Development and Validation of a Test to Identify Drugs That Cause Idiosyncratic Drug-Induced Liver Injury

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BACKGROUND & AIMS: **Idiosyncratic drug-induced liver injury (iDILI) is one of the most challenging diagnoses in hepatology. It is frequently impossible to identify the agent that has caused iDILI in patients who take multiple medicines. We developed an in vitro method to identify drugs that cause liver injury in patients, based on drug toxicity to monocyte-derived hepatocyte-like (MH) cells from patient blood samples. We then collected data on patients who were re-exposed to drugs found to be toxic in the MH test to validate test performance.**

METHODS: **We performed a prospective study of patients referred to the University Hospital in Munich, Germany, with acute liver injury believed to be caused by medications (300 patients were enrolled in the study and we present data from 40 patients with iDILI and re-exposure to implicated drugs). We collected data from patients on medical history, laboratory test and imaging results, findings from biopsy analyses, and medications taken. Blood samples were collected from all patients and MH cells were isolated and cultured for 10 days. MH cells were then incubated with drugs to which each patient had been exposed, and toxicity was measured based on release of lactate dehydrogenase. Agents found to be toxic to MH cells were considered as candidates for the cause of liver injury. Patients were followed up for up to 6 months after liver injury and data on drug re-exposures and subsequent liver damage within the following 3 to 24 months were associated with findings from MH tests.**

RESULTS: **Our test identified 10 drugs that were toxic to MH cells from 13 patients (amoxicillin/clavulanate to cells from 2 patients; diclofenac to cells from 2 patients; methylprednisolone to cells from 2 patients; and atorvastatin, metamizole, pembrolizumab, piperacillin/tazobactam, moxifloxacin, duloxetine, or sertraline each to cells from 1 patient). Thirteen patients had a recurrence of liver injury after inadvertent re-exposure to a single drug, and the MH test correctly identified 12 of the 13 drugs that caused these liver re-injury events. All 86 drugs that were not toxic to MH cells in our assay were safely resumed by patients and were not associated with liver re-injury in 27 patients. Therefore, the MH test identifies drugs that cause liver injury with 92.3% sensitivity and 100% specificity (1 false-negative and 12 true-positive results).**

CONCLUSIONS: **We developed a test to identify drugs that cause liver injury in patients based on their toxicity to MH cells isolated from patients with DILI. We validated results from the assay and found it to identify drugs that cause DILI with 92.3% sensitivity and 100% specificity. The MH cell could be a tool to identify causes of iDILI, even in patients taking multiple medications. ClinicalTrials.gov no: NCT 02353455.**

Keywords: Antibiotic; Nonsteroidal Anti-inflammatory; Pain Reliever; Diagnostic.

Abbreviations used in this paper: **DILI, drug-induced liver injury; iDILI, idiosyncratic drug-induced liver injury; MH cells, monocyte-derived hepatocyte-like cells; RUCAM, Roussel Uclaf Causality Assessment Method; ULN, upper limit of normal.**

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Drug-induced liver injury (DILI) is a major problem in health care and in drug development: DILI is responsible for most cases of acute liver failure in the United States and Europe[1,2] and it is a major cause for project terminations, drug withdrawals, and restrictions of use.[3]

Idiosyncratic DILI (iDILI) is very challenging for clinicians and for drug developers because its occurrence is determined by individual patient characteristics, such as genetics and environmental factors.[4–7] The incidence of iDILI may be very low (eg, 1 in 10,000), but it can have potentially severe consequences: acute liver failure with the risk of death or liver transplantation.[8,9] Because iDILI events cannot be predicted by preclinical models,[10,11] much effort is made to improve the detection and diagnosis of iDILI[12–16] to avoid rechallenge and related consequences.

