Comparison of diagnostic accuracy of 3 diagnostic criteria combined with refined pathological scoring system for drug-induced liver injury

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Abstract

Drug-induced liver injury (DILI) is difficult in diagnosis, 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.

We enrolled 458 consecutive hospitalized DILI patients from January 1, 2012 to December 31, 2018, using Roussel-Uclaf Causality Assessment Method (RUCAM), Maria & Victorino scale (M&V), and Digestive Disease Week-Japan criterion (DDW-J) combined with refined pathological scoring system respectively to perform the evaluation.

A total of 458 DILI patients were enrolled, the area under receiver operating characteristics (AUROC) of the 3 clinical diagnostic criteria were 0.730 (95% confidence interval [CI]: 0.667–0.793), 0.793 (95% CI: 0.740–0.847), and 0.764 (95% CI: 0.702–0.826) respectively. Three hundred two DILI patients’ liver biopsies were included: steatosis in 204 cases (67.5%), cholestasis in 151 cases (50%), cell apoptosis in 139 cases (46%), eosinophil granulocyte infiltration in 131 cases (43.4%), central and/or portal phlebitis in 103 cases (34.1%), iron deposition in 90 cases (29.8%), and pigmented macrophages in 92 cases (30.5%). The AUROC of refined pathological scale combined with 3 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).

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.

Abbreviations: 95% CI = 95% confidence interval, ALF = acute liver failure, ALP = alkaline phosphatase, ALT = alanine transaminase, AST = aspartate aminotransferase, AUROC = area under the ROC, CK18 = caspase-cleaved keratin 18, CD68 = cluster of differentiation, CHO = total cholesterol, CIOMS = The Council for International Organizations of Medical Sciences, CK19 = cytokinin-19, DB = direct bilirubin, DDW-J = Digestive Disease Week-Japan criterion, DILI = drug-induced liver injury, DILI-PSS = DILI-pathological scoring system, DLST = drug lymphocyte stimulation test, GGT = γ-glutamyltransferase, Glu = blood-glucose, HbV = hepatitits B virus, HBCAg = hepatitis B virus core antigen, HBsAg = hepatitis B virus surface antigen, HMGB1 = high mobility group box 1, H&E = hematoxylin-eosin staining, LR+ = positive likelihood ratio, LR− = negative likelihood ratio, M&V = Maria & Victorino scale, NPV = negative predictive value, NSAIDs = Non-steroidal anti-inflammatory drugs, PPV = positive predictive value, RUCAM = Roussel-Uclaf Causality Assessment Method, ROC curve = receiver operating characteristic curve, TB = total bilirubin, TG = triglyceride, ULN = upper limits of normal.

Keywords: drug-induced liver injury, diagnostic accuracy, liver pathology, Roussel-Uclaf Causality Assessment Method

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1. 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.11–13 Studies showed that DILI occurs with an annual incidence of approximately 13.9 per 100,000 inhabitants in France compared with 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 assess 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.10 were the first who divided DILI into 6 patterns: zonal necrosis, simple cholestasis, hepatitis with/without cholestasis, acute hepatitis-like with/without massive necrosis, reactive hepatitis, and steatosis. However, a prospective study showed that liver biopsy was performed in only approximately 50% of patients.11

Thus, we compared the diagnostic accuracy of 3 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 analyzed 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.

2. Method
2.1. Patients
Consecutive DILI inpatients at Tianjin Second People’s Hospital from January 1, 2012 to December 31, 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 201712) 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.

2.2. 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.

2.3. 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 3 different diagnostic rating scales by 3 physicians. RUCAM14 has 5 degrees: score = 0, relationship “excluded”; 1–2: “unlikely”; 3–5: “possible”; 6–8: “probable”; and ≥9: “highly probable.” M&V15 has 5 degrees: score < 6, “excluded”; 6–9: “unlikely”; 10–13: “possible”; 14–17: “probable”; and ≥18: “definite.” DDW-J16,17 has 3 degrees: ≤2: “possible”; 3–4: “probable”; and ≥5 “highly probable.”

2.4. 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, NJ) 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 studies in China18,19 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 Abcam (Abcam Co., Cambrige, UK). Blinded to the clinical data, 2 experienced hepatic pathologists independently reviewed the histologic findings. Consensus was reached in cases of disagreement.

2.5. Statistical analysis
Continuous variables were compared using the Mann–Whitney U test for 2 nonnormal datasets and the Kruskal-Wallis H test for >2 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 <.05 was...
considered indicative of significance. Statistical analyses were performed using SPSS 21.0 (SPSS, Chicago, IL) and MedCalc 15. (MedCalc Software, M&Vkerke, Belgium).

