ORIGINAL ARTICLE

Monocyte-derived hepatocyte-like cells for causality assessment of idiosyncratic drug-induced liver injury

Andreas Benesic,[1,2] Alexandra Leitl,[1] Alexander L Gerbes[1]

▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2015-309528).

[1]Liver Center Munich, Department of Internal Medicine II, University Hospital Munich, Campus Grosshadern, Munich, Germany
[2]MetaHeps GmbH, Planegg/Martinsried, Germany

Correspondence to
Dr Andreas Benesic, Liver Center Munich, Department of Internal Medicine II, University Hospital Grosshadern, Ludwigs-Maximilians-University Munich, Germany, Marchioninistr. 15, Munich 81377, Germany; andreas.benesic@med.uni-muenchen.de

Received 6 March 2015
Revised 20 April 2015
Accepted 8 May 2015
Published Online First 4 June 2015

ABSTRACT

Background Idiosyncratic drug-induced liver injury (iDILI) is a frequent cause of acute liver injury and a serious problem in late stage drug-development. Its diagnosis is one of the most challenging in hepatology, since it is done by exclusion and relies on expert opinion. Until now no reliable in vitro test exists to support the diagnosis of iDILI. In some instances it is impossible to determine the causative drug in polymedicated patients.

Aim To investigate if monocyte-derived hepatocyte-like (MH) cells might be a tool supporting clinical judgment for iDILI diagnosis and causality assessment.

Methods This prospective study included 54 patients with acute liver injury and intake of at least one drug. Thirty-one patients were diagnosed with iDILI based on causality likelihood. MH cells were generated from every patient and in vitro toxicity of the respective drugs was assessed by lactate-dehydrogenase release. The results from MH cells and RUCAM, the most widely used scoring system as methods to support clinical judgement were compared.

Results MH cells showed enhanced toxicity in 29 of the 31 patients with iDILI, similar to RUCAM score. MH cells exhibited negative results in the 23 non-DILI cases, whereas RUCAM indicated possible iDILI in six cases. Analysis of the comedications also showed superior specificity of MH cells. No MH cell toxicity of the drugs showing toxicity in patients with iDILI was observed in MH cells of healthy donors.

Conclusions In this pilot study in vitro testing using MH cells derived from patients with acute liver injury was able to identify patients with iDILI with an excellent sensitivity and a higher specificity than RUCAM, the most widely used current causality assessment score. Therefore, MH cells could be useful to identify the causative drugs even in polymedicated patients by adding objective data to causality assessment.

Trial registration number : NCT02353455

Significance of this study

What is already known on this subject?

- Idiosyncratic drug-induced liver injury (iDILI) is the most challenging diagnosis in hepatology requiring exclusion of other causes for liver injury.
- The gold standard for iDILI diagnosis and causality assessment is expert opinion.
- Methods to positively diagnose iDILI cases and to identify the causative agent are urgently needed.

What are the new findings?

- In this pilot study monocyte-derived hepatocyte-like (MH) cells were generated from blood of patients with acute liver injury.
- MH cells showed sensitive and highly specific toxicity responses towards the implicated drugs in iDILI cases.
- MH cell toxicity identified the causative agent in iDILI cases with higher specificity than RUCAM, the most widely used scoring system.
- No MH cell toxicity was observed in cells derived from patients with acute liver injury not caused by DILI or from healthy donors.

How might it impact on clinical practice in the foreseeable future?

- MH cell testing can help to further clarify iDILI suspicion.
- Positive identification of iDILI cases by MH cells may facilitate the development of drug specific iDILI biomarkers.

INTRODUCTION

Drug-induced liver injury (DILI) is a rare event with a huge impact on healthcare: It is by far the most common cause for acute liver failure in the USA and in Europe.[1 2] Apart from its clinical relevance, DILI is also a major issue during the development of novel drugs and one of the most common causes for drug withdrawals, restrictions and project terminations.[3]

DILI is commonly classified as either dose-dependent (intrinsic) or idiosyncratic. The most important example for intrinsic DILI is acetaminophen (APAP).[4] Since dose-dependent toxicity can be reproduced in animals and in vitro models, the underlying mechanisms of APAP-hepatotoxicity are increasingly better understood.[4 5] Idiosyncratic DILI (iDILI) is responsible for about 10–15% of acute liver failures in the USA[6] but the mechanisms leading to this reaction are only poorly identified. iDILI is characterised by variable latency to onset (weeks to months), lack in clear dose-dependency and rare incidence (eg, 1 in 10.000).[7] Multiple approaches have been made to identify iDILI potential of a drug in early preclinical development: cell based assays using primary human hepatocytes, immortalised hepatocytes, hepatoma cell lines or stem cell derived hepatocytes.[8–10] Yet, current in vitro and animal models have only limited possibilities of individualisation

To cite: Benesic A, Leitl A, Gerbes AL. *Gut* 2016;**65**:1555–1563.

and predictive value for iDILI. Therefore, iDILI often is not detected before late stage clinical trials or marketing of the drug. Most regulatory actions on drugs related to hepatotoxicity in the recent years have been due to iDILI and often only few cases (3–20 patients) were observed.

In addition to being a risk for patients and a threat for drug development, iDILI is also a very challenging diagnosis. It requires exclusion of other possible aetiologies for acute liver injury and expert opinion,[11] based on patient data and the typical 'signatures' associated with iDILI by a certain drug. Additional causality assessment methods, such as the RUCAM score[12 13] were designed in an attempt to provide a more objective method supporting or excluding DILI suspicion and supporting causality assessment in case of polymedication. Most recently expert opinion systems[14] have been proposed as a gold standard in the absence of a suitable in vitro test. However, in the case of novel drugs a typical signature of injury is mostly not available, increasing the difficulty to find the right diagnosis. Polymedication is a huge challenge in patients with suspected DILI, since it may be impossible to identify the causative agent in this setting.[15]

Since immune mechanisms seem to be important in iDILI,[16 17] immunological tests have been developed to support clinical expertise with in vitro tests.[18] In Japan, the drug lymphocyte stimulation test, measuring proliferation of patient lymphocytes upon stimulation with the suspected drug and its metabolites is part of the causality assessment. Further methods are in development, such as the lymphocyte migration test or cytokine release of CD14 positive cells from patients with iDILI when incubated with lysates of drug-pulsed HepG2 cells.[19] Yet, the use of the drug lymphocyte stimulation test is limited by low sensitivity and false positive results.[20 21] The use of patient derived hepatocytes for testing of individual susceptibility and causality is extremely limited by invasiveness, especially in the setting of drug-induced liver failure. To our best knowledge, there are no data available for the use of primary hepatocytes, 3D systems, spheroids or stem-cell derived hepatocytes from patients with iDILI in individual causality assessment.