As yet, there is no reliable in vitro test that can be used to confirm or exclude iDILI or that helps to identify the causative drug.[17,18] Recent investigations using the lymphocyte transformation test showed that this test could neither reliably and reproducibly diagnose iDILI nor identify the causative drug, with the possible exception of isoniazid.[19] Therefore, the gold standard for diagnosis and causality assessment of iDILI remains expert review of available clinical information, including patient history and review of implicated drugs.[20–22] A positive re-exposure is considered as solid evidence for drug causality,[23,24] but harbors risks for the patient and therefore is recommended only for critical medicines.[25] Thus, in most cases re-exposure occurs inadvertently.[26]

The problem of iDILI is even more challenging in patients taking several drugs. In these situations, known drug signature and associations with DILI are important tools for causality assessment; this approach biases causality assessment toward drugs well known to cause iDILI.[26,27] Moreover, the pattern and course of liver injury also may be influenced by patient characteristics, rendering the identification of the causative drug more difficult.[28–31] Complex situations include novel drugs, such as those in clinical development for which no typical signature is established yet.[32] In drug development, iDILI suspicion often leads to development stops or regulatory actions that aim to avoid damage to patients,[23] and incorrect diagnosis or causality assessment may lead to nonapproval of a beneficial drug. Another unmet need is causality assessment in complex patients taking several drugs[33] (between 4%[34] and 40% of cases[35,36]), in whom idiosyncratic drug–drug interactions could play an additional role. Thus, to improve patient safety, allowing for pharmaceutical innovation and effective treatments, further improvement in causality assessment of iDILI by objective methods is urgently needed.[37] We have developed a test using monocyte-derived hepatocyte-like (MH) cells from patients with iDILI suspicion that shows promising results in improving the diagnosis of complex iDILI cases. In a pilot study we showed that MH cell testing supported clinical causality assessment and outperformed the Roussel Uclaf Causality Assessment Method (RUCAM) in terms of specificity.[38] The aim of the present study was to investigate the performance of the MH cell test in iDILI patients with available data on drug re-exposure.

Patients and Methods

Patient Selection

Patients referred to the Ludwig Maximilians University Hospital Munich with acute liver injury were prospectively recruited for the study (study identifier: ClinicalTrials.gov: NCT 02353455). Written informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committee of the Faculty of Medicine, LMU Munich (project number 55-13). All authors had access to the study data and reviewed and approved the final manuscript. Inclusion criteria were a positive drug history within 6 months before liver injury and the following[39]: alanine aminotransferase $\geq$5 times the upper limit of normal (ULN) and/or alanine aminotransferase $\geq$3 times the ULN in combination with total bilirubin $\geq$2 times the ULN and/or alkaline phosphatase $\geq$2 times the ULN. After informed consent was obtained, patient data were collected including a comprehensive history for drug intake to perform causality assessment[40] and a blood sample (20–40 mL EDTA blood) was withdrawn for MH cell generation and testing. In total, 112 iDILI patients were included. Of these, we specifically selected the subgroup of iDILI patients with documented re-exposure to drugs as described later.

Diagnosis of Idiosyncratic Drug-Induced Liver Injury and Causality Assessment

Causality assessment was performed for each case and each drug involved in the respective case. Clinical causality assessment was based on exclusion of other causes, such as a history of shock, heart failure, sepsis, alcohol, and so forth; testing for hepatitis A, B, C, and E; human immunodeficiency virus; Epstein–Barr virus; cytomegalovirus; antinuclear antibodies; antimitochondrial antibodies; antineutrophil cytoplasmic antibodies; antisoluble liver antigen antibodies; anti–liver-kidney microsomal antibodies; anti–smooth-muscle antibodies; IgG; IgM; ceruloplasmin; α1-antitrypsin; ferritin; transferrin; transferrin saturation; thyroid hormones; as well as imaging of the liver and, if clinically warranted, a liver biopsy (Supplementary Table 1). Data on the typical signature of each drug involved and its association with iDILI were collected from the literature, the LiverTox website,[18,41] and drug labels. The clinical causality assessment was performed by