Drug-Induced Liver Injury: An Analysis of 461 Incidences Submitted to the Spanish Registry Over a 10-Year Period

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Background & Aims: **Progress in the understanding of susceptibility factors to drug-induced liver injury (DILI) and outcome predictability are hampered by the lack of systematic programs to detect bona fide cases.** ***Methods:*** **A cooperative network was created in 1994 in Spain to identify all suspicions of DILI following a prospective structured report form. The liver damage was characterized according to hepatocellular, cholestatic, and mixed laboratory criteria and to histologic criteria when available. Further evaluation of causality assessment was centrally performed.** ***Results:*** **Since April 1994 to August 2004, 461 out of 570 submitted cases, involving 505 drugs, were deemed to be related to DILI. The antiinfective group of drugs was the more frequently incriminated, amoxicillin-clavulanate accounting for the 12.8% of the whole series. The hepatocellular pattern of damage was the most common (58%), was inversely correlated with age ($P < .0001$), and had the worst outcome (Cox regression, $P < .034$). Indeed, the incidence of liver transplantation and death in this group was 11.7% if patients had jaundice at presentation, whereas the corresponding figure was 3.8% in nonjaundiced patients ($P < .04$). Factors associated with the development of fulminant hepatic failure were female sex (OR = 25; 95% CI: 4.1–151; $P < .0001$), hepatocellular damage (OR = 7.9; 95% CI: 1.6–37; $P < .009$), and higher baseline plasma bilirubin value (OR = 1.15; 95% CI: 1.09–1.22; $P < .0001$).** ***Conclusions:*** **Patients with drug-induced hepatocellular jaundice have 11.7% chance of progressing to death or transplantation. Amoxicillin-clavulanate stands out as the most common drug related to DILI.**

Chemical hepatic injury caused by medicaments, recreational drugs, or nonstandardized medical remedies (such as herbal products) is an increasing health problem. Actually, hepatotoxicity remains the main reason for postmarketing regulatory decisions, including drug withdrawal.[1] However, only scattered data regarding the epidemiology of toxic liver disease are currently available. The bulk of information is derived from the cases reported to the regulatory agencies by the spontaneous reporting system (*yellow card*) and those published in medical journals,[2] but this is very probably only "the tip of the iceberg." This has recently been emphasized in a community prospective study performed in France over a 3-year period,[3] which found an annual incidence of hepatic reactions to drugs 16 times higher than the number reported to the French Pharmacovigilance System. In addition, the lack of an accurate diagnosis is an important limitation of the spontaneous reporting system; approximately 50% of the reactions have been found to be unrelated to the incriminated drug when evaluated carefully thereafter.[4] Therefore, efforts to enhance identification of adverse hepatic reactions and to improve certainty and reliability are clearly needed. These include the establishment of registries with a more

Abbreviations used in this paper: DILI, drug-induced liver injury; FHF, fulminant hepatic failure.

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rigorous and uniform approach to causality assessment. Such registries could help to define the character of the hepatic injury and serve to guide epidemiologic studies.[5]

The present study overviews the incidences of hepatotoxicity recorded over a 10-year period in a regional registry in work in southern Spain. This registry has allowed identifying/amplifying some signals of risk with the use of new and old drugs that, when further analyzed by the Spanish Pharmacovigilance System, ultimately led to the adoption of regulatory measures.

Materials and Methods

The study population was all cases of toxic liver disease assembled in the Regional Registry of Hepatotoxicity in southern Spain since its foundation in April 1994. The registry is coordinated by 2 of the authors (R.J.A. and M.I.L.). The operational structure of the registry, data recording, and case ascertainment have been summarily reported elsewhere.[6] In addition, several of the cases reported here have been published in peer-reviewed journals as case reports or case series.[6–8]

The project was intended to create a collaborative network of specialists in liver and digestive diseases, internal medicine, and clinical pharmacology at several hospitals located in the autonomic community of Andalusia and was later opened to those at other Spanish hospitals who wished to participate in the study. The main objective of this network was to identify in a standardized and prospective manner either inpatients or outpatients attending the participants units whose liver diseases were highly suspicious of being related to drugs or toxins. A secondary aim was to identify/evaluate the existence of effect modification factors.