There is evidence that monocytes and monocyte derived cells are linked to liver injury and regeneration: In the setting of APAP toxicity the course of liver injury is influenced by recruitment of monocytes/macrophages to the liver[22–24] and there is also evidence that these cells may acquire hepatocyte characteristics.[25] We therefore have developed a standardised method to generate monocyte-derived hepatocyte-like (MH) cells, which exhibit several donor specific hepatocyte characteristics: MH cells are derived from peripheral monocytes and retain several of their characteristics (eg, low level expression of CD14). Additionally, MH cells show inducible activities of CYP450 enzymes 1A2, 2C9, 3A4 reflecting the activities in primary human hepatocytes of the same donor.[26] Additional data on phase II metabolism as uridine diphosphate (UDP)-glucuronosyltransferase activity as well as expression of several sulfotransferases and glutathione-S-transferases have been obtained. MH cells also express transporter proteins involved in excretion of xenobiotics (data on phase II and transporter activities are given in online supplementary figure S1). In order to investigate novel methods to diagnose iDILI and assign causality by the reactions of patient derived cells, we investigated whether hepatocyte-like cells derived from patient monocytes are capable of reflecting clinical iDILI in vitro.

PATIENTS AND METHODS

Patients

Patients with acute liver injury and intake of at least one drug at onset of liver injury were prospectively included in this study. Acute liver injury was defined[26] as elevation of alanine aminotransferase activity (ALT) ≥5×ULN (upper limit normal), alkaline phosphatase activity (AP) ≥2×ULN or ALT ≥3×ULN accompanied by total bilirubin (Bili) ≥2×ULN. After informed consent of the patients, data on gender, age, ethnicity, height, weight, alcohol and nicotine consumption as well as the results of the clinical investigations were recorded in order to comply with recommendations from expert consensus meetings.[28 29] A detailed history of each patient was obtained including comorbidities, previous and current prescription medications, over-the-counter drugs and herbals/dietary supplements. The onset of liver injury was defined as the first documented abnormalities in liver parameters or unequivocal symptoms of liver disease, such as jaundice or dark urine. ALT, aspartate aminotransferase activity, AP and total bilirubin (Bili) are expressed as x-fold ULN. These ULN values were for aspartate aminotransferase activity and ALT 35 U/L for women and 50 U/L for men, for AP 105 U/L for women and 135 U/L for men and 1 mg/dL (17.1 μmol/L) for total bilirubin. Unspecific symptoms such as nausea, abdominal pain or fatigue were also recorded. The time course and outcome of liver injury in relation to discontinuation of the suspected medications were documented. Liver injury was classified using R-values (R=ratio ALT×ULN/AP×ULN) as 'hepatocellular' (R≥5), 'mixed' (R>2 and R<5) and 'cholestatic' (R≤2), respectively. The RUCAM Score was calculated for up to five drugs in each patient. Data on typical signatures of the respective drugs were used for diagnostic assessment of the patients whenever available. These data were obtained from the LiverTox website[30] or using case reports (PubMed). Fifty-four patients were included in the study, 31 iDILI cases and 23 cases with other cause of liver injury (non-DILI): acute hepatitis A (n=2), acute hepatitis E (n=1), idiopathic autoimmune hepatitis (n=4), Morbus Still of the adult (n=1), coeliac disease (n=1), alcoholic steatohepatitis (n=5), extrahepatic cholestasis (n=3), acute heart failure/cardiac shock (n=3), secondary sclerosing cholangitis (n=3).

The diagnosis of iDILI and causality assessment was based on clinical judgement and the results of RUCAM and MH cells as two possibilities to support the clinical assessment were compared. Likelihood of iDILI causality for each drug was classified as 'definite' (no other cause for liver injury and a clear role of the respective drug), 'highly likely' (no other cause for liver injury but atypical signature and/or intake of at least one other drug with compatible signature), 'probable' (no compelling evidence for liver injury other than iDILI, but atypical presentation and polymedication), 'possible' (other cause of liver injury likely, but drug toxicity cannot be excluded from presentation and signature) or 'unlikely' (other cause of liver injury very likely, no compatible drug signature) in analogy to previously published classification systems.[31] The diagnosis of iDILI was assigned for cases with a likelihood of 'definite', 'highly likely' or 'probable' (table 1).

Blood sampling, cell generation and toxicity testing

Patient blood for cell generation was collected in standard haematology EDTA-tubes (Saarstedt, Germany) and stored at 2–8°C for up to 48 h. Monocytes were isolated using gradient centrifugation and adherence separation. MH cells were generated as described.[26] MH cells of healthy donors were generated from residual blood that could be obtained anonymously after thrombocyte donation in the department of transfusion medicine, University Hospital Munich. After the generation process, MH cells were incubated with the respective drugs in 96-well plates using $1\times C_{max}$ and $10\times C_{max}$. Data on drug pharmacokinetics were obtained from literature and drug information sheets.

Table 1 Classification system used to diagnose iDILI and non-DILI and to assess causality likelihood for the respective drugs in the individual patients

	iDILI			Non-DILI	
Diagnosis classification	**'definite'**	**'highly likely'**	**'probable'**	**'possible'**	**'unlikely'**
Other causes of liver injury	Definitely excluded	Definitely excluded	Unlikely	Probable	Highly probable
Drug signature	Typical	Atypical OR comedication with compatible signature	Atypical AND comedication with compatible signature	Compatible with iDILI	Atypical

iDILI, idiosyncratic drug-induced liver injury.

After 48 h cytotoxicity was determined by release of lactate-dehydrogenase (LDH): LDH-activity was measured in cell lysates and supernatants using the Cyto Tox 96 non-radioactive assay (Promega, USA). The assay was performed as end point measurement after initial experiments in MH cells showed similar results for kinetic and end point determination (data not shown). The LDH assay has been chosen for this study since it reflects actual loss in cell integrity and its results allowed best comparison between the individual patients. Other end points that were evaluated included Caspase, ATP-content, CellTiter Blue and JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide) staining for mitochondrial toxicity (data not shown). LDH-release was calculated as ratio $LDH_{supernatant}/(LDH_{lysate}+LDH_{supernatant})$. Results were normalised to vehicle control (0%) and positive control (100%) (lysis with 1% TWEEN®20 (Polyethylene glycol sorbitan monolaurate)). To compensate for individual differences in the assays, toxicity was expressed as toxicity/(2×SD of vehicle control). Figure 1A shows an example of LDH-release in MH cells of a patient with iDILI, the corresponding toxicity is shown in figure 1B. To allow a better interpretation of the results, the respective drugs were tested in MH cells of healthy donors, as exemplified in figure 1C. The data presented on MH cell toxicity are results obtained at $10\times C_{max}$ except for simvastatin, atorvastatin, lamotrigine, fluspirilene, piperacilline/tazobactam and roxithromycin. The latter drugs showed dose dependent toxicity in MH cells at $10\times C_{max}$ and $1\times C_{max}$ was used for analysis. The incubation of MH cells and measurement of toxicity was performed independently of assessment of causality likelihood and RUCAM.

Data analysis and statistics

Data are shown as median and range, statistical analysis was performed with analysis of variance (ANOVA), Welch test and Brown Forsythe test where appropriate using SPSS software (V.22.0.0.1, IBM). Significant differences were assumed at $p<0.05$. The cut-off values for RUCAM (≥6) and MH cells (≥2) were determined from the study population and chosen for providing maximal sensitivity and specificity in the cases investigated in this study.

