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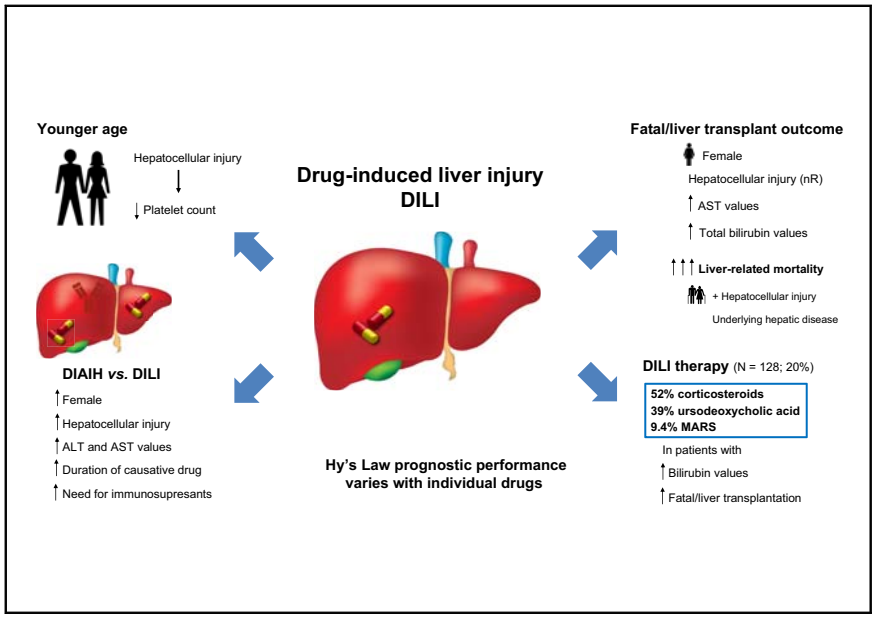
Comprehensive analysis and insights gained from long-term experience of the Spanish DILI Registry

Graphical abstract



Highlights

- Clinical parameters can help predict DILI phenotype and outcome.
- Older patients with cytolitic DILI and those with liver disease have worse outcome.
- Serum AST at DILI onset should be assessed as it strongly predicts poor outcome.
- Prognostic potential of Hy's law in DILI varies between causative agents.

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Lay summary

Clinical information on drug-induced liver injury (DILI) collected from enrolled patients in the Spanish DILI Registry can guide physicians in the decision-making process. We have found that older patients with hepatocellular type liver injury and patients with additional liver conditions are at a higher risk of mortality. The type of liver injury, patient sex and analytical values of aspartate aminotransferase and total bilirubin can also help predict clinical outcomes.

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Comprehensive analysis and insights gained from long-term experience of the Spanish DILI Registry

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Background & Aims: Prospective drug-induced liver injury (DILI) registries are important sources of information on idiosyncratic DILI. We aimed to present a comprehensive analysis of 843 patients with DILI enrolled into the Spanish DILI Registry over a 20-year time period.

Methods: Cases were identified, diagnosed and followed prospectively. Clinical features, drug information and outcome data were collected.

Results: A total of 843 patients, with a mean age of 54 years (48% females), were enrolled up to 2018. Hepatocellular injury was associated with younger age (adjusted odds ratio [aOR] per year 0.983; 95% CI 0.974–0.991) and lower platelet count (aOR per unit 0.996; 95% CI 0.994–0.998). Anti-infectives were the most common causative drug class (40%). Liver-related mortality was more frequent in patients with hepatocellular damage aged ≥65 years (p = 0.0083) and in patients with underlying liver disease (p = 0.0221). Independent predictors of liver-related death/transplantation included nR-based hepatocellular injury, female sex, higher onset aspartate aminotransferase (AST) and bilirubin values. nR-based hepatocellular injury was not associated with 6-month overall mortality, for which comorbidity burden played a more important role. The prognostic capacity of Hy's law varied between causative agents. Empirical therapy (corticosteroids, ursodeoxycholic acid and MARS) was prescribed to 20% of patients. Drug-induced autoimmune hepatitis patients (26 cases) were mainly females (62%) with hepatocellular damage (92%), who more frequently received immunosuppressive therapy (58%).

Conclusions: AST elevation at onset is a strong predictor of poor outcome and should be routinely assessed in DILI evaluation. Mortality is higher in older patients with hepatocellular damage and patients with underlying hepatic conditions. The Spanish DILI Registry is a valuable tool in the identification of causative drugs, clinical signatures and prognostic risk factors in DILI and can aid physicians in DILI characterisation and management.

Lay summary: Clinical information on drug-induced liver injury (DILI) collected from enrolled patients in the Spanish DILI Registry can guide physicians in the decision-making process. We have found that older patients with hepatocellular type liver injury and patients with additional liver conditions are at a higher risk of mortality. The type of liver injury, patient sex and analytical values of aspartate aminotransferase and total bilirubin can also help predict clinical outcomes.

Keywords: Hepatotoxicity; DILI; epidemiology; liver-related death; causative agents; outcome; risk factors; therapy in DILI; drug-induced autoimmune hepatitis.
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Introduction

Drug-induced liver injury (DILI), particularly unpredictable idiosyncratic DILI, can have a significant impact, which is not limited to patient safety but also affects healthcare costs, drug development and the range of marketed drug treatments. Hence, there has been a growing interest in enhancing the understanding of DILI both from a clinical, epidemiological and molecular point of view over the last decades.

Improved medication safety analyses and toxicological studies have led to a notable reduction in hepatotoxicity-related post-marketing drug withdrawals issued by the US Food and Drug Administration since 1997, reflecting a greater emphasis on hepatotoxicity in drug development.[1] The ability to predict who is at risk of developing idiosyncratic DILI is in line with the concept of personalized medicine and would enhance drug safety by enabling patients without DILI risk to securely benefit from an effective treatment (including treatments with black box warnings), while providing alternative medications to those at increased risk of DILI. A better understanding of DILI and its implication for the individual patient is required to reach this goal.[2] Considerable progress has been made in understanding the pathophysiological mechanisms of idiosyncratic hepatotoxicity,[3] however, translation to DILI prediction and treatment is still a work in progress.[4]

Because of the lack of reliable animal models that can reproduce the complexity of idiosyncratic DILI, prospective collection of phenotypic information and biological samples from identified DILI cases is still the most valuable resource for DILI research. Idiosyncratic DILI is relatively rare, with an estimated yearly incidence rate of approximately 14-19 cases per 100,000 (based on prospective population-based studies in Europe), or 23.8 per 100,000 (based on a more recent retrospective Chinese study).[5–7] The low incidence rate makes it difficult for individual hospital units/research groups to obtain sufficient numbers of cases to perform studies with high statistical power. To circumvent this issue a number of prospective DILI registries have emerged. The establishment of national and international DILI registries over the last 30 years has provided a significant step forward in understanding DILI.[8–12] The Spanish DILI Registry, founded in 1994, was a pioneer initiative to strengthen DILI epidemiological figures, phenotypic characterisations, risk factor identification, and prognosis. Our group has also contributed to facilitating standardised diagnostic and causality assessment procedures over more than 20 years.[13–19]

An analysis of 461 DILI cases enrolled into the Spanish DILI Registry over the first 10-year period was published in 2005,[8] and many of the findings have since been replicated in other large DILI cohorts.[6,7,9–12] With a near doubling of enrolled cases in the Spanish DILI Registry since 2005 it is timely to undertake a new analysis. In the present study, we aimed to provide an updated description of clinical features, outcomes of special populations, management and main therapeutic groups featured in 843 prospectively recruited individuals in the Spanish DILI Registry.

Material and methods

Design

The Spanish DILI Registry, established in 1994, is a prospective multicentre study focusing on prospectively identifying *bona fide* DILI cases, mainly idiosyncratic DILI cases. The operational structure and procedures of the registry, data collection and case enrolment have been published elsewhere.[8] Clinical data corresponding to each patient with DILI is collected using a standardised protocol to ensure that information necessary to adjudicate DILI is collected: (1) detailed medication history including herbal and dietary supplements (HDS) and over-the-counter medications; (2) biochemistry, detailed viral serology work-up (including viral hepatitis E on a routine basis since 2016), imaging and, if available, histological data to exclude alternative causes of liver injury; (3) outcome. The biochemical criteria for DILI used in this registry were initially those established by the CIOMS and later adapted to those of Aithal *et al.* (alanine aminotransferase [ALT] ≥5 x upper limit of normal [ULN], alkaline phosphatase [ALP] ≥2 xULN or ALT ≥3 xULN together with total bilirubin [TBL] >2 xULN).[20,21] In the study cohort, 86% fulfilled the more stringent criteria of Aithal *et al.* at detection.