3. Results

3.1. 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, and female patients were more than male patients: 290 (63.32%) patients had the hepatocellular type injury, 188 (64.83%) were women; 71 (15.5%) patients had the cholestatic type, 52 (73.24%) were women; 97 (21.18%) patients had the mixed type, 68 (70.10%) were women. The patient ages (mean ± SD) in the 3 types were 47.47 ± 13.87, 51.82 ± 12.77, and 50.71 ± 12.03, respectively. The median (range) values of ALT, AST, GGT, ALP, TB, DB, Glu, TG, and CHO were shown in Table 2. Except for Glu and TG, other biochemical data were statistically significant (P < .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 Fig. 1.

Table 1

| Description                                                                 | Count   |
|----------------------------------------------------------------------------|---------|
| Patients hospitalized with a principal diagnosis of DILI and suspected DILI in Tianjin Second People’s Hospital from 1 January 2012 to 31 December 2017 (n=634). |         |
| Exclude:                                                                   |         |
| 1. Match the guidelines’ exclusion criteria. (n=107)                        |         |
| 2. Daily alcohol consumption >20g. (n=53)                                  |         |
| 3. Immune suppressive treatment within 1 year. (n=12)                      |         |
| Patients were included in analysis and demographics, medication details, laboratory data and histological characteristics were extracted (n=458), patients under liver biopsy (n=306). |         |
| Exclude:                                                                   |         |
| Liver biopsy specimen <15 mm length or <0.8 mm diameter (n=4).             |         |
| The RUCAM, M&V and DDW-J score were calculated by three designated hepatologist (n=458) and rDILI-PSS was calculated independently by two pathologists (n=302). |         |
Serological characteristics of 458 DILI patients.

| Characteristics | Hepatocellular type (n = 290) | Mixed type (n = 97) | Cholestatic type (n = 71) | P value |
|-----------------|-------------------------------|-------------------|--------------------------|---------|
| Demographic variables |                               |                   |                          |         |
| Age (year, mean ± SD) | 47.47 ± 13.87                 | 50.71 ± 12.03     | 51.62 ± 12.77           | .009    |
| Female gender [%/n] | 188 (64.83%)                  | 68 (70.10%)       | 52 (73.24%)             | .319    |
| Biochemical data [median (range)] |                   |                   |                          |         |
| ALT, IU/L | 1018.5 (131, 4435)           | 253 (48, 1920)    | 151 (12, 477)           | <.001   |
| AST, IU/L | 612.5 (53, 3045)            | 175 (28, 1513)    | 135 (18, 823)           | <.001   |
| GGT, IU/L | 207.5 (17, 1516.4)          | 255 (39, 2625)    | 295 (25, 868)           | <.001   |
| ALP, IU/L | 713.5 (20, 769)            | 204 (64, 1240)    | 265 (64, 1399)          | <.001   |
| TB, µmol/L | 74.75 (9.3, 492.9)         | 22.8 (5.3, 213.9) | 54.6 (48, 439.2)        | <.001   |
| DB, µmol/L | 56.1 (3, 365)              | 12.3 (1.142)      | 11.1 (1, 358)           | <.001   |
| Glu, mmol/L | 5.33 (3, 16)                | 5.53 (4, 29)      | 5.42 (4, 17)            | .112    |
| TG, mmol/L | 1.74 (1, 8)                 | 1.51 (1.22)       | 1.64 (0, 5)             | .263    |
| CHO, mmol/L | 4.02 (2, 8)               | 4.82 (3, 24)      | 4.91 (2, 17)            | <.001   |
| Interval days, d | 19 (2, 47)                 | 16 (2, 45)        | 12 (1, 38)              | <.001   |

3.2. 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 in 158 (34.50%) patients, and the top 3 individually used herbal medicines were Polygonum multiflorum, Alismae rhizome, and Radix bupleuri, used in 46 (10.04%), 7 (1.53%), and 5 (1.09%) patients respectively. 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 were shown in Table 3.