RESULTS

Patient characteristics

Characteristics of the study population are summarised in table 2.

No significant differences were found for gender distribution, ethnicity, age, height, weight and body mass index. The predominant pattern of liver injury in both groups was hepatocellular (iDILI 71% vs non-DILI 57%, not significant; $p>0.05$). A minor proportion in either group took only one single drug (iDILI 8/31 and non-DILI 7/23; 25% vs 30%, n.s.). More than one comedication was present in 58% of patients (14/31) in the iDILI group versus 52% (12/23) in the non-DILI group (n.s.; figure 2).

Eighty-four different medications were tested in patients with iDILI and 62 in the non-DILI group (see online supplementary tables S1A,B). Most frequently agents suspected causative in iDILI cases were non-steroidal anti-inflammatory drugs (NSAIDs) (8/31; 25.8%), oral anticoagulants (4/31; 12.9%), anti-infectives (2/31; 6.5%), immunomodulators (2/31; 6.5%) and antithyroid medications (2/31; 6.5%) (see online supplementary table S2).

The criteria for 'Hy's law' (ALT ≥3×ULN and total bilirubin ≥2×ULN)[32 33] were fulfilled in 19 iDILI cases (61.3%) and 16 non-DILI cases (69.6%; n.s.). A summary of the laboratory findings is given in table 3 together with the calculated R-ratios at onset and peak level of total bilirubin.

The RUCAM score was calculated for each case and for up to five medications in order to investigate iDILI-diagnosis and causality for the implicated agents. However, in nine of the iDILI cases RUCAM score did not differ between the drug with the highest causality likelihood and at least one comedication. In five of these cases analysis of drug information and signature did not allow to assign causality to a single agent.

Toxicity testing in patient-derived MH cells

MH cells were generated from patients with iDILI and those without DILI and toxicity of drugs 1–5 were compared with RUCAM score results. Since the gold standard for the diagnosis of iDILI is expert opinion, we analysed 11 iDILI cases and 12 non-DILI cases in which a definite diagnosis could be identified without any reasonable doubt (likelihood 'definite' for iDILI cases and 'unlikely' for non-DILI cases, respectively). In case of iDILI this included that the causative agent had a typical signature. Moreover in two cases a positive rechallenge was observed. The results of the RUCAM score and toxicity testing for the drug with the highest causality likelihood in the respective patient are presented in figures 3A, B. In this subgroup, toxicity testing in MH cells showed high sensitivity and specificity. Only in one case with unequivocal iDILI no increased toxicity at $10\times C_{max}$ could be observed. In the 12 non-DILI cases there was no increased MH cell toxicity of the involved compounds, whereas RUCAM indicated possible iDILI in two patients (one coeliac disease and one M. Still of the adult).

Following this subgroup analysis, the results of MH cell toxicity and RUCAM score were compared in the total study population (figure 4A, B, results for the drug with the highest causality likelihood in the respective patient).

Targeting the issue of polymedication, MH toxicity in iDILI cases was compared with the calculated RUCAM scores.