Cases induced by acetaminophen and occupational exposure to toxins were excluded from the study cohort. In addition, cases of drug-induced autoimmune hepatitis (DIAIH) were analysed as a distinct cohort. Diagnosis of DIAIH was based on the following: a temporal relationship between drug intake and the appearance of an autoimmune hepatitis (AIH) phenotype; no prior evidence of AIH; and cases fulfilled the simplified AIH criteria.[22] For patients with multiple episodes (re-exposure to the causative agent or a second episode induced by a different drug, recurrent DILI) data pertaining to one of the episodes only were included in the current study to avoid duplication of demographic data. All patients underwent follow-up until liver profile normalisation, when possible. Therapy for DILI, if any, was decided by the physician in charge, recorded and analysed.

The pattern of liver injury (hepatocellular, cholestatic and mixed) was determined by calculating the ratio (R) of ALT to ALP from the first available blood analysis after DILI recognition, using multiples of the ULN for both values.[21] Severity was assessed using the severity index defined by Aithal *et al.*[21] Death and need for liver transplantation were assessed within a maximum of 6 months from DILI onset.

Eosinophilia was defined as serum eosinophils exceeding 4-6% of total leukocyte count depending on the normal range of individual hospitals, and lymphopenia as serum lymphocytes <10%. Patients with hypersensitivity features were those who presented with at least one of the following features: rash, fever, eosinophilia or lymphopenia. The Charlson comorbidity index (CCI) was calculated as the total of a patient's weighted comorbid conditions according to Charlson *et al.*[23] Heavy alcohol consumption was defined as ≥60 g (men) or ≥40 g (women) of alcohol per day.

The study protocol was approved by the local Ethics Committee at the Virgen de la Victoria University Hospital in Málaga, Spain, and all subjects gave informed consent.

Statistical analysis

Variables were examined using descriptive statistics. Continuous variables were presented as mean ± SD or median (IQR), as appropriate. Qualitative variables were described using frequency distributions. Inferential statistics were used to compare groups. Differences in continuous variables between groups were assessed using Mann-Whitney *U* test or Kruskal-Wallis test (*post hoc*: Dunn's test) or ANOVA, as appropriate. Qualitative variables were compared using Pearson χ^2 or Fisher's exact test.

Table 1. Comparison of demographics, clinical characteristics, laboratory parameters and outcome between different patterns of liver injury in 843 Spanish DILI cases.

	Total registry n = 843	Hep n = 482 (57%)	Chol n = 173 (21%)	Mix n = 188 (22%)	*p* value*
Age (yr), mean ±SD (range)	54±18 (11-91)	51±18 (11-88)	61±17 (16-90)	55±18 (14-91)	<0.0001
Female, %	48	50	43	46	0.2140
BMI (kg/m^2), mean ±SD	26 ±3.8	26 ±3.8	26 ±3.9	26 ±3.7	0.6294
Diabetes mellitus, %	12	11	17	11	0.1192
Hypertension, %	20	21	37	31	0.0007
Dyslipidaemia, %	14	11	17	16	0.0748
Underlying hepatic disease, %	6.3	5.6	6.4	7.9	0.5223
History of drug allergy, %	15	19	7.3	13	0.009
DILI episode characteristics					
Jaundice, %	69	66	77	70	0.0327
Rash, %	7.9	7.0	8.8	8.8	0.5411
Hospitalisation, %	60	57	69	59	0.0317
Total oral daily dose (mg), mean ±SD	925 ±1,056	786 ±995	1,222 ±1,257	1,068 ±1,127	0.0018
Duration of therapy (d), mean/median (IQR)	63/27 (8-64)	71/32 (9-82)	43/16 (9-41)	61/16 (8-56)	<0.0001
Time to onset (d), mean/median (IQR)	58/25 (10-62)	66/30 (12-73)	40/22 (9-39)	56/20 (8-45)	<0.0001
Concomitant drugs, %					
None	26	28	20	28	0.0667
1-2 drugs	40	41	40	36	
3-4 drugs	21	20	22	22	
≥5 drugs	13	10	18	13	
Laboratory parameters at onset x ULN, mean ±SD					
TBL	7.0 ±6.9	6.4 ±6.8	8.9 ±7.3	6.8 ±6.4	<0.0001
AST	15 ±21	23 ±25	3.3 ±3.0	6.1 ±7.5	<0.0001
ALT	19 ±22	28 ±25	3.9 ±3.8	7.7 ±6.3	<0.0001
ALP	2.2 ±2.1	1.3 ±0.9	4.2 ±3.2	2.4 ±1.9	<0.0001
INR	1.3 ±0.7	1.4 ±0.7	1.2 ±0.6	1.1 ±0.4	0.0001
Glucose (mg/dl)	113 ±52	110 ±52	124 ±66	110 ±31	0.0025
eGFR (ml/min/1.73 m^2)	96 ±65	100 ±76	94 ±63	90 ±31	0.1949
Haemoglobin (g/dl)	14 ±1.8	14 ±1.7	13 ±1.8	14 ±1.7	<0.0001
Platelets x10^3/μl	233 ±90	223 ±89	253 ±103	240 ±73	0.0003
Lymphopenia, %	24	19	32	26	0.0047
Peripheral eosinophilia, %	23	20	26	27	0.0800
Positive autoantibody titres, %	20	25	17	12	0.0021
Severity, %					
Mild	31	36	20	28	<0.001
Moderate	59	51	73	66	
Severe	6.2	7.3	5.8	3.7	
Fatal/transplantation	3.7	5.4	1.2	1.6	
Outcome					
Time to resolution (d), median (IQR)	108 (56-218)	103 (50-192)	132 (68-272)	107 (59-199)	0.2275
Liver-related death, n (%)	18 (2.1)	14 (2.9)	1 (0.6)	3 (1.6)	0.1765
Liver transplantation, n (%)	13 (1.5)	12 (2.5)	1 (0.6)	0	0.0309
Death due to other causes§, n (%)	14 (1.7)	5 (1.0)	8 (4.6)	1 (0.5)	0.0058

Statistical tests: Pearson chi-squared test or Fisher's exact test, as appropriate, for qualitative variables; Kruskal-Wallis test or ANOVA, as appropriate, for quantitative variables. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Chol, Cholestatic; DILI, drug-induced liver injury; eGFR, estimation glomerular filtration rate according to the Modification of Diet in Renal Disease study; Hep, hepatocellular; INR, international normalised ratio; Mix, mixed; TBL, total bilirubin; ULN, upper limit of normal.
*comparison between Hep, Chol and Mix groups.
§During time of follow-up.

Multivariable logistic regression models were fitted to study associations of clinical and demographic characteristics on DILI phenotypic expression. All statistical tests were 2-sided hypotheses performed at the 0.05 level of significance using STATA v 13.0 (College Station, TX: StataCorp LP).