After obtaining the consent of the patient, in each participating hospital, the physician in charge of the study prospectively collected information on all patients with a suspicion of drug-related liver disease who were attended. For all patients, a detailed history was obtained concerning antecedents of liver or biliary tract disease, drug addiction and/or alcohol abuse, transfusion of blood products, or surgery within the 6 months preceding the onset of hepatitis. A structured report form was proposed and agreed on to record the patient's data. This report form contains different codes to record the following: (1) the temporal relationship between the beginning of drug intake or toxin exposure and the onset of the liver disease and between the discontinuation of suspicious agent and improvement in or recovery of liver dysfunction; (2) a screen to rule out alternative liver diseases; (3) the presence of known risk factors of hepatotoxicity such as alcohol intake, measured as standard drink units of 10 g for all beverages,[9] or pregnancy; and (4) the outcome of the liver damage.

Liver disease that needed to be excluded before ascribing the hepatotoxicity to the drug were as follows: recent viral hepatitis with hepatitis A virus (HAV) (IgM anti-HAV) or hepatitis B virus (HBV) (IgM anti-HBV) or hepatitis C virus (HCV) (anti-HCV and RNA positive by PCR), autoimmune liver disease (test for antinuclear, antimitochondrial antismooth muscle and anti-LKM-1 antibodies), and biliary obstruction (routine abdominal ultrasonography as well as complementary nuclear magnetic resonance of biliary tracts and/or endoscopic colangiography if a cholestatic pattern was present). In a suggestive clinical context, a search for cytomegalovirus, Epstein-Barr virus, herpes virus infection or hepatitis E and bacterial serology for salmonella, campylobacter, and listeria was also performed. In alcoholic patients, an alcoholic liver disease was excluded. Wilson disease was ruled out in patients less than 40 years of age. Other metabolic liver disorders such as hemochromatosis, alfa-1 antitrypsin deficiency, and, in patients with recent hypotension history, ischemic hepatitis were discarded. In dubious cases, such as strongly positive markers for autoimmunity, alcoholic patients, preexistent liver disease, or systemic diseases that may affect the liver, a liver biopsy was usually indicated to ascertain further the cause.

A thorough check for present and previous use of drugs, herbal remedies, and over-the-counter medications was done. Information regarding drug use was obtained by asking all patients about the treatment followed in the previous months and newly introduced drugs. To minimize errors in the ascertainment of medicines that the patients were actually taking, all available sources of information were used, which included the following: (1) questioning the patients to identify drugs used for other medical problems that might not be recorded in the medical record and to provide information on any herbal remedy use or consumption of illicit drugs, (2) interviewing family members when patients were not able to collaborate, (3) requesting medication containers or a written medication plan, when available, to reduce the possibility of recall errors by the patient.

The definition and criteria for a case were those established by the International Consensus Meeting for liver injury.[10] In summary, liver injury was considered if there was an increase over 2 N (upper limit of normal range) in alanine aminotransferase (ALT) or conjugated bilirubin or a combined increase in aspartate aminotransferase (AST), alkaline phosphatase (AP), and total bilirubin, provided one of them was above 2 N. The pattern of liver damage was classified according to the International Consensus Meeting criteria,[10] which use ALT and alkaline phosphatase activity, expressed as a multiple of the upper limit of normality, to determine the ratio (R) of ALT/AP. The pattern of liver damage is hepatocellular when $R > 5$, cholestatic when $R < 2$, and mixed when $R > 2$ but < 5. The liver tests used for the classification of liver damage were the first blood test available after liver injury. Alternatively, liver damage was determined on the basis of liver biopsy specimen findings when available. Liver pathologic lesions were coded into 9 basic diagnoses in an attempt to gain uniformity among centers. The drugs thought to be responsible for hepatic reactions were classified according to the Anatomical Therapeutic Classification (ATC) recommended by WHO-Europe.[11] In every case, it was attempted to establish the presumable mechanism of toxic liver injury. Cases were classified as hypersensitivity or immunologic in nature if they presented with any of

