

LR-: negative likelihood ratio

ALF: acute liver failure

Declarations

Ethics approval and consent to participate: The Study protocol was approved by the Ethics Committee of Tianjin Second People's Hospital and conformed to the Declaration of Helsinki. All patients signed an informed consent form before enrolment in this study.

Consent for publication: Not applicable.

Availability of data and materials: Not applicable.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: Liu YQ analyzed and interpreted the patient data, also a major contributor in writing the manuscript, Li P put forward constructive

revisions to the paper, Liu YQ and Li P carried out the idea and study design, Wang FF, Liu L and Zhang YL participated in the data collection and coordination of the analysis work, Liu YG and Shi RF performed the histological examination of the liver. All authors read and approved the final manuscript.

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Tables

NOTE: Due to technical limitations, Table 1 could not be shown here. Please see the supplementary files to access the table.

Table 2. Serological characteristics of 458 DILI patients.

Characteristics	hepatocellular type	mixed type	cholestatic type	P value
	(n=290)	(n=97)	(n=71)	
Demographic variables				
Age (year, mean±SD)	47.47±13.87	50.71±12.03	51.82±12.77	0.009
Female gender[n(%)]	188(64.83%)	68(70.10%)	52(73.24%)	0.319
Biochemical data[median(range)]				
ALT(IU/L)	1018.5(131, 4435)	253(48, 1920)	151(12, 477)	<0.001
AST(IU/L)	612.5(53, 3045)	175(28, 1510)	135(18, 823)	<0.001
GGT(IU/L)	207.5(17, 1516.4)	255(39, 2652)	295(25, 888)	<0.001
ALP(IU/L)	171.35(20, 765)	204(54, 1240)	265(64, 1399)	<0.001
TB(μmol/L)	74.75(9.3, 492.9)	32.8(5.3, 213.3)	34.6(4.8, 439.2)	<0.001
DB(μmol/L)	56.1(3, 365)	12.3(1, 142)	11.1(1, 358)	<0.001
Glu(mmol/L)	5.33(3, 16)	5.53(4, 29)	5.42(4, 17)	0.112
TG(mmol/L)	1.74(1, 8)	1.51(1, 22)	1.64(0, 5)	0.263
CHO(mmol/L)	4.02(2, 8)	4.82(3, 24)	4.91(2, 17)	<0.001
Interval days(day)	19(2, 47)	16(2, 45)	12(1, 38)	<0.001

Table 3. Pathogenicity drugs[n(%)] used by the 458 DILI patients.

	Hepatocellular type (n=290)	mixed type (n=97)	cholestatic type (n=71)
Antibiotics			
Cephalosporin	8(2.76%)	5(5.16%)	2(2.82%)
Macrolid antibiotic	3(1.04%)	0	1(1.41%)
Floxacina antibiotics	2(0.69%)	0	0
Antifungal	2(0.69%)	4(4.12%)	0
Tetracycline	0	2(2.06%)	0
NNRTIs	4(1.38%)	2(2.06%)	2(2.82%)
NSAIDs	22(7.59%)	9(9.28%)	6(8.45%)
Aminoglucoſe	2(0.69%)	2(2.06%)	0
Antiallergic drugs	2(0.69%)	2(2.06%)	2(2.82%)
Antituberculoſic drugs	7(2.41%)	5(5.16%)	5(7.04%)
Acyeterion	0	1(1.03%)	0
Healthcare products	13(4.48%)	4(4.12%)	8(11.26%)
Immunosuppreſſors	2(0.69%)	1(1.03%)	2(2.82%)
Chemotherapenſics	16(5.53%)	13(13.42%)	11(15.49%)
Nervous ſyſtem drugs			
Sedative-hypnotic drug	1(0.34%)	3(3.09%)	0
Flupentixol-melitracen	5(1.72%)	0	0
Antiepileptic drug	2(0.69%)	0	0
Cardiovascular ſyſtem drugs			
Calcium antagoniſts	2(0.69%)	1(1.03%)	0
ACEI	2(0.69%)	0	0
Digetiſtiſe ſyſtem drugs			
Proton pump inhibitor	4(1.38%)	0	0
Endocrinology and metabolic Drugs			
Antithyroid drug	4(1.38%)	3(3.09%)	2(2.82%)
Diabetes drug	0	0	2(2.82%)
glucocorticoid	2(0.69%)	2(2.06%)	0
ſtatins	5(1.72%)	4(4.12%)	0
luteoſterone	0	0	2(2.82%)
Chinese traditional herbals			
Croton	1(0.34%)	0	0
Rhizome atractylodis	4(1.38%)	0	0
Radix bupleuri	2(0.69%)	0	3(4.22%)
Radix ſalviae miltiorrhizae	2(0.69%)	0	1(1.41%)
Poria cocos	2(0.69%)	0	0
Herba rhodiolae	2(0.69%)	0	0
Tripterygium wilfordi	1(0.34%)	2(2.06%)	0
Ginſeng	0	0	2(2.82%)
Garter ſnake	0	1(1.03%)	0
Asarum ſieboldi Mig.	1(0.34%)	0	0
Monkſhood	0	0	3(4.22%)
Alismae rhizoma	5(1.72%)	2(2.06%)	0
Herba epimedium	2(0.69%)	0	0
Polygonum multiflorum	37(12.76%)	5(5.16%)	4(5.64%)
Multiple herbal uſe	121(41.73%)	24(24.74%)	13(18.30%)