Results

Clinical and demographic characteristics

A total of 843 DILI cases enrolled in the Spanish DILI Registry between 1994 and 2018 fulfilled the inclusion criteria for the current study. Using the CIOMS/RUCAM scale, 33% of the cases were classified as highly probable, 52% as probable and 15% as possible. The mean age of the patients with DILI was 54 years with a similar distribution between males and females (Table 1). 791 patients (94%, mean age 54 years) had a CCI ≤2 (none to mild comorbidity), while 52 patients (6%, mean age 64 years) had significant comorbidity with a CCI >2. The most frequent conditions contributing to CCI were diabetes (12%), chronic pulmonary disease (6.4%) and congestive heart failure (5.8%). The majority of DILI cases (86%) were judged to have a single causative agent, while 2 culprit drugs were attributed to 14% of the cases. The causative agents were mainly taken orally (94%; mean daily dose 925 mg over a median duration of 27 days). Of the cases caused by oral conventional drugs, 25% involved a daily dose of <100 mg, 18% <50 mg and 6.4% ≤10 mg (Table S1). Drugs were given parenterally in 48 cases (60% intravenously, 23% intramuscularly, 10% cutaneously/subcutaneously, 4.2% inhaled and 2.1% sublingually). The most common parenterally given causative agents were antibacterials 25%, immunomodulating agents 17% and antineoplastics 15%. The median latency (time

from drug initiation to detection of symptoms/elevated liver profile) was 25 days and the median time to resolution 108 days. Of the 843 patients with DILI, 23% had peripheral eosinophilia, 69% presented with jaundice and 60% required hospitalisation. Thirty-two cases with eosinophilia and/or lymphopenia also presented with rash and were consequently diagnosed as DRESS (drug reaction with eosinophilia and systemic symptoms) cases and 3 as Stevens-Johnson syndrome. Eighty-four patients (15%) reported a history of drug allergy. Biopsy was performed in 141 (17%) patients. The most common features included cholestatic hepatitis (37%), hepatocellular necrosis (7.8%) and cholestasis (7.1%). In total, 18 patients (2.1%) died from liver-related causes and 13 (1.5%) underwent liver transplantation. These 31 patients had significantly higher comorbidity with 12.9% having a CCI >2 compared to 5.9% among those with a favourable outcome (p = 0.016).

The distribution of liver injury pattern according to age and sex is depicted in Fig. 1. The peak DILI age was 60-69 years for both males and females. Hepatocellular injury was the predominant phenotype (57%) in all age groups except for patients ≥80 years in whom cholestatic injury predominated. Patients did not differ in age, sex, BMI, type or severity of liver injury between those enrolled in the first 10 years and thereafter, but prevalence of hypertension, dyslipidaemia and the use of immunosuppressants, immunostimulants and antineoplastic agents was significantly higher in the later period (data not shown).

Comparison of clinical and demographic characteristics between different patterns of liver injury

Patients with a cholestatic pattern of injury were older (mean age of 61 years compared to 51 and 55 years for patients with hepatocellular and mixed pattern, respectively [p <0.0001]) (Table 1). Similar sex distributions were seen across the groups. However, men had a significantly higher risk of developing amoxicillin-clavulanate-induced cholestatic type liver injury (adjusted odds ratio [aOR] 2.249; 95% CI 1.342-3.769; p = 0.002). Comorbid conditions such as diabetes mellitus, hypertension and dyslipidaemia, and correspondingly polypharmacy (≥5 drugs) were more frequently seen in cholestatic patients. Platelet count differed significantly between the 3 groups (p = 0.0003). Hepatocellular injury was independently associated with younger age

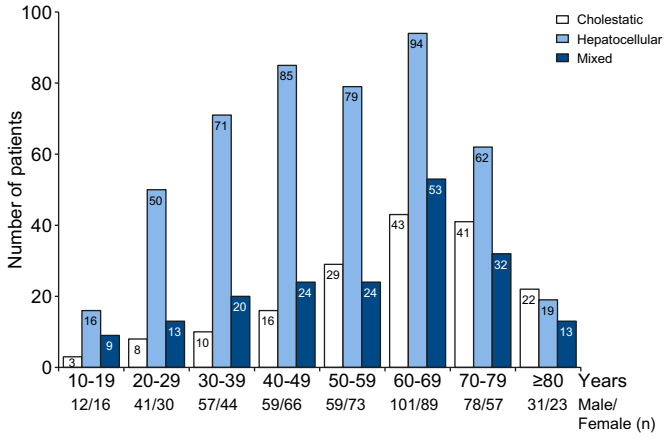

Fig. 1. Distribution of 836 DILI patients in the Spanish DILI Registry according to pattern of liver injury and age.

(aOR 0.983 per year; 95% CI 0.974-0.991; p <0.001) and lower platelet count at DILI recognition (aOR 0.996 per unit; 95% CI 0.994-0.998; p <0.001). The hepatocellular group had lower oral daily dose (p = 0.0018), but longer time to onset (p <0.0001) than the other groups. Hospitalisation was more common in cases with a cholestatic pattern of injury (p = 0.0317) Nevertheless, fatal/transplantation outcomes were more frequent among hepatocellular cases (p = 0.0082). Survival curves for the 3 different types of liver injury likewise demonstrated a significant difference (p = 0.0118), with hepatocellular cases being 4 times more likely to develop the worst outcome within 90 days than cholestatic cases (Fig. S1).

Influence of age and injury pattern on the clinical presentation and outcome of DILI

A comparison between age groups (young: ≤45 years old; middle-aged: 46-64 years old; and old: ≥65 years old) according to injury pattern (hepatocellular and cholestatic/mixed) was performed (Table 2). Sex distribution was equal across all groups. As expected, increased age was associated with higher BMI (p <0.0004), diabetes mellitus (p <0.0001), hypertension (p <0.0001), dyslipidaemia (p <0.0001) and polypharmacy (p <0.0001) in both liver injury groups. The frequency of jaundice was highest in the oldest age category in both the hepatocellular (73% *vs.* 60% and 68%) and cholestatic/mixed group (83 % *vs.* 65% and 67%). Younger age was linked to shorter duration of therapy in hepatocellular cases, ranging from a median of 25 days in the youngest patients to 40 and 43 days in the older age groups (p = 0.0080), while the opposite was found for the cholestatic/mixed cases with a median of 31 days in the youngest patients *vs.* 16 and 15 days in the older age groups (p = 0.0251). Hepatocellular patients aged ≥65 years had the highest proportion of liver-related fatalities (7.2%) compared with the younger age groups (1.2% and 1.6%; p = 0.0083).

Hepatocellular injury based on the definition of the new Ratio (nR) >5 (nR = AST or ALT in ULN (whichever highest)/ALP in ULN)[18] (aOR 4.914; 95% CI 1.316-18.350; p = 0.018), AST elevation (aOR 1.015; 95% CI 1.002-1.028; p = 0.024), female sex (OR 2.744; 95% CI 1.180-6.380; p = 0.019) and TBL value at recognition (aOR 1.102; 95% CI 1.054-1.152, p <0.001) were independent predictors of liver-related mortality and liver transplantation.

Interestingly, an nR-based hepatocellular injury pattern was not associated with 6-month overall mortality, while patients who died within 6 months had higher comorbidity burden compared to those who survived (mean CCI 2.25 *vs.* 0.6, p <0.001). This finding highlights the greater relevance of comorbidity burden over liver injury pattern when focusing on overall mortality. Regarding the influence of alcohol consumption, there was no difference between the proportion of heavy and no/light drinkers with regards to liver-related death/liver transplantation (5.7% *vs.* 3.8%, p = 0.641) or 6-month overall mortality (8.6% *vs.* 2.9%, p = 0.099).

Comparison of clinical and demographic characteristics between DILI patients with and without pre-existing liver conditions

As shown in Table 3, 53 patients suffered from underlying hepatic conditions before the DILI episode. These conditions included chronic viral hepatitis (55%), alcohol-related liver disease (23%), fatty liver disease (11%), idiopathic AIH (5.7%), alpha-1 antitrypsin deficiency (1.9%), iron metabolism disorder (1.9%), primary biliary cholangitis (1.9%), primary sclerosing cholangitis

Table 2. Comparison of demographics, clinical characteristics, laboratory parameters and outcome between different age groups in hepatocellular and cholestatic/mixed DILI.