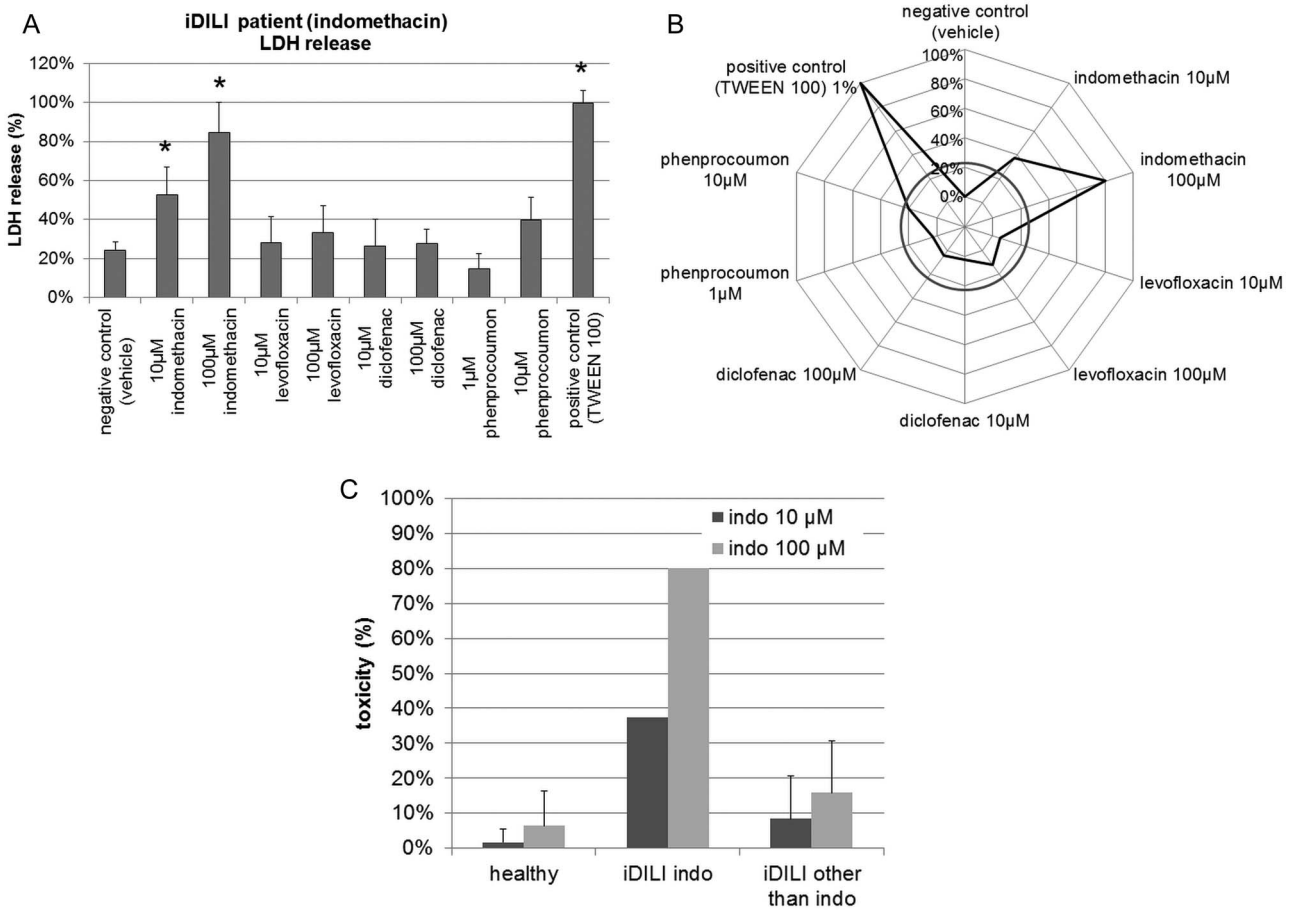

Figure 1 (A) Example of monocyte-derived hepatocyte-like (MH) testing of patients with idiosyncratic drug-induced liver injury (iDILI). Lactate-dehydrogenase (LDH) release from a patient with indomethacin as likely cause of iDILI. Indomethacin at 1×C_{max} and 10×C_{max} induces significant increase in LDH release. (triplicates±SD; *p<0.05 vs control). (B) Example of a spiderweb graph of toxicity results in a patient with iDILI. Grey circle represents cut-off (2× double SD of control). Indomethacin as the likely cause of iDILI in this case (causality likelihood: highly likely RUCAM: 8), induces toxicity of 80% (MH: 7.2) at 10×Cmax. No toxicity of the comedication levofloxacin is observed in MH cell testing. Indomethacin at 100 µM did not induce any toxicity in healthy controls (n=25, data not shown). Diclofenac and phenprocoumon, drugs that caused iDILI in other patients did not cause toxicity in this patient. (C) Example of MH testing of indomethacin toxicity in MH cells of healthy donors (n=22), the indomethacin patient with iDILI and patients with iDILI with other causes than indomethacin (n=8). MH cells of the indomethacin patient with iDILI show individual susceptibility.

Similar to the comparison of MH cell testing and RUCAM for iDILI diagnosis, we first analysed the subgroup of patients with iDILI in whom clinical likelihood as gold standard supports causality of one drug in the presence of at least one comedication: This subgroup consisted of 15 patients with iDILI taking 57 drugs (42 comedications). Sixteen of the 42 comedications reached RUCAM ≥6 (38%), whereas only two comedications where tested positive with MH cells (5%).

In the analysis of the whole study population, MH cell toxicity also showed better concordance with causality likelihood than RUCAM for DILI-drugs and for the respective comedications (drugs with the greatest likelihood for causality in patients with iDILI are listed in online supplementary table S2). The results of RUCAM score and MH testing for the respective comedications are shown in figures 5A, B (results for all comedications).

The RUCAM score assessed positive causality for 27 of the 53 comedications (51%) and additionally was false positive for 2 of the comedications in non-DILI cases (clinical diagnosis of alcoholic steatohepatitis, but drug and comedications as cause for liver injury could not be ruled out definitely). MH cell testing showed toxicity in only four of the comedications in iDILI cases and no MH cell toxicity was observed in non-DILI cases. These findings suggest that MH cell testing could improve causality assessment of iDILI in the presence of comedication.

From the four patients testing positive for two drugs in MH cells, in one case a combination medication of synthetic oestrogen and gestagen was the putative DILI drug and the in vitro reaction could be seen for both components. Another patient had intake of two different neuroleptics and showed a transient increase of ALT (maximal 3.4×ULN) without clinical symptoms when taking a drug with less likely causality (risperidone). He experienced clinical significant liver injury (abdominal pain, nausea, ALT_{max} 19.8×ULN; no bilirubin increase) after starting treatment with the drug with highest causality likelihood (olanzapine). In the other two cases all medications have been discontinued and no additional information like, for example, rechallenge is available to support the interpretation of the test results.

Since MH cell testing is independent from clinical causality assessment, the test should provide additional information supporting clinical judgement. Figure 6 depicts the comparison of correct and incorrect results of RUCAM and MH cells for all drugs and comedications in the total study population in

Table 2 Case characteristics expressed as median and range (minimum and maximum)

Characteristic	iDILI (n=31)	Non-DILI (n=23)	p Value
Age, years	55 (14–79)	55 (22–74)	n.s.
Female, N(%)	15 (48%)	9 (39%)	n.s.
Ethnicity			
Caucasian, N(%)	30 (97%)	23 (100%)	n.s.
Hispanic, N(%)	1 (3%)		
BMI (kg/m^2)	22.0 (16.8–34.7)	25.4 (19.2–40.8)	n.s.
Latency*, days	36 (1–370)	22 (0–1470)	n.s.
Latency to blood sample, days	10 (2–254)	11 (3–338)	n.s.
Pattern of liver injury at onset			
Hepatocellular, N(%)	22 (71%)	13 (57%)	n.s.
Mixed, N(%)	2 (7%)		
Cholestatic, N(%)	7 (23%)	10 (43%)	
Outcome			
Remission, N(%)	22 (71.3%)	18 (78.3%)	n.s.
Chronicity, N(%)	1 (3.2%)	4 (17.3%)	
Death from liver disease, N(%)	1 (3.2%)		
Death, not liver related, N(%)	1 (3.2%)	1 (4.3%)	
Liver transplant, N(%)	6 (19.4%)		

*Latency: time to onset of liver injury from start of the drug most likely causal according to RUCAM and injury pattern. Latency from liver injury to blood sampling was less than 21 days in 23 patients with iDILI (74%) and in 18 non-DILI cases (78%).
BMI, body mass index; iDILI, idiosyncratic drug-induced liver injury.

Table 3 Laboratory parameters from cases expressed as x-fold upper limit normal (ULN)

		iDILI (n=31)			Non-DILI (n=23)			
		Median	Min	Max	Median	Min	Max	p Value
ALT	Onset	27.8	0.8	108.2	15.1	0.9	107.5	0.58
	Max	35.5	1.8	108.2	26.0	1.1	107.5	0.31
	PTBL	19.3	0.8	80.0	9.3	0.9	84.7	0.69
AST	Onset	12.8	1.0	202.2	9.1	0.9	139.2	0.40
	Max	17.8	1.7	202.2	13.0	0.9	139.2	0.33
	PTBL	6.2	1.0	42.4	5.2	1.1	63.1	0.94
AP	Onset	1.6	0.5	5.3	1.6	0.6	8.6	0.60
	Max	2.0	1.0	9.0	2.0	1.0	33.8	0.10
	PTBL	1.7	0.5	9.0	1.9	0.5	33.8	0.12
Bili	Onset	2.2	0.0	48.8	7.0	0.3	27.3	0.70
	Max	3.2	0.5	50.9	11.0	0.7	41.9	0.30
R-	Onset	21.8	0.3	76.0	12.7	0.2	66.2	0.51
	PTBL	13.5	0.3	76.0	7.0	0.1	47.4	0.40

Onset: values at first diagnosis of liver enzyme abnormalities.
max: maximal levels.
PTBL: values at the peak of total bilirubin levels
ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; Bili, total bilirubin; iDILI, idiosyncratic drug-induced liver injury; R, ratio ALT×ULN/AP×ULN.

relation to the respective causality likelihood. Performance of RUCAM is best in more unequivocal situations ('definite', 'highly likely' and 'unlikely'). If causality likelihood is less distinct ('probable' and 'possible'), RUCAM shows a relevant percentage of incorrect results. MH cell testing by contrast yields a stable high rate of correct test results throughout the causality likelihood scale providing additional information even in more ambiguous cases.