(NNRTIs: new non-nucleoſide reſerſe tranſcriptaſe inhibitorſ; NSAIDs: non-ſteroidal antiinflammatoſy drugſ; ACEI: angiotenſion conſerſing enzyſe inhibitorſ.)

Table 4. Diagnostic performance of RUCAM, M&V, DDW-J and new parameterſ(Pre1, Pre2, Pre3).

		Sen	Spe	PPV	NPV	LR+	LR-
RUCAM	>8	46.54%	89.04%	92.66%	35.91%	4.25	0.60
	≥6	97.70%	12.33%	76.81%	64.29%	1.11	0.19
	≥8	74.19%	60.27%	84.74%	44.00%	1.87	0.43
AUROC: 0.730 (95%CI: 0.667-0.793) Z=7.147 P<0.001							
Maria	≥14	39.63%	1.00	1.00	35.78%		0.60
	≥10	86.18%	45.21%	82.38%	52.38%	1.57	0.31
	>11	72.35%	68.49%	87.15%	45.05%	2.28	0.41
	≥11	78.80%	60.27%	85.50%	48.89%	1.98	0.35
AUROC: 0.793 (95%CI: 0.740-0.847) Z=10.753 P<0.001							
DDW-J	≥5	99.08%	1.37%	74.91%	33.33%	1.00	0.67
	≥3	99.54%	0.00	74.74%	0.00	1.00	
	>8	51.15%	87.67%	92.50%	37.43%	4.13	0.56
	≥8	88.43%	53.42%	84.89%	60.94%	1.90	0.22
AUROC: 0.764 (95%CI: 0.702-0.826) Z=8.303 P<0.001							
Pre1	>4.992	77.94%	85.71%	96.36%	44.44%	5.45	0.26
	AUROC: 0.843(95%CI: 0.747-0.914) Z=7.653 P<0.001						
Pre2	>6.056	77.94%	92.86%	98.15%	46.43%	10.92	0.24
	AUROC: 0.907(95%CI: 0.822-0.960) Z=10.467 P<0.001						
Pre3	>7.046	77.49%	85.71%	96.36%	44.44%	5.45	0.26
	AUROC: 0.881(95%CI: 0.790-0.942) Z=9.352 P<0.001						

(Sen: sensitivity; Spe: specificity; PPV: positive predictive value; NPV: negative predictive value; LR+: positive likelihood ratio; LR-: negative likelihood ratio; AUROC: area under the receiver operating characteristic curve; 95%CI: 95% confidence intervals.)

Table 5. Histological characteristics in 302 DILI patients.