	Hepatocellular				Cholestatic/Mixed			
	≤45 yr n = 182	46-64 yr n = 169	≥65 yr n = 125	*p* value	≤45 yr n = 84	46-64 yr n = 124	≥65 yr n = 152	*p* value
Female, %	50	52	46	0.6188	38	52	41	0.0774
BMI (kg/m^2); mean ±SD	25 ±4.0	26 ±3.2	27 ±3.8	<0.0001	25 ±4.0	26 ±3.4	27 ±3.7	0.0004
Diabetes mellitus, %	2.8	14	18	<0.0001	3.6	9.7	23	<0.0001
Hypertension, %	5.2	23	38	<0.0001	3.5	33	50	<0.0001
Dyslipidaemia, %	2.2	15	19	<0.0001	3.6	22	20	0.0010
Underlying hepatic disease, %	6.0	5.3	5.6	0.9578	9.5	10	3.3	0.0464
History of drug allergy, %	17	19	21	0.732	3.9	14	11	0.154
DILI episode characteristics, %								
Jaundice	60	68	73	0.0568	67	65	83	0.0010
Rash	7.2	8.3	5.2	0.6086	5.6	14	7.0	0.0695
Hospitalisation	53	53	67	0.0334	64	52	73	0.0019
Total oral daily dose (mg), mean ±SD	832 ±975	724 ±897	730 ±940	0.6383	816 ±925	1,122 ±1,127	1,307 ±1,287	0.0126
Duration of therapy (d), mean/median (IQR)	53/25 (8-62)	89/40 (10-95)	72/43 (8-93)	0.0080	74/31 (9-76)	56/16 (8-47)	38/15 (8-33)	0.0251
Time to onset (d), mean/median (IQR)	52/25 (11-61)	79/34 (11-81)	70/33 (13-76)	0.1998	69/26 (9-62)	48/19 (8-38)	37/21 (8-37)	0.1734
Concomitant dugs, %								
None	37	31	12	<0.0001	37	27	15	<0.0001
1-2 drugs	41	41	42		44	37	36	
3-4 drugs	18	19	25		18	23	23	
≥5 drugs	4.4	8.9	22		1.2	13	26	
Laboratory parameters at onset x ULN, mean ±SD								
TBL	5.8 ±7.3	5.7 ±5.6	7.9 ±7.3	0.0057	7.4 ±7.9	7.1 ±7.1	8.5 ±6.1	0.0074
AST	25 ±28	23 ±22	22 ±23	0.9452	4.4 ±6.2	4.6 ±5.2	5.1 ±6.4	0.0826
ALT	30 ±27	30 ±26	24 ±20	0.3159	5.1 ±3.5	5.8 ±5.5	6.4 ±6.5	0.2428
ALP	1.2 ±0.7	1.4 ±1.0	1.4 ±0.9	0.0153	2.4 ±2.1	3.3 ±2.9	3.7 ±2.9	<0.0001
INR	1.4 ±0.7	1.3 ±0.7	1.5 ±0.8	0.1664	1.2 ±0.5	1.1 ±0.4	1.1 ±0.6	0.4104
Glucose (mg/dl)	98 ±39	116 ±57	119 ±55	<0.0001	96 ±18	119 ±51	126 ±60	<0.0001
eGFR (ml/min/1.73 m^2)	117 ±61	92 ±35	86 ±118	<0.0001	105 ±31	96 ±55	83 ±50	<0.0001
Hemoglobin (g/dl)	14 ±1.7	14 ±1.6	13 ±1.8	0.0098	14 ±1.7	13 ±1.8	13 ±1.9	0.0030
Platelets x10^3/μl	243 ±92	218 ±98	202 ±68	0.0005	268 ±109	244 ±87	235 ±76	0.1322
Lymphopenia, %	15	15	32	0.0013	30	28	29	0.9383
Peripheral eosinophilia, (%)	19	23	17	0.4132	24	25	29	0.7090
Positive autoantibody titres, %	17	29	31	0.0156	4.2	21	15	0.0071
Severity, %								
Mild	45	34	25	0.0004	27	33	15	0.0073
Moderate	41	59	58		64	61	80	
Severe	9.3	4.7	8.0		4.8	4.0	5.3	
Fatal/transplantation	5.4	3.0	8.8		3.6	1.6	0	
Outcome								
Time to resolution (d); median (IQR)	96 (48-172)	97 (52-182)	117 (61-327)	0.1881	124 (72-218)	106 (60-198)	122 (62-340)	0.4365
Liver-related death, n (%)	3 (1.6)	2 (1.2)	9 (7.2)	0.0083	3 (3.6)	1 (0.8)	0	0.0336
Liver transplantation, n (%)	7 (3.8)	3 (1.8)	2 (1.6)	0.5866	0	1 (0.8)	0	0.5778
Death due to other causes[§], n (%)	1 (0.5)	1 (0.6)	3 (2.4)	0.3143	1 (1.2)	3 (2.4)	5 (3.3)	0.6345

Statistical tests: Pearson chi-squared test or Fisher's exact test, as appropriate, for qualitative variables; Kruskal-Wallis test or ANOVA, as appropriate, for quantitative variables. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DILI, drug-induced liver injury; eGFR, estimation glomerular filtration rate according to the Modification of Diet in Renal Disease study; INR, international normalised ratio; TBL, total bilirubin; ULN, upper limit of normal.

[§]During time of follow-up.

Table 3. Comparison of demographics and clinical characteristics between subjects with and without underlying hepatic conditions.

	With underlying hepatic condition* n = 53	Without underlying hepatic condition n = 790	*p* value
Age (yr), mean ±SD (range)	52 ±15 (26-83)	54 ±18 (11-91)	0.1706
Female, %	30	49	0.0087
BMI (kg/m^2), mean ±SD	26 ±4.7	26 ±3.7	0.7206
Diabetes mellitus, %	7.6	13	0.3864
Hypertension, %	16	28	0.1079
Dyslipidaemia, %	11	14	0.6282
Pattern of DILI (Hep/Chol/Mix, %)	51/21/28	58/21/22	0.5223
DILI episode characteristics			
Jaundice, %	65	70	0.4709
Rash, %	4.3	8.1	0.3401
Hospitalisation, %	49	60	0.1270
Laboratory parameters at onset x ULN, mean ±SD			
TBL	6.9 ±6.8	7.0 ±6.9	0.9301
AST	15 ±27	15 ±21	0.5710
ALT	14 ±13	19 ±23	0.2689
ALP	2.0 ±1.8	2.2 ±2.2	0.4819
INR	1.6 ±0.9	1.3 ±0.6	0.0341
Platelets x 10^3/μl	209 ±100	235 ±90	0.0413
Albumin, mg/dl	3.7 ±1.0	3.98 ±1.7	0.2930
Outcome			
Liver-related death due to DILI	4 (7.5)	14 (1.8)	0.0221
Liver transplantation	0	13 (1.6)	0.6216

Statistical tests: Pearson chi-squared test or Fisher's exact test, as appropriate; for qualitative variables; Mann–Whitney U test for quantitative variables.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Chol, Cholestatic; DILI, drug-induced liver injury; Hep, hepatocellular; INR, international normalised ratio; TBL, total bilirubin; ULN, upper limit of normal.
*Underlying hepatic diseases include alcoholic liver disease, alpha-1 antitrypsin deficiency, chronic viral hepatitis, fatty liver disease, idiopathic autoimmune hepatitis, iron metabolism disorder, primary biliary cholangitis, primary sclerosing cholangitis and prior liver transplantation.

Table 4. Comparison of demographics, clinical characteristics and outcome between treated (corticosteroids, UDCA and MARS) and non-treated DILI patients.

	Corticosteroids (n = 66)	UDCA (n = 50)	MARS (n = 12)	No treatment (n = 497)	*p* value
Age (yr), mean ±SD (range)	53 ±20 (16-88)	55 ±18 (17-91)	41 ±18 (20-73)	54 ±18 (11-90)	0.170
Female, %	55	46	33	48	0.628
Diabetes mellitus, %	12	10	8.3	11	0.983
Hypertension, %	17	26	17	20	0.844
Dyslipidaemia, %	9.1	10	17	16	0.669
Underlying hepatic disease, %	7.6	4.0	0	6.2	0.855
DILI episode characteristics					
Type of liver injury, % (Hep/Chol/Mix)	55/26/20	48/22/30	67/17/17	59/18/23	0.521
Jaundice, %	89	88	100	65[a,b,c]	<0.001
Hospitalisation, %	91	67[a,c]	100	46[a,b,c]	<0.001
Hypersensitivity features, %	48[c]	51[c]	83	40[c]	0.010
Rash, %	12	16	17	5.7[b]	0.021
Lymphopenia, %	26	12	33	18	0.111
Eosinophilia, %	29	26	17	22	0.602
Peak laboratory parameters xULN, mean ±SD					
TBL	14.7 ±11.4	16.6 ±12.0	29.8 ±16.9[a]	7.8 ±8.7[a,b,c]	<0.001
AST	21.2 ±23.0	20.1 ±31.0	10.6 ±14.6	17.8 ±24.7	0.317
ALT	24.4 ±30	21.3 ±25.0	12.4 ±9.2	20.9 ±24.4	0.824
ALP	2.8 ±3.0	3.3 ±3.4	2.8 ±2.3	2.2 ±2.1[a,b]	0.001
INR	1.4 ±0.7	1.4 ±0.8	1.3 ±0.7	1.3 ±0.6	0.312
Severity, %					<0.001
Mild	12	6.0	0	35[a,b,c]	
Moderate	62	76	67	57[b]	
Severe	17	10	17	5.6[a]	
Fatal/transplantation	9.1	8.0	17	2.0[a,b,c]	
Outcome					
Time to resolution (d), median (IQR)	112 (79-183)	142 (79-288)	147 (92-741)	96 (49-178)	0.193
Liver-related death, n (%)	4 (6.1)	3 (6.0)	1 (8.3)	9 (1.8)[a]	0.011
Liver transplantation, n (%)	2 (3.0)	1 (2.0)	1 (8.3)	1 (0.2)[a,b,c]	0.011
Chronicity[†], n (%)	3 (4.6)	3 (6.0)	1 (8.3)	38 (7.7)	0.751