3.3. The diagnostic value of 3 clinical criteria

Among all 458 DILI patients, 340 were ultimately diagnosed with definite DILI, 118 were suspected DILI. For the 3 diagnostic criteria of DILI, the scores (mean ± 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 < .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 < .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 < .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 Fig. 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 < .001; 0.741 (95% CI: 0.673–0.802), Z = 6.297, P < .001; and 0.759 (95% CI: 0.692–0.818), Z = 6.552, P < .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 = .042, 0.807 (95% CI: 0.649–0.915), Z = 4.283, P < .001 and 0.656 (95% CI: 0.487–0.800), Z = 1.606, P = .108 respectively. In the mixed type, the AUROCs of RUCAM, M&V, and DDW-J were 0.765 (95% CI: 0.673–0.865), Z = 4.173, P < .001; 0.886 (95% CI: 0.777–0.933), Z = 8.528, P < .001 and 0.794 (95% CI: 0.670–0.888), Z = 4.444, P < .001, respectively.

3.4. Histological findings and its diagnostic value combined with clinical criteria

We used immunohistochemistry HBsAg(−), HBeAg(−), 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 couldn’t perform liver biopsy because of physical condition. Finally, 302 DILI patients’ liver biopsies were included (Fig. 1), 248 were diagnosed 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 (67.5%) cases, χ² = 4.487, P = .106; cholestasis in 151(50.9%) cases, χ² = 3.886, P = .143; cell apoptosis in 139(46%) cases, χ² = 0.840, P = .657; eosinophil granulocyte infiltration in 131 (43.4%) cases, χ² = 0.30, P = .985; central and/or portal phlebitis in 103 (34.1%) cases, χ² = 25.948, P < .001; iron deposition in 90 (29.8%) cases, χ² = 5.737, P = .057; and pigmented macrophages in 92(30.5%) cases, χ² = 6.616, P = .037. Table 5 showed the results of the characteristics of histological findings according to injury type. The mean ± 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. 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 < .001, with a sensitivity of 77.94%, specificity of 85.71%; 0.907 (95% CI: 0.822–0.960), Z = 10.467, P < .001, with a sensitivity of 77.94%, specificity of 92.86%; and 0.881 (95% CI: 0.790–0.942), Z = 9.352, P < .001, with a sensitivity of 77.94%, specificity of 85.71%, respectively. The ROC curves were shown in Fig. 2 and the diagnostic performance of pre1, pre2, and pre 3 were also shown in Table 4.
Figure 1. The distribution of characteristics in DILI patients. DILI = drug-induced liver injury.
In the hepatocellular type, the AUROCs of pre1, pre2, and pre3 were 0.894 (95% CI: 0.787–0.959), Z = 9.086, P < .001, 0.960 (95% CI: 0.857–0.994), Z = 19.015, P < .001, and 0.940 (95% CI: 0.847–0.985), Z = 14.544, P < .001, respectively; in the cholestatic type, the AUROCs were 0.750 (95% CI: 0.466 to 0.931), Z = 2.000, P = .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 = .032, 0.869 (95% CI: 0.619–0.981), Z = 3.058, P = .002 and 0.762 (95% CI: 0.498–0.930), Z = 2.052, P = .040, respectively.

### 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%)             |
| Floxacin antibiotics                                     | 2 (0.69%)                  | 0                | 0                     |
| Antifungal                                               | 2 (0.69%)                  | 4 (4.12%)        | 0                     |
| Tetracycline                                             | 0                          | 2 (2.06%)        | 0                     |
| NNRIs                                                   | 4 (1.38%)                  | 2 (2.06%)        | 2 (2.82%)             |
| NSAIDs                                                  | 22 (7.59%)                 | 9 (9.28%)        | 6 (8.45%)             |
| Antimicrobial drugs                                       | 2 (0.69%)                  | 2 (2.06%)        | 0                     |
| Antihistamine drugs                                      | 2 (0.69%)                  | 2 (2.06%)        | 2 (2.82%)             |
| **Antimicrobial drugs**                                  |                            |                  |                       |
| Calcium antagonons                                        | 1 (0.34%)                  | 3 (3.09%)        | 0                     |
| Fluoxetine-melitracen                                     | 5 (1.72%)                  | 0                | 0                     |
| Antiepileptic drug                                       | 2 (0.69%)                  | 0                | 0                     |
| **Cardiovascular system drugs**                          |                            |                  |                       |
| **Cardiovascular system drugs**                          |                            |                  |                       |
| Calcium antagonons                                        | 2 (0.69%)                  | 1 (1.03%)        | 0                     |
| ACEI                                                    | 2 (0.69%)                  | 0                | 0                     |
| **Diuretics**                                            |                            |                  |                       |
| 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**                                         |                            | 2 (2.82%)        |                       |
| Chinese traditional herbs                                 |                            |                  |                       |
| Croton                                                  | 1 (0.34%)                  | 0                | 0                     |
| Rhizome atracylodis                                      | 4 (1.38%)                  | 0                | 0                     |
| RADX bupeuri                                             | 2 (0.69%)                  | 0                | 3 (4.22%)             |
| RADX salviae millontrhiae                                | 2 (0.69%)                  | 0                | 1 (1.41%)             |
| Poria cocos                                             | 2 (0.69%)                  | 0                | 0                     |
| Hera miobada                                            | 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                     |
| Arslam sieboldi Mig.                                     | 1 (0.34%)                  | 0                | 0                     |
| Monkshood                                               | 0                          | 0                | 3 (4.22%)             |
| Alismiae rhizome                                         | 5 (1.72%)                  | 2 (2.06%)        | 0                     |
| Herba epimedium                                          | 2 (0.69%)                  | 0                | 0                     |
| Polygonum multiflorum                                    | 37 (12.76%)                | 5 (5.16%)        | 4 (5.84%)             |
| Multiple herbal use                                      | 121 (41.73%)               | 24 (24.74%)      | 13 (18.30%)           |