the classical clinical or laboratory features of allergy (fever, rash, serum eosinophilia, cytopenia) and/or there was accompanying suggestive pathologic findings (eosinophil-rich inflammatory infiltrate and/or granuloma formation). In the remaining cases, the mechanism was presumed to be metabolic idiosyncrasy. The liver damage was classified as intrinsic in nature when there was obvious overdosage of a known intrinsic hepatotoxin such as acetaminophen or damage induced by chemical agents.

For chronologic purposes, cases were considered reliable if symptoms or laboratory abnormalities occurred within 15 days from cessation of the suspected drug for the hepatocellular pattern of injury or within 30 days for cholestatic/mixed pattern.[10] From the beginning of therapy, no specific time exposure was required to establish the responsibility of a particular agent (eg, the suspicious drug could have been taken for more than 3 months), but, if exposure to several drugs was recorded, the latest agent introduced was thought to be responsible, except when intake of a known hepatotoxic drug antedated the latest medication. In these situations and when a combination of drugs was started simultaneously, the case was ascribed to the combination of both drugs. Also, at the coordinating center, cases were further evaluated for the potential pharmacokinetic and dynamic interactions among the drugs prescribed.

Case ascertainment was first left to the interpretation of the attending physician and thereafter was centrally evaluated by at least 3 independent experts at the coordinating center, who assessed causality, first by clinical judgment[12] and then by applying the Council for International Organizations of Medical Sciences (CIOMS) scale,[13] which was found to be more accurate in attributing causality in a previous study.[14] Actually, when a disagreement in causality assessment among experts arises, the concerns are posed and discussed, complementary data are requested if needed, and a final consensus is reached. Only cases considered drug-related by experts' clinical judgments were assessed by the CIOMS scale. Of these, only cases assessed as definite or highly probable, probable or possible were included in the database.

Outcome was assessed by clinical, analytical, imaging tests and histologic methods when available. Cases were defined as *resolved* when liver tests had returned to normal within 3 months for hepatocellular pattern of damage or 6 months for cholestatic/mixed pattern of injury or *chronic* when liver test remained otherwise altered. If follow-up was incomplete to ascertain outcome, cases were classified as *undetermined*.

Forms were checked for completeness at the coordinating center before data entry into an access database created ad hoc. Physicians submitting the cases were periodically contacted by telephone, fax, or e-mail to maintain adherence to the project and every time that additional information to ascertain the causality was needed. The study protocol was approved by the local ethics committee of the coordinating center at Virgen de la Victoria University Hospital of Malaga.

Data Management and Statistical Analysis

Data were analyzed with the Statistical Package for Social Sciences (SPSS; version 12.0; SPSS Inc. Chicago, IL) for Windows software. Variables were examined using descriptive statistics. Bivariate associations were measured using *t* tests for continuous variables and χ^2 test for categoric items. Analysis of variance (ANOVA) was used for comparisons of groups. Where variables did not follow a normal distribution, nonparametric analyses (Kruskal-Wallis test) were performed. Differences were reported as statistically significant if the *P* value was less than .05.

Variables that were associated with the development of fulminant hepatic failure (FHF) on univariate analysis or that were considered clinically relevant were included as potential covariates in a multiple logistic regression model. The risk for developing FHF in a given patient with hepatotoxicity can be formulated as follows:

$$P = \frac{1}{1 + e^{-(\beta_0+\beta_1 X_1+\beta_2 X_2+\cdots+\beta_x X_x)}}$$

in which *P* is the probability to develop FHF; X_1, X_2, ... ,X_x represent the risk factors analyzed or independent variables; and β_0, β_1, ... β_x the unknown coefficients to be estimated. Calibration of the model was assessed using the Hosmer and Lemeshow χ^2 statistics ($P < .05$). The survival curve was estimated with the use of the Cox regression model.