Statistical tests: Pearson chi-squared test or Fisher's exact test, as appropriate, for qualitative variables; Kruskal-Wallis test and Dunn's test (post hoc) for quantitative variables.
ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; DILI, drug-induced liver injury; INR, international normalised ratio; MARS, molecular adsorbents recirculation system; TBL, total bilirubin; UDCA, ursodeoxycholic acid.
a: p <0.05 *vs.* corticosteroids; b: p <0.05 *vs.* ursodeoxycholic acid; *c*: p <0.05 *vs.* MARS.
[†]Defined as cases who failed to resolve (normalisation of liver biochemistry, imaging test or histology) within 365 days.

Table 5. Comparison of demographics, clinical characteristics, laboratory parameters and outcome between DILI-autoimmune hepatitis (DIAIH) and DILI cases.

	DIAIH n = 26	DILI n = 843	*p* value
Age (yr), mean ±SD (range)	57±17 (15-86)	54±18 (11-91)	0.5504
Female, %	62	48	0.162
BMI (kg/m^2), mean ±SD	25 ±5.0	26 ±3.8	0.1592
Diabetes mellitus, %	15	12	0.550
Hypertension, %	28	20	0.318
DILI episode characteristics			
Type of liver injury, %			0.002
Hepatocellular	92	57	
Cholestatic	4.0	21	
Mixed	4.0	22	
Jaundice, %	69	69	0.953
Rash, %	4.5	7.9	1.000
Hospitalisation, %	39	54	0.205
Duration of therapy* (d), median (IQR)	65 (27-274)	27 (8-64)	0.0044
Laboratory parameters at onset xULN, mean ±SD			
TBL	5.7 ±5.5	7.0 ±6.9	0.6656
AST	24 ±17	15 ±21	0.0001
ALT	28 ±19	19 ±22	0.0002
ALP	2.2 ±2.8	2.2 ±2.1	0.8643
Autoantibodies			
ANA, %	88	12	<0.001
ASMA, %	44	8.9	<0.001
AMA, %	4.0	1.9	0.397
Anti-LKM-1, %	0	1.1	1.000
IgG (g/L), mean (SD)	19.5 ±10.7	11.9 ±4.6	<0.001
Treatment, %			<0.001
Corticosteroids/azathioprine	58	9.9	
Ursodeoxycholic acid	3.8	7.5	
MARS	0	1.8	
Severity, %			0.784
Mild	35	31	
Moderate	54	59	
Severe	7.7	6.2	
Fatal/transplantation	0/1 (3.8)	18/13 (3.7)	

Statistical tests: Pearson chi-squared test or Fisher's exact test, as appropriate, for qualitative variables; Mann–Whitney U test for quantitative variables.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANA, anti-nuclear antibodies; anti-LKM-1, anti-liver kidney microsome type 1 antibodies; ASMA, anti-smooth muscle antibodies; AMA, anti-mitochondrial antibodies; AST, aspartate aminotransferase; DILI, drug-induced liver injury; MARS, molecular adsorbents recirculation system; TBL, total bilirubin; ULN, upper limit of normal.
*Duration of causative agent before DILI detection.

(1.9%) and prior liver transplantation (1.9%). Fourteen patients (26%) had cirrhosis. The most frequently implicated drugs in patients with DILI and underlying hepatic conditions were anti-TB medications (11, 21%), amoxicillin-clavulanate (4, 7.5%) followed by atorvastatin, fluvastatin, norfloxacin, flutamide, azathioprine and ibuprofen (2, 3.8% each).

The patients with pre-existing liver conditions were predominantly males (70%), with a similar age to those without pre-existing conditions. No significant differences were found in the clinical presentation between the 2 groups, except for international normalised ratio (INR), which was higher in patients with underlying hepatic diseases (1.6 *vs.* 1.3, $p = 0.0341$). Furthermore, liver-related death due to DILI was significantly more common in patients with underlying hepatic diseases (4 [7.5%] *vs.* 14 [1.8%], $p = 0.0221$).

Analysis of DILI cases according to therapy received for the episode

Recorded information on therapy received for the DILI episode was available for 625 cases. In total 128 patients received specific therapy to ameliorate DILI at discretion of the physician in charge, which included corticosteroids (52%), ursodeoxycholic acid (UDCA, 39%), or molecular adsorbents recirculation system (MARS, 9.4%) (Table 4). These patients did not differ from those without DILI treatment with regards to demographics and underlying conditions. Hepatocellular injury predominated in all groups, with MARS-treated patients presenting the highest proportion (67%) and UDCA-treated patients the lowest (48%). Jaundice and hospitalisation were both significantly more frequent in the treatment groups (p <0.001). This was also reflected in higher values of TBL and a higher rate of death/liver transplantation in patients treated with corticosteroids (9.1%), UDCA (8.0%) and MARS (17%), compared to non-treated patients (2.0%). Although a large proportion of patients receiving MARS treatment presented with moderate severity injury, this group had the highest values of TBL and anabolic androgenic steroids (AAS) were the culprit agents in a large fraction of cases (58%). Amoxicillin-clavulanate (20%) and anti-TB treatments (11%) predominated as culprit agents among the corticosteroid-treated patients. Similarly, amoxicillin-clavulanate was highly represented among the UDCA-treated patients (34%).

Comparison of clinical and demographic characteristics between DILI and DIAIH patients

The 843 DILI cases were also assessed against an independent cohort of 26 DIAIH cases. These cases were mainly induced by

Table 6. Number of DILI patients stratified by age attributed to specific causative agents.

ATC	Main pharmacological groups, n (%)	Total registry n = 843	≤45 yr n = 266	46-64 yr n = 293	≥65 yr n = 277
A	Alimentary tract and metabolic agents excluding anabolic agents	46 (5.5)	16 (6)	19 (6.5)	11 (4)
	Drugs for peptic ulcer drugs	28	9	12	7
B	Antithrombotic agents	18 (2.1)	2 (0.7)	5 (1.7)	11 (4)
C	Cardiovascular agents	89 (11)	8 (3)	40 (14)	39 (14)
	ACE inhibitors+angiotensin II antagonists	20	1	8	10
	Statins	44	3	20	20
	Fibrates	7	1	5	1
D	Dermatologicals	6 (0.7)	3 (1.1)	2 (0.7)	1 (0.4)
G	Genito-urinary system and sex hormones	20 (2.4)	10 (3.8)	8 (2.7)	2 (0.7)
H	Thyroid therapy	10 (1.2)	3 (1.1)	6 (2)	1 (0.4)
J	Anti-infectives	337 (40)	95 (36)	112 (38)	127 (46)
	Antibacterials for systemic use	266	64	90	110
	Amoxicillin-clavulanate	193	42	66	84
	Penicillins/cephalosporins excluding amoxicillin-clavulanate	16	8	2	5
	Macrolides	18	6	6	6
	Fluoroquinolones	31	5	11	15
	Antimycobacterials	64	26	20	17
L	Antineoplastic and immunomodulating agents	66 (7.8)	18 (6.8)	22 (7.5)	26 (9.4)
	Antineoplastic agents	17	5	8	4
	Endocrine therapy	26	4	4	18
	Immunosuppressants	17	4	9	4
M	Musculoskeletal system	90 (11)	31 (12)	27 (9.2)	31 (11)
	Nonsteroidal anti-inflammatory drugs	78	26	25	26
N	Central nervous system	99 (12)	43 (16)	31 (11)	24 (8.7)
	Antiepileptics	25	15	6	4
	Antipsychotics	12	6	1	5
	Antidepressants	28	12	10	5
–	Herbal products and dietary supplements	29 (3.4)	10 (3.8)	15 (5.1)	4 (1.4)
–	Anabolic androgenic steroids	22 (2.6)	21 (7.9)	1 (0.3)	0

ACE, angiotensin converting enzyme; DILI, drug-induced liver injury.

statins (31%) and antibacterials (23%). A comparison between the 2 groups revealed a predominance of females (62% *vs.* 48%) and hepatocellular injury (92% *vs.* 57%) in DIAIH. This was reflected in significantly higher ALT (28 xULN *vs.* 19 xULN, $p = 0.0002$) and AST (24 xULN *vs.* 15 xULN, $p = 0.0001$) in the same group. Duration of therapy was found to be significantly longer in the DIAIH cases (65 *vs.* 27 days, $p = 0.0044$). However, no significant differences were detected with regards to severity and outcome (Table 5).