Since iDILI is a rare event any useful test should provide excellent specificity. Therefore, we also tested several drugs that showed toxic effects in MH cells of the iDILI cases (phenprocoumon, metamizole, diclofenac, indomethacin and carbimazole) from 25 up to 81 healthy donors. In these healthy donors a median toxicity of 0.19 was observed (minimum: 0.01, maximum: 1.85). As an example, the results for diclofenac are shown in figure 7. Moreover, no MH toxicity of diclofenac was observed in the non-DILI cases and iDILI cases with another drug as causative agent. These results provide further evidence for reflection of individual susceptibility to drugs by MH cells. As shown for diclofenac, none of the other drugs with positive results in MH cells from patients with iDILI did induce toxic responses in MH cells in this group of healthy donors.

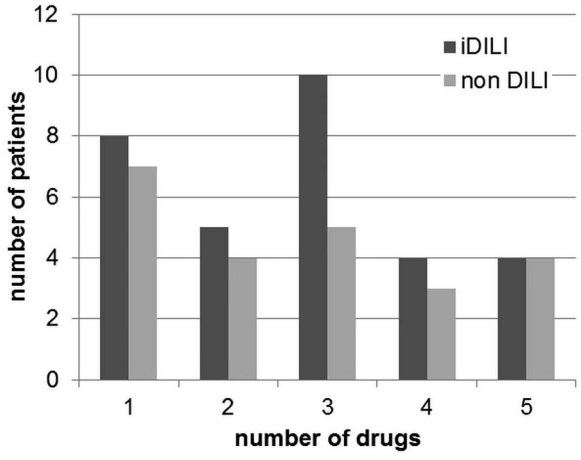

Figure 2 Medications in patient groups. In the idiosyncratic drug-induced liver injury (iDILI) group 74% of patients were taking more than one medication versus 70% in the non-DILI group (n.s.).

DISCUSSION

As yet, the diagnosis of iDILI is a diagnosis of exclusion. Thus, additional methods are needed which positively support a suspicion of iDILI and at the same time provide sufficient specificity. This also will improve understanding of underlying mechanisms, since false positive iDILI cases in research may lead to indistinct results on the search for novel safety biomarkers and can cause costly failure in drug development.[34 35]

In the present study we investigated the use of patient derived hepatocyte-like cells from monocytic origin as an individual model for iDILI and as a possible tool to support diagnosis and causality assessment. The study included 54 patients with acute liver injury of whom 31 were diagnosed with iDILI. The agents most likely implicated in DILI episodes were NSAIDs, oral anticoagulants (all cases phenprocoumon), anti-infectives, immunomodulators and antithyroid medications. These findings are consistent with previous reports showing NSAIDs, anti-infectives and immunomodulatory drugs as frequent causes of iDILI.[36–40] Phenprocoumon is an oral anticoagulant used predominantly in Germany, yet also linked to iDILI with a potential autoimmune mechanism.[41 42] Metamizole has been rarely linked to liver injury, yet immunological mechanisms are suspected.[43]

In our study, clinical judgement of causality likelihood was used as standard for iDILI diagnosis and causality assessment.

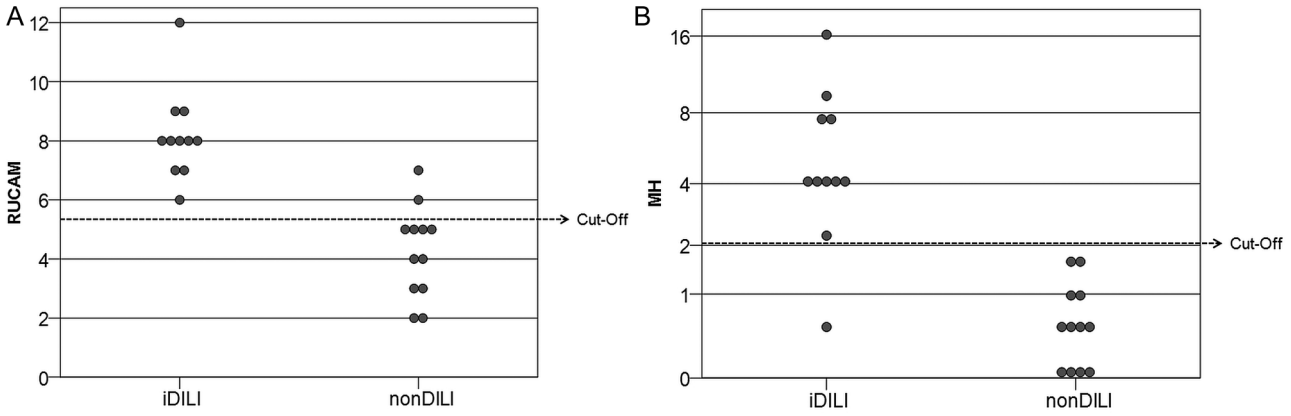

Figure 3 (A) RUCAM scores from 11 unequivocal idiosyncratic drug-induced liver injury (iDILI) cases and 12 patients with liver injury due to other causes (non-DILI). (B) Test results from patient-derived monocyte-derived hepatocyte-like (MH) cells exposed to the suspected DILI drugs. Maximum result obtained from non-DILI cases was 1.8. MH cells of one patient with iDILI did not show toxicity in MH cells after 48 h incubation. Dots represent the drug with the highest causality likelihood in the respective patient.

Analysis of the results was done using two different approaches: To analyse the utility of RUCAM and MH cell testing to support the diagnosis of iDILI, results for single drugs with the highest causality likelihood in the individual patients were used (figures 3 and 4). In order to gain information on the capability to discriminate the causative agent in individual patients, all drugs were used for the analysis, resulting in up to five RUCAM scores and MH signals per patient (figures 5 and 6). In the latter analysis, the correctness of RUCAM test results were dependent on clinical causality likelihood, while MH cell testing yielded stable test correctness regardless of causality likelihood. Thus, MH cell testing could add objective test results to improve causality assessment in complex cases.

This study provides evidence that MH cells can help to positively diagnose iDILI with a similar sensitivity as clinical causality assessment in 29 of the 31 patients. Importantly, specificity of our in vitro test seems to be superior to RUCAM, even with the cut-off ≥6, as chosen in this study. RUCAM of 3–5 indicates 'possible' DILI and using a cut-off ≥3 would increase sensitivity to 100% in our population, yet specificity would be reduced to 17%. Thus, high specificity could be an additional benefit of MH testing in the diagnostic workup of suspected iDILI cases, since false positives are a relevant problem in drug development and impair the discovery and validation of novel safety biomarkers.[44–46]

Expert opinion is the gold standard for diagnosis of iDILI and has been shown to provide better differentiation than RUCAM.[31] However, in a small proportion of cases a definite diagnosis cannot be established, especially in polymedication when clinical presentation and drug information do not provide additional clues to the causative agent.[47] In the population investigated in this study more than 70% of patients were taking two or more drugs, underlining the need for methods that allow reliable causality assessment. In four of the cases in this study the causative agent could not be definitely identified at the initial presentation, yet in two cases positive rechallenge was observed. In these two cases the MH cell test provided positive results for the respective drug. Moreover, a subgroup analysis in 15 polymedicated iDILI cases showed that MH cell testing can identify the causative agent more reliably than RUCAM. These findings might have impact on patient care and late stage drug development.