An approach to the incidence of toxic liver disease (per million person-years) was handled at the coordinating center, at which continuous reporting over the years occurred, and was estimated as follows: the number of patients with the diagnosis, divided by the number of persons served by the hospital and the duration of the time period in years.

Results

Demographic and Clinical Characteristics

From April 1994 to August 2004, 570 cases were submitted by 32 participant clinical units to the coordinating center belonging to 9 autonomous regions. One hundred nine were excluded: 36 because of unreliable chronologic criteria, 59 because an alternative cause of injury could be identified, including choledocholithiasis (13), viral hepatitis (11), underlying malignancy (10), autoimmune hepatitis (6), ischemic hepatitis (6), nonalcoholic steatohepatitis (4), alcoholic hepatitis (3), systemic sepsis (3), hypothyroidism (2), and Wilson disease (1); and 14 cases did not fulfill the criteria of liver damage (biologic alterations).

A total of 461 cases, which involved 505 drugs, was considered to be related to hepatotoxicity and, therefore, included in the database. In 44 (9%) cases, 2 drugs were suspected. The estimated annual incidence of hepatotox-

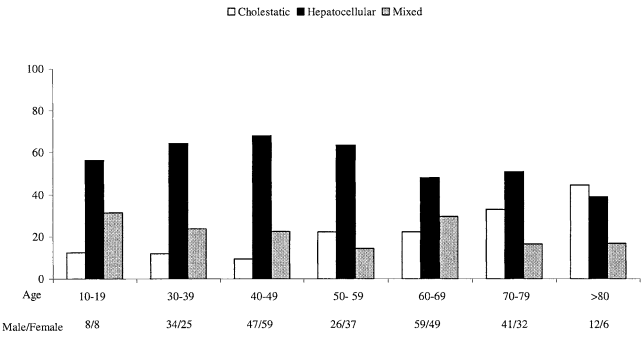

Figure 1. Type of damage according to age and sex distribution of 446 cases of drug-induced liver injury reported to a registry of hepatotoxicity between 1994 and 2004. Patients with a cholestatic pattern were significantly older than patients with other patterns of liver damage ($P < .0001$).

icity at the coordinating center from 1998 to 2003 was 34.2 ± 10.7 cases per 10^6 inhabitants per year, whereas the annual incidence rate for serious adverse hepatic reactions (those that are life-threatening, require hospitalization, prolong hospitalization, result in permanent incapacity or death) was 16.6 ± 6.7 cases per 10^6 inhabitants per year. The CIOMS diagnostic scale classified the cases as definite or highly probable in 268 cases, probable in 174, and possible in 19. In 15 cases (3.3%), hepatotoxicity was deemed to be intrinsic. Acetaminophen overdosage accounted for 13 (87%) of these cases. Among the cases with idiosyncratic hepatotoxicity, in 106 (23%) cases, any of the hallmarks of hypersensitivity (fever, rash, eosinophilia, cytopenia) were present.

Of the 446 cases of idiosyncratic hepatotoxicity, 229 (51%) were men. The overall mean age was 53 years. Type of liver damage according to age and sex distribution is shown in Figure 1. There was an overall similar sex distribution. The patients whose hepatotoxicity was due to an intrinsic mechanism were significantly younger (mean age 36 years; range, 16–69 years) than the patients classified in the idiosyncratic group ($P < .0001$).

The mean duration of drug intake was 105 days (95% CI: 63–146 days) with a mean latency period of 93 days. Jaundice was the most frequent manifestation when the case was first identified (315 cases, 71%), raised liver enzymes accounting for the remaining cases. Eosinophilia was present in 81 (18%) patients. Thirty-eight patients (8.5%) had a history of low (less than 40 g/day) alcohol consumption, 19 (4%) patients reported a moderate (between 40 and 70 g/day) intake, and 23 (5%) patients reported severe (more than 70 g/day) alcohol consumption. In 22 patients (5%), there was an underlying chronic liver disease, cirrhosis being the most frequent diagnosis (8 cases), followed by alcoholic hepatitis (3 cases). A history of positive (inadvertent) rechallenge was elicited in 26 (6%) patients. Further analysis of the information collected in the Summary Product Characteristics of the suspected drugs with regard to their hepatotoxic potential was unknown or missing in 134 (30%) leaflets. In addition, drugs were marketed for less than 3 years in 59 (13%) cases when the hepatotoxic reaction first appeared.