Therapeutic classes and individual drugs most commonly implicated in the Spanish DILI Registry

A total of 221 different causative drug treatments were implicated in the 843 DILI cases in this study, with 791 cases caused by conventional medications, 51 by HDS (including AAS products for body building purposes), and 1 by a compound under investigation. Anti-infectives (337 cases) were the most common therapeutic class followed by central nervous system drugs (99 cases), musculoskeletal drugs (90 cases, of which 78 were caused by non-steroidal anti-inflammatory drugs) and cardiovascular agents (89 cases) (Table 6). A comparison of clinical characteristics between individual causative agents associated with at least 8 DILI cases in the overall Spanish DILI Registry is presented in Table 7. Drugs with a predominant hepatocellular phenotype were isoniazid (95%, mainly as TB prophylaxis) and diclofenac (93%). While no drugs had a strong predominance for a cholestatic or mixed phenotype, azathioprine (64%) and fluvastatin (64%) were the drugs with the highest proportion of cholestatic and mixed cases, respectively. Among the most frequent causative drugs, fatal cases were reported for amoxicillin-clavulanate, anti-TB, ibuprofen, flutamide, levofloxacin, nimesulide and carbamazepine (Table 7).

Cases caused by AAS, nimesulide and flutamide demonstrated the highest mean TBL values at recognition, while paroxetine and atorvastatin (hepatocellular) had highest mean ALT values. In addition, atorvastatin (cholestatic) and amoxicillin-clavulanate (cholestatic) led to the highest mean ALP values (Table S2).

The most frequent causative agents associated with at least 8 DILI cases were examined with regards to Hy's law and its prognostic value (Table 7). Of the 38 anti-TB cases, 16 (43%) met nR-based Hy's law criteria and 4 of these cases (25%) led to death/liver transplantation. Similarly, 12 of the 22 cases attributed to flutamide (57%) met the same criteria and 4 patients (33%) died or required a liver transplantation. In contrast, cases attributed to isoniazid, atorvastatin, diclofenac and AAS that fulfilled nR-based Hy's law criteria (57%, 44%, 50% and 47%, respectively) had favourable outcomes without fatalities/liver transplantation.

Discussion

In this study we have characterised 843 patients with DILI enrolled into the Spanish DILI Registry from the initiation in 1994 to 2018, with a particular focus on the influence of age and presence of underlying liver conditions on phenotype and outcome of the DILI episode. Age and sex distributions in the current study population were similar to those previously reported from our registry, with a mean age of 54 years and 48% females compared to 53 years and 49% in a previous analysis.[8] Thirty-three percent of the total patient population were 65 years or older, which is a substantially higher proportion than in a recent report from the US Drug-Induced Liver Injury Network (DILIN), in which only 17% of patients with DILI were ≥65 years

Table 7. Comparison of demographic and clinical DILI episode characteristics of individual causative agents registered in the Spanish DILI Registry.

	n	Pattern of DILI, %			Female %	Lymphopenia %	Eosinophilia %	Fulfil nR-based Hy's law criteria, n (%)	True nR-based Hy's law (death/liver Tx), n (%)
		Hep	Chol	Mix					
Amoxicillin-clavulanate	193	39	30	31	46	23	30	54 (29)	3 (5.6)
Anti-TB	38	76	13	10	32	33	33	16 (43)	4 (25)
Ibuprofen	25	44	12	44	48	23	17	6 (25)	2 (33)
Flutamide	22	64	9.1	27	14	5.6	4.8	12 (57)	4 (33)
Isoniazid	21	95	0	4.8	43	5.9	9.5	12 (57)	0
Atorvastatin	16	44	25	31	44	0	19	7 (44)	0
Diclofenac	15	93	6.7	0	40	14	7.7	7 (50)	0
Ticlopidine	12	42	50	8.3	17	30	55	4 (33)	0
Azathioprine	11	9.1	64	27	54	20	36	0	0
Fluvastatin	11	18	18	64	54	0	18	0	0
Simvastatin	11	82	9.1	9.1	64	30	9.1	3 (30)	0
Levofloxacin	11	55	36	9.1	45	44	0	1 (9.1)	0
Paroxetine	10	70	10	20	80	20	33	3 (33)	0
Nimesulide	9	78	22	0	89	25	33	7 (78)	1 (14)
Carbamazepine	8	62	12	25	75	40	50	2 (29)	1
Valproic acid	8	75	25	0	100	29	29	3 (38)	0
Erythromycin	8	50	0	50	38	76	12	3 (50)	0
AAS	22	50	27	23	0	20	9.5	9 (47)	0
HDS	29	93	6.9	0	62	19	11	21 (75)	2 (9.5)

Fulfilment of nR-based Hy's law criteria was based on blood analysis values at DILI recognition. AAS, anabolic androgenic steroids; Anti-TB, tuberculosis treatments consisting of rifampicin, isoniazid, pyrazinamide and ethambutol combinations; Chol, cholestatic; DILI, drug-induced liver injury; HDS, herbal and dietary supplements; Hep, hepatocellular; Mix, mixed; Tx, transplantation. Causative agents attributed to less than 8 cases in the Spanish DILI Registry, but with at least 1 case resulting in fatal/liver transplantation included: bicalutamide, calcium carbimide, clomethiazole, clopidogrel, disulfiram, ebrotidine, mianserin, nefazodone, retinol and sevoflurane.

old.[10] Differences in patient age between DILI cohorts can lead to variations in causative agent frequency and comorbidities based on age-related differences in the use of many treatments.

Consistent with previous reports, hepatocellular type of liver injury predominated in the current study population. Hepatocellular damage was associated with lower platelet counts. Reduced platelet number has been associated with increased severity in DILI and acute liver failure (ALF) in general.[24–26] A lower mean platelet count in the hepatocellular patients may therefore reflect the higher proportion of more severe cases in this subgroup, similar to INR with a higher mean value in hepatocellular cases. The role of platelets in acute liver injury is still debated, with evidence supporting both injury exacerbation and recuperation depending on the level of platelet count deviation (thrombocytopenia/thrombocythemia) and degree of platelet activation.[27] Interestingly, platelets are a major source of circulating extracellular vesicles, as demonstrated in, for example, patients with ALF,[28,29] and could be of interest in the search for predictive biomarkers in liver conditions including DILI.

Sixty percent of patients with DILI in the current study required hospitalisation, which highlights the fact that DILI, despite its rareness, is an economic burden on healthcare systems. The proportion of hospitalisation was even higher, reaching up to 73%, in older patients with jaundice and cholestatic/mixed type of liver injury, possible because this is a more vulnerable population.[30] Nevertheless, the hepatocellular DILI cases were associated with a significantly higher proportion of death/liver transplantation, with older patients (≥65 years old) having the highest rate of fatal outcome. The reason for this is unclear. Aside from less frequent liver transplantation due to age restriction criteria, it could be associated with general age-related deficiency in recovery due to diminished capacity for tissue repair. The increased prevalence of comorbid conditions in older patients may also contribute to increased severity. This is supported by the higher fraction of patients with CCI >2 detected among patients with DILI who died or required liver transplantation. In contrast, patients aged 65 years and older in the DILIN population were not found to have a higher mortality and liver transplantation rate than the younger patients with DILI.[10] Interestingly, the Spanish DILI registry demonstrates slightly better outcome data than the DILIN registry,[10] with 2.1% *vs.* 3% liver-related death, 1.5% *vs.* 4% liver transplantation and 1.7% *vs.* 3.2% non-liver-related death, respectively. Reasons explaining these differences could be the lower proportion of females (48% *vs.* 59%), diabetes mellitus (12% *vs.* 25%) and pre-existing liver diseases (6.3% *vs.* 10%) in the Spanish cohort.