The lack of MH cell toxicity of several drugs tested in up to 81 healthy donors further suggests excellent sensitivity and specificity of the test. However, since the samples of healthy donors were obtained anonymously, there is no clinical

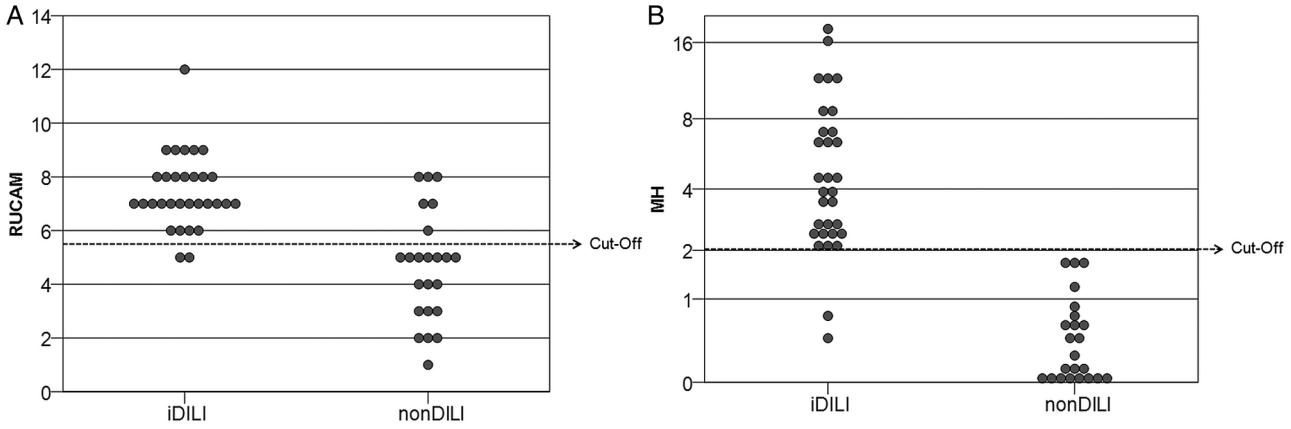

Figure 4 (A) RUCAM scores for the drug with highest causality likelihood from 31 idiosyncratic drug-induced liver injury (iDILI) and 23 non-DILI cases. Two of the patients with iDILI and six of the patients without DILI were wrongly classified by RUCAM. (B) Test results from patient derived monocyte-derived hepatocyte-like (MH) cells exposed to the drugs with the highest causality likelihood. In MH cells of two patients with iDILI no toxicity was observed. Dots represent the drug with the highest causality likelihood in the respective patient.

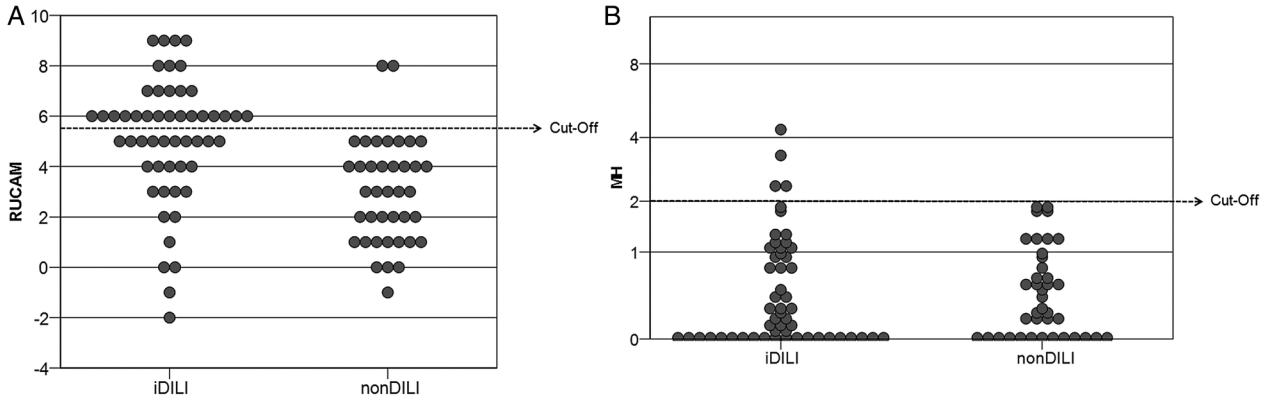

Figure 5 (A) RUCAM score results for comedications in idiosyncratic drug-induced liver injury (iDILI) and non-DILI cases. Twenty-seven of 53 comedications are classified by RUCAM as potentially causative for iDILI. (B) Test results derived from patients with iDILI monocyte-derived hepatocyte-like (MH) cells exposed to the respective comedications in iDILI and non-DILI cases. In four iDILI cases MH cell toxicity is also found for comedications, whereas no toxicity of comedication is observed in non-DILI cases.

information on drug intake or liver injury that could be compared with the in vitro results in MH cells from these healthy donors. However, the results of this pilot study should be validated in a larger and independent patient group. It will be necessary to also include patients who tolerate potential iDILI drugs without signs of liver injury.

Our present investigations focused on the correspondence of MH cells toxicity with iDILI diagnosis and clinical causality likelihood. The toxicity induced in MH cells derived from patients with iDILI by the suspected causative agents could be due to the individual metabolic capabilities of these cells.[26] Current concepts of iDILI suggest that altered dose response could lead to subclinical hepatocyte damage triggering an immune response that most likely determines the severity of the injury. An important observation is that short-term incubation using $10\times C_{max}$ sufficed to induce toxicity in MH cells of most patients with iDILI, whereas the latency of the clinical event was weeks to months. It can be hypothesised that MH cells reflect metabolic upstream events of iDILI without modelling the response of the adaptive immune system. This is supported by the finding that in most iDILI cases $10\times C_{max}$ was necessary to induce a toxic response. Therefore, MH cells could reflect altered dose response of the patient and thus perhaps model the initial damage. Due to differences of exposure of hepatocytes in vivo and MH cells in vitro (eg, accumulation of toxic metabolites due to lack of removal from culture media) the effect may be observed earlier in the in vitro model. MH cells might also retain functions of innate immune cells that could contribute to their toxicity response. It remains to be investigated if the test performs equally well in iDILI cases where the main mechanism of damage is mediated by adaptive immunity, for example, amoxicillin/clavulanate.

Thorough investigation of the underlying mechanisms was beyond the scope of this manuscript. However, if it could be shown that the mechanisms in MH cells relate to the clinical situation these findings could have major impact on the understanding of iDILI and furthermore allow the development of novel tests for iDILI potential in drug development.