Characteristics	Hepatocellular type (n=220)	Mixed type (n=38)	Cholestatic type (n=44)	P value	Definite DILI (n=248)	Suspected DILI (n=54)	P value
Steatosis	142(64.5%)	31(81.6%)	31(70.5%)	0.106	175(69.7%)	29(56.9%)	0.074
Cholestasis	118(53.6%)	14(36.8%)	20(45.5%)	0.143	129(51.4%)	23(40.1%)	0.412
Cell apoptosis	100(45.5%)	20(52.6%)	19(43.2%)	0.657	117(46.6%)	22(43.1%)	0.650
Eosinophil* ¹	95(43.2%)	16(42.1%)	20(45.5%)	0.985	104 (41.4%)	27(52.9%)	0.131
Phlebitis* ²	73(33.2%)	18(47.4%)	12(27.3%)	<0.001	89(35.5%)	14(27.5%)	0.271
Iron deposition	74(33.6%)	7(18.4%)	9(20.5%)	0.057	81 (32.3%)	9(17.7 %)	0.037
Pigmented macrophages	75(34.1%)	10(26.3%)	7(15.9%)	0.037	76 (30.3%)	16(31.4%)	0.877

*1.eosinophil infiltration

*2. central and/or portal phlebitis

Figures

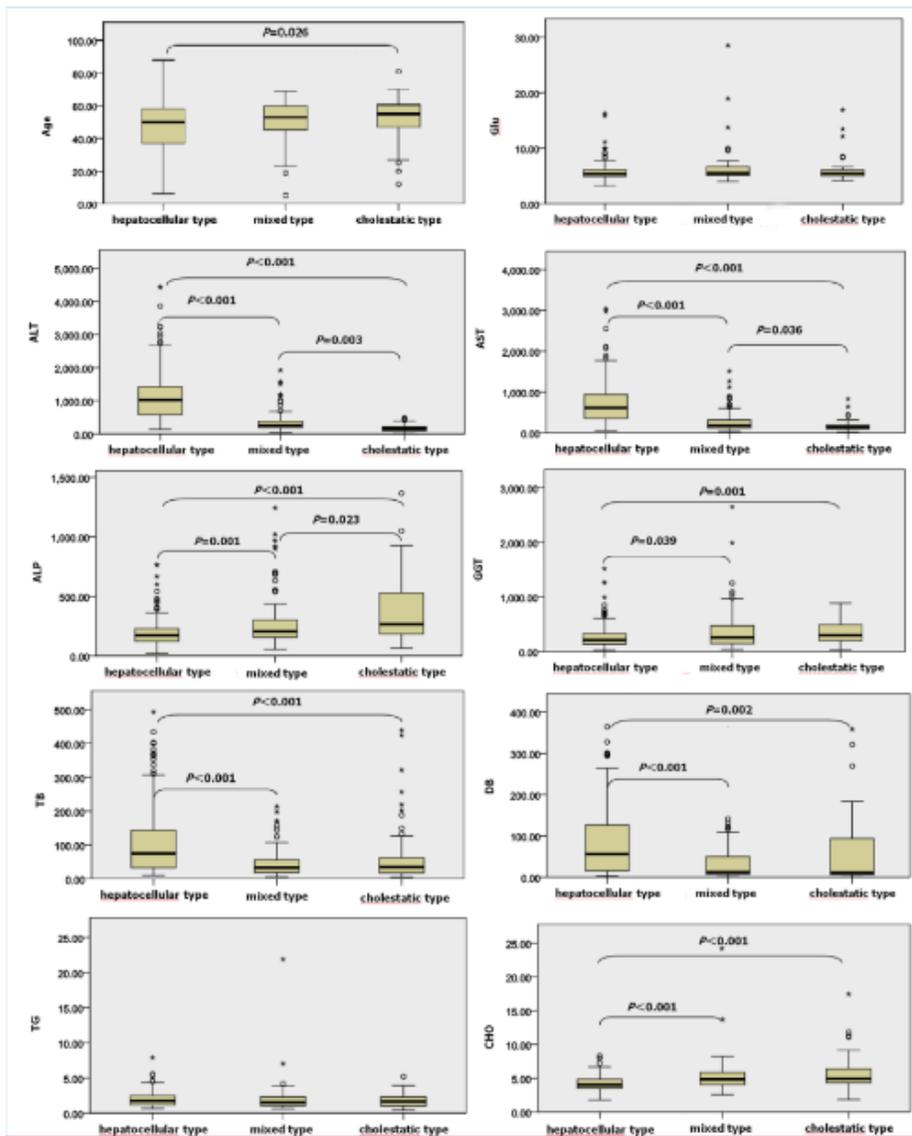


Figure 1

The distribution of characteristics in DILI patients.