The AST value at DILI onset was independently associated with the risk of ALF, as was hepatocellular injury, but only when calculated using nR.[18] This underscores that AST has a higher sensitivity than ALT in ALF prediction, and nR consequently performs better than the classical R for this purpose as previously reported by the Spanish DILI Registry and validated by the US DILIN group.[18,25] Thus, we suggest that AST should be included in the biochemical criteria for DILI assessment and be performed routinely in DILI evaluation. Our findings may also support the use of nR-based Hy's law instead the traditional Hy's law in drug development. Hepatocellular damage, however, was only found to be a predictor of liver-related death, but not of overall mortality. This confirms earlier findings in the DILIN cohort by Ghabril *et al.*[31] Thus, prediction of liver-related death based on type of liver damage is useful in order to refer the

patient to a liver transplant centre, while the comorbidity burden instead of type of liver injury would be useful when assessing the risk of overall mortality. The impact of pre-existing liver disease on DILI susceptibility and outcome is not yet fully elucidated. Only 6.3% of the patients with DILI in the current study had underlying hepatic conditions, but DILI was more frequently associated with a fatal outcome in these patients. Similarly, 10% of North American patients with DILI have been reported to have pre-existing liver conditions and higher mortality, although liver-related mortality did not differ significantly between the North American patients with and without pre-existing liver disease.[10] The discrepancy between our data and those of the DILIN cohort may come from differences in types of underlying liver conditions. While viral hepatitis was highly represented in both registries, alcohol-related liver conditions were more frequent in the Spanish registry and non-alcoholic fatty liver disease in the American registry. Anti-TB drugs were found to be implicated in 21% of cases with underlying hepatic conditions, which is considerably higher compared to 7.6% in the entire study cohort. Most of these cases were positive for chronic viral hepatitis, which is often related to parenteral drug abuse and subsequently a population at higher risk of TB infection. Anti-TB drugs are also among the drugs associated with the highest rate of poor outcomes in our registry, which may also contribute to increased severity among cases with underlying hepatic conditions. The reduced proportion of amoxicillin-clavulanate cases, on the other hand, could be related to physicians' decisions to avoid this known hepatotoxic drug in patients with chronic liver conditions. Hence, our data show that chronic liver disease increases the likelihood of mortality related to liver dysfunction in patients suffering from hepatotoxicity. Presumably, underlying hepatic conditions diminish the liver's capacity to recuperate from a DILI episode and subsequently increase the probability of a more severe outcome. Interestingly, we did not find evidence to support that heavy alcohol consumption significantly enhances the risk of a poor DILI outcome. This corroborates earlier findings from the DILIN cohort, but also comes with the same limitation of self-reported alcohol intake that may be underestimated.[32]

Therapeutic approaches for DILI are used in some cases, although limited evidence is available to determine efficacy. Due to the lack of uniform clinical guidelines for the use of DILI treatments, the decision to initiate such treatments is often left to the physician in charge. The effect of pharmaceutical treatments in the current study is not assessable as the criteria for treatment initiation varied between the corresponding physicians. As expected, the treated patients presented a more severe episode reflected in higher degree of jaundice and hospitalisation. This, however, increases the probability of a fatal/liver transplantation outcome in these patients compared to the non-treated group, independent of the treatment effect. Despite the wide use of treatments for managing DILI, limited evidence is available to demonstrate effectiveness. Our analysis, while providing a picture of what clinicians do in real practice, also highlights the necessity to undertake well-designed clinical trials to determine the true effect of DILI treatments.

DIAIH is a distinct form of DILI that has been increasingly recognised in the last decade. Twenty-six DIAIH cases are currently enrolled in the Spanish DILI registry. Compared with the 843 conventional DILI cases, a higher proportion of females and hepatocellular liver injury were noted in the former group. This confirms previous findings for DIAIH.[33] The DIAIH group also had significantly longer latency. The reason for this is unknown and may be related to the attributed agents. In fact, notably long latency has been reported previously for DIAIH, particularly due to nitrofurantoin and infliximab.[34,35] Patients with DIAIH in the current study received immunosuppressive treatments more frequently but showed similar outcome with regards to mortality and liver transplantation. In fact, DIAH outcome is generally good after withdrawal of the causative agent and immunosuppression, with low risk of relapses or progression to chronic liver injury.[33]

In the current analysis, 5.7% of the causative agents were given parenterally, which in part accounted for a growing representation of immunomodulating agents. This is an emerging area where drug metabolism is not a consideration. We continue to observe that most cases had a daily dose of the attributed drug exceeding 50 mg. Anti-infectives, in particular antibacterials, remain the main causative drug class in the Spanish DILI Registry, independent of patient age. The proportion of antibiotics was highest among the cholestatic cases in the study population, with amoxicillin-clavulanate being responsible for a third of all cholestatic cases. This may explain why the cholestatic group had a significantly shorter time to onset as well as higher total daily dose compared with the hepatocellular cases.

Antimycobacterials represented a large subgroup that mainly included cases caused by combined anti-TB treatments and isoniazid alone. These causative agents, in particular isoniazid, have long been associated with increased DILI susceptibility in older patients.[36,37] Interestingly, our findings do not corroborate this. A relatively young median age (49 years) was also found for 60 North American patients with hepatotoxicity due to isoniazid in a report from the DILIN registry.[38] Some of the causative agents were unequally represented with regards to patient sex. It is unknown if this is due to biological differences increasing susceptibility or in some instances is simply due to the nature of the condition that the treatment is prescribed for.

The prognostic value of Hy's law has been validated in various large DILI cohorts, but little is known about its applicability to specific causative drugs. We found considerable differences when comparing the proportion of cases attributed to a specific causative agent that fulfils nR-based Hy's law criteria and the proportion of these with a fatal/liver transplantation outcome. Almost half of the anti-TB cases fulfilled nR-based Hy's law and 25% of these had the worst outcome, which is in line with Hyman Zimmerman's observations. Several other causative agents for which up to 57% of cases met nR-based Hy's law did not have any fatal/liver transplantation outcomes. Overall, these findings show that Hy's law performance differs between different causative agents.

While providing important information on DILI, our study also has limitations. These include the lack of information on paediatric DILI because paediatric units do not participate in the Spanish DILI Registry. Secondly, DILI caused by immune checkpoint inhibitors, an emerging issue over recent years, is not covered in this study because the use of these drugs was still limited in Spain at the time of this analysis.

In conclusion, we have presented hypothesis-generating findings on phenotypic variations, outcome and causative agents in DILI based on cases enrolled in the Spanish DILI Registry over 20 years. The ever-expanding Spanish DILI Registry, while confirming many findings of the prior smaller cohort from

the first decade, has identified new findings and insights not previously appreciated, which can aid physicians in DILI case characterisation and management, and provide a basis for decision making by regulatory authorities.

Abbreviations

AAS, anabolic androgenic steroids; AIH, autoimmune hepatitis; ALF, acute liver failure; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCI, Charlson comorbidity index; DIAIH, DILI-autoimmune hepatitis; DILI, drug-induced liver injury; DILIN, drug-induced liver injury network; HDS, herbal and dietary supplements; INR, international normalised ratio; MARS, molecular adsorbents recirculation system; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.

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Conflict of interest

The authors have no conflict of interest to disclose.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study concept and design: CS, MRD, MIL, RJA; Case recruitments: MRD, MGC, AOA, JSC, MJP, RGG, MCF, MC, GS, ER, HH, MRG, AC, EM, AMB, IC, MP, JMMP, AG; Case diagnosis: MRD, MCG, AOA, MIL, RJA; Data acquisition: IMC; Statistical analyses: AGJ, IAA, MS; Analysis and interpretation of data: CS, MRD, IMC, JSC, AGJ, IAA, MIL, RJA; Drafting of the manuscript: CS, MRD, MIL, RJA; Critical revision of the manuscript: NK, MIL, RJA

Data availability statement

All data analysed in this article were obtained from the Spanish DILI Registry database, a privately owned database maintained by the registry's coordinating center in Malaga, Spain. The database is not publicly available.