Our study provides evidence that MH cells could be a useful tool in future research on iDILI and might provide significant improvement in clinical diagnosis or exclusion of iDILI. Moreover, MH testing may allow identification of the causative agent in polymedication by supporting the clinical judgement with additional unprecedented information that cannot be matched by RUCAM. The MH cell test seems to be an improvement in diagnosis or exclusion of iDILI in individual patients, allowing correct assessment of causality in polymedicated patients. Despite the 2 week interval between blood sampling and MH cell assay results, the test may still have relevance for the clinical scenario and consequences for future medication of the

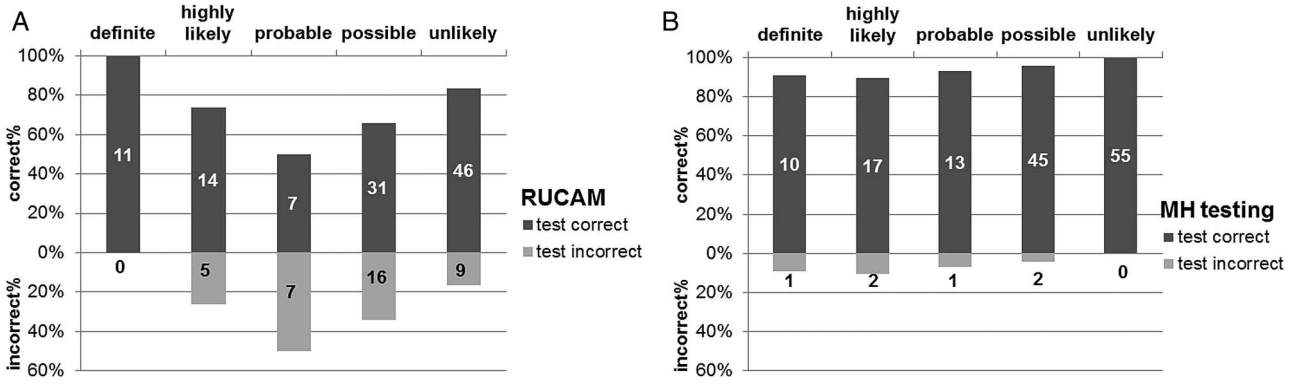

Figure 6 (A) Correct and incorrect RUCAM score results versus causality likelihood: Correctness of RUCAM score results depends on clinical causality likelihood, with effective classification for 'definite', 'highly likely' and 'unlikely' drugs, whereas in drugs with 'probable' or 'possible' there is a relevant percentage of wrong test results. (B) Correct and incorrect monocyte-derived hepatocyte-like (MH) test results versus causality likelihood: MH testing is independent on clinical causality likelihood, producing stable results.

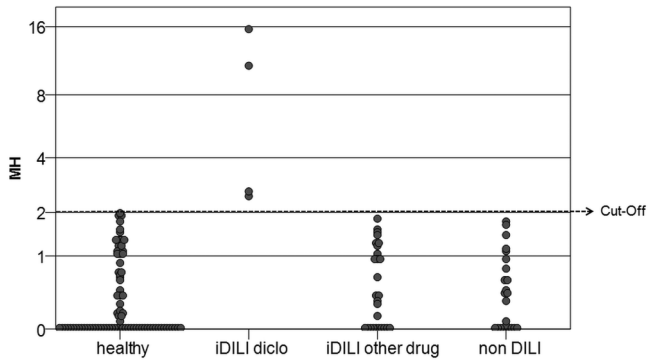

Figure 7 Monocyte-derived hepatocyte-like (MH) cell toxicity of diclofenac in 81 healthy donors, in 4 patients with idiosyncratic drug-induced liver injury (iDILI) with diclofenac as most likely cause for liver injury, in n=27 patients with iDILI with iDILI caused by another drug than diclofenac and in n=23 patients without DILI. Dots represent toxicity responses from individual patients and healthy donors.

patient. Especially in polymedication MH cell testing may help to clarify causality and avoid inadvertent rechallenge of patients.

Our data provide evidence that MH cell testing can be used to diagnose or exclude iDILI in individual patients and moreover could be of great value for causality assessment in iDILI. We see three major applications of the test: (1) In the clinical setting MH cell testing can help to support the diagnosis and identify the causative drug in polymedication, thus preventing inadvertent re-exposure with impact on patient care. (2) Pharmacovigilance can be supplemented with unprecedented information on drug causality leading to improved assessment of risk and benefit in a regulatory setting or even allowing definition of high-risk populations for a given drug. (3) Clinical development of novel drugs is often halted if a liver signal occurs, especially when no or little previous information on liver safety of the drug complicate clinical judgement on causality.[48 49] MH cell testing could help to diagnose or exclude iDILI without the need for a typical drug signature and support causality assessment to differentiate between effects of the study drug or comedication.

Acknowledgements The authors thank Maria Escobar and Sabine Pirsig for excellent technical support, and the department of transfusion medicine of the University Hospital Munich for providing blood samples of anonymous healthy donors. The authors also thank all patients who made this study possible by their participation.

Contributors AB: Wrote the paper, planned and performed experiments, analysed data, performed statistics, obtained funding. AL: Collected and analysed data, performed statistics. The presented data are part of her doctoral thesis. ALG: Critically revised the manuscript, planned experiments, supervised the study, obtained funding.

Funding German Federal Ministry for Economic Affairs and Energy (EXIST grant no. 03EFT9BY56), Bavarian Ministry of Economic Affairs and Media, Energy and Technology, Germany (m^4-award) grant no. 1330/68362/34/2013.

Competing interests The authors disclose potential financial conflicts, since patent applications have been filed for the generation of MH cells as well as for possible applications.