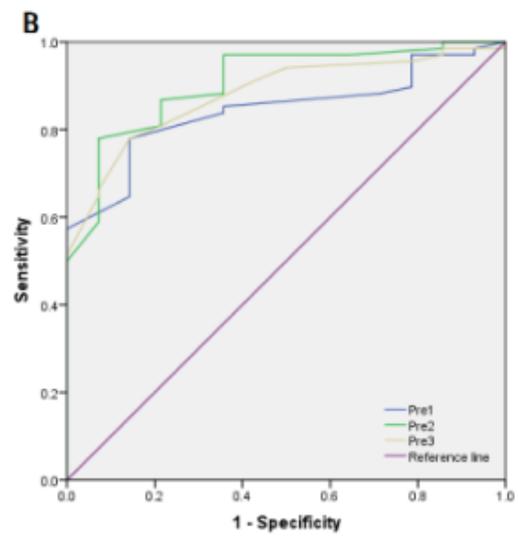
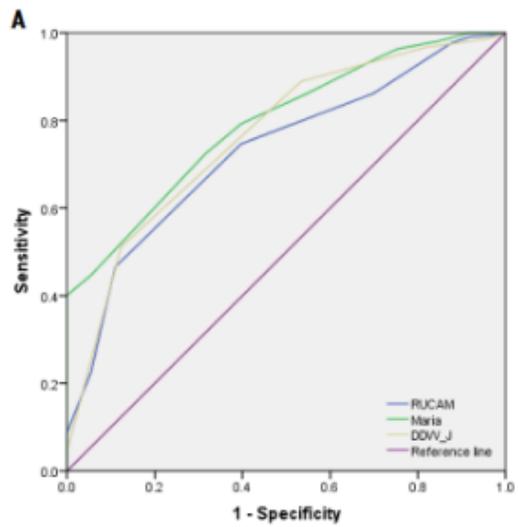


Figure 2

The ROC curve of RUCAM, Maria, DDW-J and new parameters(pre1, pre2, pre3).

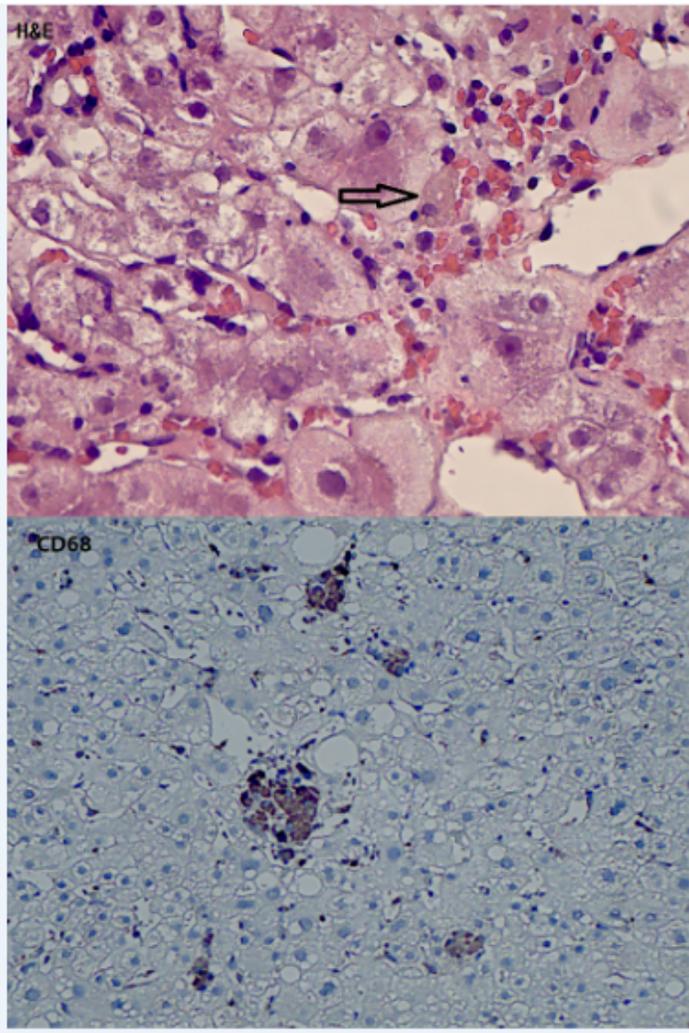


Figure 3

Histological lesions of pigmented macrophages.

Supplementary Files

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- [Table1.docx](#)