Two hundred thirty-seven patients (53%) required hospitalization. The overall outcome was death in 24 cases (5%) and liver transplantation in 8 patients (2%). Forty-six (10%) patients fulfilled the criteria of chronicity.

Therapeutic Groups Involved in Idiosyncratic Liver Injury

The main causative pharmacologic group of drugs was antiinfectious (32%), followed by central nervous system (17%), musculoskeletal (17%), and gastrointestinal drugs (10%). Among the main therapeutic class, the rank order was systemic antibiotics (98), nonsteroidal anti-inflammatory drugs (53), H2-receptor antagonists (33), antituberculous drugs (33), antidepressants drugs (20), analgesics (19), platelet aggregation inhibitors (18), lipid-lowering drugs (18), anxiolytics (10), and medicinal herbs (9). Table 1 lists the distribution of the main causative drugs according to type of liver damage and severity. Amoxicillin-clavulanate was the individual drug responsible for the highest number (59) of cases. Annual sales data (2001-2004) obtained by the Andalusian Health Service as number of items sold were expressed as defined daily dose (DDD)/1000 inhabitants per day. The corresponding figures for each year were 33.87; 42.0; 57.69, and 67.84 DDD/1000 inhabitants/day, respectively.

Comparison of Demographics and Clinical and Laboratory Findings by Type of Liver Damage

The predominant pattern of lesion was hepatocellular (258 patients, 58%). Comparison of the demographics characteristics and clinical and laboratory findings according to the type of liver injury is shown in Table 2. Patients with the cholestatic type of injury presented more frequently with jaundice (81%; $P < .03$) and had the highest mean plasma bilirubin values ($P < .0001$). Among patients with the hepatocellular type of damage, 96 cases (22%) had ALT values 30 times above the upper limit of normal. The results did not change when the analysis was conducted in the subgroup of patients labeled as definite or highly probable. Table 3 lists the mean values of bilirubin, ALT, and AP by drug class.

Table 1. Distribution of the Main Drugs Suspected in 446 Cases of Drug-Induced Liver Disease Reported to the Registry Between 1984 and 2004 According to the Type of Liver Damage, Severity of Hepatic Injury, and Presence of Eosinophilia

Drug	Total cases (N)	Type of liver injury (N)			Eosinophilia (N)	Liver-related hospitalization N (%)	ALF/liver tx (N)	Death N
		Hepatocellular	Cholestatic	Mixed				
Amoxicillin-clavulanate	59	22	16	21	19	40 (68%)	2[a]/1	1
Ebrotidine	22	21	—	1	2	13 (59%)	1/0	1
INH + RIP + PIZ	22	15	5	2	2	14 (64%)	3/1	5
Ibuprofen	18	8	1	9	2	10 (56%)	2/1	1
Flutamide	17	11	1	5	—	9 (53%)	2/1	4
Ticlopidine	13	7	5	1	5	8 (62%)	—	—
Diclofenac	12	10	2	—	1	6 (50%)	—	—
Isoniazid	9	8	—	1	1	5 (56%)	—	1
Medicinal herbs	9	8	1	—	1	5 (56%)	—	1
Nimesulide	9	7	2	—	2	3 (33%)	2/1	1
Carbamazepine	8	4	1	3	4	3 (38%)	1/0	1
Bentazepam	7	5	—	2	—	3 (43%)	—	—
Tetrabamate	7	6	1	—	—	2 (29%)	—	—
Azathioprine	6	1	4	—	1	1 (17%)	—	—
Erythromycin	6	—	4	2	1	3 (50%)	—	—
Paroxetine	6	3	1	2	—	3 (50%)	—	—
Valproic acid	5	4	1	—	1	2 (40%)	—	—
Trovafloxacin	5	4	—	1	2	3 (60%)	—	—
Thiamazole	5	1	4	—	1	3 (60%)	—	—