Ethics approval Ethikkommission der Med. Fakultät der LMU München.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Lee WM. Drug-induced acute liver failure. *Clin Liver Dis* 2013;17:575–86, viii.
2 Larrey D, Pageaux GP. Drug-induced acute liver failure. *Eur J Gastroenterol Hepatol* 2005;17:141–3.
3 Stevens JL, Baker TK. The future of drug safety testing: expanding the view and narrowing the focus. *Drug Discov Today* 2009;14:162–7.
4 McGill MR, Jaeschke H. Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. *Pharm Res* 2013;30:2174–87.
5 McGill MR, Sharpe MR, Williams CD, *et al*. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. *J Clin Invest* 2012;122:1574–83.
6 Reuben A, Koch DG, Lee WM, Acute Liver Failure Study Group. Drug-induced acute liver failure: results of a U.S. multicenter, prospective study. *Hepatology* 2010;52:2065–76.
7 Yuan L, Kaplowitz N. Mechanisms of drug-induced liver injury. *Clin Liver Dis* 2013;17:507–18, vii.
8 LeCluyse EL, Alexandre E, Hamilton GA, *et al*. Isolation and culture of primary hepatocytes. *Methods Mol Biol* 2005;290:207–29.
9 Gómez-Lechón MJ, Lahoz A, Gombau L, *et al*. In vitro evaluation of potential hepatotoxicity induced by drugs. *Curr Pharm Des* 2010;16:1963–77.
10 Anson BD, Kolaya K, Kamp TJ. Opportunities for human iPS cells in predictive toxicology. *Clin Pharmacol Ther* 2011;89:754–8.
11 Chalasani NP, Hayashi PH, Bonkovsky HL, *et al*. ACG clinical guideline: the diagnosis and management of idiosyncratic drug-induced liver injury. *Am J Gastroenterol* 2014;109:950–66.
12 Danan G, Benichou C. Causality assessment of adverse reactions to drugs—I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. *J Clin Epidemiol* 1993;46:1323–30.
13 García-Cortés M, Stephens C, Lucena MI, *et al*. Causality assessment methods in drug induced liver injury: strengths and weaknesses. *J Hepatol* 2011;55:683–91.
14 Rockey DC, Seeff LB, Rochon J, *et al*. Causality assessment in drug-induced liver injury using a structured expert opinion process: comparison to the Roussel-Uclaf causality. *Hepatology* 2010;51:2117–26.
15 Verma S, Kaplowitz N. Diagnosis, management and prevention of drug-induced liver injury. *Gut* 2009;58:1555–64.
16 Adams DH, Ju C, Ramaiah SK, *et al*. Mechanisms of immune-mediated liver injury. *Toxicol Sci* 2010;115:307–21.
17 Stephens C, Andrade RJ, Lucena MI. Mechanisms of drug-induced liver injury. *Curr Opin Allergy Clin Immunol* 2014;14:286–92.
18 Maria V, Victorino R. Diagnostic value of specific T cell reactivity to drugs in 95 cases of drug induced liver injury. *Gut* 1997;41:534–40.
19 Tajiri K, Shimizu Y. Immunological aspects of drug-induced liver injury. *World J Immunol* 2014;4:149–57.
20 Berg PA, Becker EW. The lymphocyte transformation test—a debated method for the evaluation of drug allergic hepatic injury. *J Hepatol* 1995;22:115–l18.
21 Mantani N, Kogure T, Tamura J, *et al*. Lymphocyte transformation test for medicinal herbs yields false-positive results for first-visit patients. *Clin Diagn Lab Immunol* 2003;10:479–80.
22 Hogaboam CM, Bone-Larson CL, Steinhauser ML, *et al*. Exaggerated hepatic injury due to acetaminophen challenge in mice lacking C-C chemokine receptor 2. *Am J Pathol* 2000;156:1245–52.
23 Dambach DM, Watson LM, Gray KR, *et al*. Role of CCR2 in macrophage migration into the liver during acetaminophen-induced hepatotoxicity in the mouse. *Hepatology* 2002;35:1093–103.
24 Zigmond E, Samia-Grinberg S, Pasmanik-Chor M, *et al*. Infiltrating monocyte-derived macrophages and resident kupffer cells display different ontogeny and functions in acute liver injury. *J Immunol* 2014;193:344–53.
25 Stadtfeld M, Graf T. Assessing the role of hematopoietic plasticity for endothelial and hepatocyte development by non-invasive lineage tracing. *Development* 2005;132:203–13.
26 Benesic A, Rahm NL, Ernst S, *et al*. Human monocyte-derived cells with individual hepatocyte characteristics: a novel tool for personalized in vitro studies. *Lab Invest* 2012;92:926–36.
27 Aithal GP, Watkins PB, Andrade RJ, *et al*. Case definition and phenotype standardization in drug-induced liver injury. *Clin Pharmacol Ther* 2011;89:806–15.
28 Agarwal VK, McHutchison JG, Hoofnagle JH, *et al*. Important elements for the diagnosis of drug-induced liver injury. *Clin Gastroenterol Hepatol* 2010;8:463–70.
29 Avigan MI, Bjornsson ES, Pasanen M, *et al*. Liver safety assessment: required data elements and best practices for data collection and standardization in clinical trials. *Drug Saf* 2014;37(Suppl 1):S19–31.
30 Hoofnagle JH, Serrano J, Knoben JE, *et al*. LiverTox: a website on drug-induced liver injury. *Hepatology* 2013;57:873–4.
31 Rockey DC, Seeff LB, Rochon J, *et al*. Causality assessment in drug-induced liver injury using a structured expert opinion process: comparison to the Roussel-Uclaf causality assessment method. *Hepatology* 2010;51:2117–26.
32 Regev A, Björnsson ES. Drug-induced liver injury: morbidity, mortality, and Hy's law. *Gastroenterology* 2014;147:20–4.
33 Robles-Diaz M, Lucena MI, Kaplowitz N, *et al*. Use of Hy's law and a new composite algorithm to predict acute liver failure in patients with drug-induced liver injury. *Gastroenterology* 2014;147:109–18.
34 Regev A. Drug-induced liver injury and drug development: industry perspective. *Semin Liver Dis* 2014;34:227–39.
35 Stephens C, Lucena MI, Andrade RJ. Genetic variations in drug-induced liver injury (DILI): resolving the puzzle. *Front Genet* 2012;3:253.

36 Björnsson ES, Bergmann OM, Björnsson HK, *et al*. Incidence, presentation, and outcomes in patients with drug-induced liver injury in the general population of Iceland. *Gastroenterology* 2013;144:1419–25, 1425.e1–3.
37 Sgro C, Clinard F, Ouazir K, *et al*. Incidence of drug-induced hepatic injuries: a French population-based study. *Hepatology* 2002;36:451–5.
38 Aithal PG, Day CP. The natural history of histologically proved drug induced liver disease. *Gut* 1999;44:731–5.
39 Chalasani N, Fontana RJ, Bonkovsky HL, *et al*. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. *Gastroenterology* 2008;135:1924–34, 1934.
40 Hernández N, Bessone F, Sánchez A, *et al*. Profile of idiosyncratic drug induced liver injury in Latin America: an analysis of published reports. *Ann Hepatol* 2014;13:231–9.
41 Douros A, Bronder E, Andersohn F, *et al*. Drug-induced liver injury: results from the hospital-based Berlin Case-Control Surveillance Study. *Br J Clin Pharmacol* 2015;79:988–99.
42 Castiella A, Zapata E, Lucena MI, *et al*. Drug-induced autoimmune liver disease: a diagnostic dilemma of an increasingly reported disease. *World J Hepatol* 2014;6:160–8.
43 Herdeg C, Hilt F, Büchtemann A, *et al*. Allergic cholestatic hepatitis and exanthema induced by metamizole: verification by lymphocyte transformation test. *Liver* 2002;22:507–13.
44 Lee WM, Senior JR. Recognizing drug-induced liver injury: current problems, possible solutions. *Toxicol Pathol* 2005;33:155–64.
45 Senior JR. Drug hepatotoxicity from a regulatory perspective. *Clin Liver Dis* 2007;11:507–24, vi.
46 Senior JR. Evolution of the Food and Drug Administration approach to liver safety assessment for new drugs: current status and challenges. *Drug Saf* 2014;37(Suppl 1):S9–17.
47 Hayashi PH, Barnhart HX, Fontana RJ, *et al*. Reliability of causality assessment for drug, herbal and dietary supplement hepatotoxicity in the Drug-Induced Liver Injury Network (DILIN). *Liver Int* 2015;35:1623–32.
48 Merz M, Lee KR, Kullak-Ublick GA, *et al*. Methodology to assess clinical liver safety data. *Drug Saf* 2014;37(Suppl 1):S33–45.
49 Kullak-Ublick GA, Merz M, Griffel L, *et al*. Liver safety assessment in special populations (hepatitis B, C, and oncology trials). *Drug Saf* 2014;37(Suppl 1): S57–62.