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Supplementary data

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References

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[1] 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–S17.

[2] Andrade RJ, Chalasani N, Björnsson ES, Suzuki A, Kullak-Ublick GA, Watkins PB, et al. Drug-induced liver injury. Nat Rev Primers 2019;5:58.

[3] McGill MR, Jaeschke H. Animal models of drug-induced liver injury. Biochim Biophys Acta Mol Basis Dis 2019;1865:1031–1039.

[4] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: drug-induced liver injury. J Hepatol 2019;70:1222–1261.

[5] Sgro C, Clinard F, Ouazir K, Chanay H, Allard C, Guilleminet C, et al. Incidence of drug-induced hepatic injuries: a French population-based study. Hepatology 2002;36:451–455.

[6] Björnsson ES, Bergmann OM, Björnsson HK, Kvaran RB, Olafsson S. Incidence, presentation, and outcomes in patients with drug-induced liver injury in the general population of Iceland. Gastroenterology 2013;144:1419–1425.

[7] **Shen T**, **Liu Y**, **Shang J**, Xie Q, Li J, Yan M, et al. Incidence and etiology of drug-induced liver injury in mainland China. Gastroenterolgoy 2019;156:2230–2241.

[8] Andrade RJ, Lucena MI, Fernández MC, Pelaez G, Pachkoria K, García-Ruiz E, et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. Gastroenterology 2005;129:512–521.

[9] Suk KT, Kim DJ, Kim CH, Park SH, Yoon JH, Kim YS, et al. A prospective nationwide study of drug-induced liver injury in Korea. Am J Gastroenterol 2012;107:1380–1387.

[10] Chalasani N, Bonkovsky HL, Fontana R, Lee W, Stolz A, Talwalker J, et al. Features and outcomes of 899 patients with drug-induced liver injury: the DILIN prospective study. Gastroenterology 2015;148:1340–1352.

[11] Bessone F, Hernandez N, Mendizabal M, Sanchez A, Paraná R, Arrese M, et al. When the creation of a consortium provides useful answers: experience of the Latin American DILI Network (LATINDILIN). Clin Liver Dis (Hoboken) 2019;13:51–57.

[12] Aiso M, Takikawa H, Tsuji K, Kagawa T, Watanabe M, Tanaka A, et al. Analysis of 307 cases with drug-induced liver injury between 2010 and 2018 in Japan. Hepatol Res 2019;49:105–110.

[13] Lucena MI, Camargo R, Andrade RJ, Perez-Sanchez CJ, Sanchez de la Cuesta F. Comparison of two clinical scales for causality assessment in hepatotoxicity. Hepatology 2001;33:123–130.

[14] Lucena MI, Kaplowitz N, Hallal H, Castiella A, García-Bengoechea M, Otazua P, et al. Recurrent Drug-Induced Liver Injury (DILI) with different drugs in the Spanish Registry: the dilemma of the relationship to autoimmune hepatitis. J Hepatol 2011;55:820–827.

[15] Andrade RJ, Lucena MI, Kaplowitz N, García-Munoz B, Borraz Y, Pachkoria K, et al. Outcome of acute idiosyncratic drug-induced liver injury: long-term follow-up in a hepatotoxicity registry. Hepatology 2006;44:1581–1588.

[16] Lucena MI, Andrade RJ, Fernández MC, Pachkoria K, Pelaez G, Durán JA, et al. Determinants of the clinical expression of amoxicillin-clavulanate hepatotoxicity: a prospective series from Spain. Hepatology 2006;44:850–856.

[17] Lucena MI, Andrade RJ, Kaplowitz N, García-Cortes M, Fernández MC, Romero-Gomez M, et al. Phenotypic characterization of idiosyncratic drug-induced liver injury: the influence of age and sex. Hepatology 2009;49:2001.

[18] Robles-Diaz M, Lucena MI, Kaplowitz N, Stephens C, Medina-Cáliz I, González-Jimenez A, 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;147:109–118.

[19] **Medina-Caliz I**, **Robles-Diaz M**, Garcia-Muñoz B, Stephens C, Ortega-Alonso A, Garcia-Cortes M, et al. Definition and risk factors for chronicity following acute idiosyncratic drug-induced liver injury. J Hepatol 2016;65:532–542.

[20] Benichou C. Criteria of drug-induced liver disorders. Report of an international consensus meeting. J Hepatol 1990;11:272–276.

[21] Aithal GP, Watkins PB, Andrade RJ, Larrey D, Molokhia M, Takikawa H, et al. Case definition and phenotype standardization in drug-induced liver injury. Clin Pharmacol Ther 2011;89:806–815.

[22] Hennes EM, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology 2008;48:169–176.

[23] Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol 1994;47:1245–1251.

[24] Lo Re 3rd V, Haynes K, Forde KA, Goldberg DS, Lewis JD, Carbonari DM, et al. Risk of acute liver failure in patients with drug-induced liver injury: evaluation of Hy's law and a new prognostic model. Clin Gastroenterol Hepatol 2015;13:2360–2368.

[25] Hayashi PH, Rockey DS, Fontana RJ, Tillmann HL, Kaplowitz N, Barnhart HX, et al. Death and liver transplantation within 2 years of onset of drug-induced liver injury. Hepatology 2017;66:1275–1285.

[26] Stravitz RT, Ellerbe C, Durkalski V, Reuben A, Lisman T, Lee WM, et al. Thrombocytopenia is associated with multi-organ system failure in patients with acute liver failure. Clin Gastroenterol Hepatol 2016;14:613–620.

[27] Lisman T, Luyendyk JP. Platelets as modulators of liver disease. Semin Thromb Hemost 2018;44:114–125.

[28] Balaphas A, Meyer J, Sadoul K, Fontana P, Morel P, Gonelle-Gispert C, et al. Platelets and platelet-derived extracellular vesicles in liver physiology and disease. Hepatol Commun 2019;3:855–866.

[29] Stravitz RT, Bowling R, Bradford RL, Key NS, Glover S, Thacker LR, et al. Role of procoagulant microparticles in mediating complications and outcome of acute liver injury/acute liver failure. Hepatology 2013;58:304–313.

[30] Lucena MI, Sanabria J, García-Cortes M, Stephens C, Andrade RJ. Drug-induced liver injury in older people. Lancet Gastroenterol Hepatol 2020;5:862–874.

[31] Ghabril M, Gu J, Yoder L, Corbito L, Ringel A, Beyer CD, et al. Development and validation of a model consisting of comorbidity burden to calculate risk of death within 6 months for patients with suspected drug-induced liver injury. Gastroenterology 2019;157:1245–1252.

[32] Dakhoul L, Ghabril M, Gu J, Navarro V, Chalasani N, Serrano J, et al. Heavy consumption of alcohol is not associated with worse outcomes in patients with idiosyncratic drug-induced liver injury compared to non-drinkers. Clin Gastroenterol Hepatol 2018;16:722–729.

[33] Czaja AJ. Drug-induced autoimmune-like hepatitis. Dig Dis Sci 2011;56:958–976.

[34] de Boer YS, Kosinski AS, Urban TJ, Zhao Z, Long N, Chalasani N, et al. Features of autoimmune hepatitis in patients with drug-induced liver injury. Clin Gastroenterol Hepatol 2017;15:103–112.

[35] Björnsson ES, Bergmann O, Jonasson JG, Grondal G, Gudbjornsson B, Olafsson S. Drug-induced autoimmune hepatitis: response to corticosteroids and lack of relapse after cessation of steroids. Clin Gastroenterol Hepatol 2017;15:135–1636.

[36] Fountain FF, Tolley E, Chrisman CR, Self TH. Isoniazid hepatotoxicity associated with treatment of latent tuberculosis infection: a 7-year evaluation from a public health tuberculosis clinic. Chest 2005;128:116–123.

[37] Bright-Thomas RJ, Gondker AR, Morris J, Ormerod LP. Drug-related hepatitis in patients treated with standard anti-tuberculosis chemotherapy over a 30-year period. Int J Tuberc Lung Dis 2016;20:1621–1624.

[38] Hayashi PH, Fontana RJ, Chalasani NP, Stolz AA, Talwalkar JA, Navarro VJ, et al. Under-reporting and poor adherence to monitoring guidelines for severe cases of isoniazid hepatotoxicity. Clin Gastroenterol Hepatol 2015;13:1676–1682.