ACEI = angiotension converting enzyme inhibitors; DILI = drug-induced liver injury; NNRIs = new non-nucleoside reverse transcriptase inhibitors; NSAIDs = non-steroidal anti-inflammatory drugs.

### 4. Discussion

The 3 diagnostic criteria were RUCAM, designed in 1993 by Danan and Benichou,[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 ethnic 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 it is DILI.

Some emerging biomarkers, such as microRNAs\(^{[28,29]}\) high mobility group box 1 (HMGBox1) and caspase-cleaved keratin 18 (cK18)\(^{[30,31]}\) 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 hemoglobin 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 7 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 3 clinical types (\(P < .05\), 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 (Fig. 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 specimens may present a bias. Third, the number of cases such as non-resident DILI patients is lacking. Moreover, the refined rDILI-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 3 diagnostic criteria in diagnosing DILI from suspected patients; this study shows that M&V has the highest diagnostic performance among the 3 criteria when a patient was suspected definitely. Further study is still needed, especially on suspected DILI patients who are difficult to diagnose definitely.

5. Conclusions

The main age of onset of DILI patients in this study is 40 to 60 years old, mostly are women. The main drug caused DILI is Chinese herbal medicine. The most common clinical subtypes is hepatocyte injury, followed by mixed subtypes and cholestasis subtypes; pathological injury patterns of DILI are not completely consistent with the clinical subtypes, which are basically consistent with previous studies, and refined pathological scoring system (rDILI-PSS) in this study can provide a scientific and objective assessment of liver pathological damage in DILI patients; this study shows that M&V has the highest diagnostic performance among the 3 criteria when a patient was suspected definitely.

| 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%)              | .106   | 175 (69.7%)            | 29 (56.9%)             | .074   |
| Cholestasis     | 118 (53.6%)                   | 16 (42.1%)        | 20 (45.5%)              | .143   | 129 (51.4%)            | 23 (40.1%)             | .412   |
| Cell apoptosis  | 100 (45.5%)                   | 18 (47.4%)        | 19 (43.2%)              | .657   | 117 (46.6%)            | 22 (43.1%)             | .650   |
| Eosinophil infiltration | 95 (43.2%) | 16 (42.1%)        | 20 (45.5%)              | .985   | 104 (41.4%)            | 27 (52.9%)             | .131   |
| Central and/or portal phlebitis | 73 (33.2%) | 18 (47.4%)        | 12 (27.3%)              | <.001  | 89 (35.5%)             | 14 (25.5%)             | .271   |
| Iron deposition | 74 (33.6%)                    | 7 (19.4%)         | 9 (20.5%)               | .057   | 81 (32.3%)             | 9 (17.7%)              | .037   |
| Pigmented macrophages | 75 (34.1%) | 10 (26.3%)        | 7 (15.9%)               | .037   | 76 (30.3%)             | 16 (31.4%)             | .877   |

DILI = drug-induced liver injury.
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Author contributions

Yiqi Liu analyzed and interpreted the patient data, also a major contributor in writing the manuscript, Ping Li put forward constructive revisions to the paper, Yiqi Liu and Ping Li carried out the idea and study design, Fangfang Wang, Liang Liu, and Yilian Zhang participated in the data collection and coordination of the analysis work, Yonggang Liu and Ruifang Shi performed the histological examination of the liver. All authors read and approved the final manuscript.

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