ALF, acute liver failure; Tx, liver transplantation; INH, isoniazid; RIP, rifampicin; PIZ, pirazinamide.
[a]One case of acute chronic liver damage (cirrhosis), another case of inadvertent rechallenge leading to cirrhosis and liver transplant.

Liver histology was available in 110 (25%) patients. Cholestasis was the most common finding, which was reported in 53 patients (48%), hepatocellular necrosis accounting for 30 cases (27%). In patients with histologic diagnosis of cholestasis, 41 (37%) had accompanying hepatitis, whereas 10 (9%) had "pure" cholestasis. Chronic hepatic damage was reported in 16 patients (15%), chronic active hepatitis accounting for 9 cases, cirrhosis for 3 cases, and fibrosis and ductopenia for 2 cases each. Other histologic diagnoses were granulomatous hepatitis in 4 cases and steatosis in 2 cases. In 5 cases, a second biopsy was performed: In 2 cases (droxicam and raloxifene-fenofibrate), the liver damage progressed to vanishing bile duct syndrome; in 1 case (vitamin A), the hepatic necrosis worsened, and a massive necrosis ensued; and, in the remaining 2 cases (irbesartan and estradiol), the liver damage remained unchanged. None of these patients had evidence of underlying liver disease. A close correlation was found between the biologic and histologic pattern of damage, with only 1 overt disagreement.

Chronic outcome of the hepatotoxicity was similar among the different groups of liver damage. However, the frequency of FHF, requirement of liver transplantation, and death was more frequent in patients presenting with hepatocellular damage. Survival curves for hepatocellular compared with cholestatic/mixed cases did significantly differ (Figure 2). The outcome was significantly better in patients with cholestatic/mixed damage as compared with patients with the hepatocellular pattern of liver damage ($P = .034$). Additionally, the incidence of liver transplantation or fatal outcome in patients with hepatocellular damage was 11.7% if they also had jaundice at presentation, whereas the corresponding figure was 3.8% in nonjaundiced patients ($P < .04$).

Characterization of Risk Factors for Development of Fulminant Liver Failure

Eighteen patients with hepatotoxicity developed FHF. None of the patients with FHF had a spontaneous recovery. Twelve patients died, and 6 received a liver transplant. Only 1 patient in this group had hypersensitivity features. Comparison of baseline demographic characteristics of these patients with that of those who had a milder immediate outcome showed that, in the former, there was a higher predominance of female sex, hepatocellular damage, and higher levels of plasma bilirubin (Table 4), which were the factors found to be independently associated with the development of FHF in a multiple logistic regression model (Table 5).

Discussion

Drug-induced idiosyncratic hepatotoxicity remains a challenge of modern hepatology. Hepatotoxicity is typically detected after marketing when several thou-

Table 2. Demographics, Clinical, and Laboratory Parameters of the 446 Cases of Idiosyncratic Hepatotoxicity According to the Type of Liver Damage

Variables	Type of liver injury		
	Hepatocellular (N = 258)	Cholestatic (N = 89)	Mixed (N = 99)
Mean age (range), *y*	51 (13–83)	61 (18–88)[a]	52 (14–83)
Men, n (%)	131 (51%)	48 (54%)	50 (51%)
Clinical presentation, n (%)			
Jaundice	179 (69%)	72 (81%)[a]	64 (65%)
Raised enzymes	79 (31%)	17 (19%)	39 (35%)
Hospital admission	129 (50%)	57 (64%)	51 (52%)
Hypersensitivity features, n (%)	53 (21%)	23 (26%)	29 (29%)
Underlying liver disease, n (%)	10 (4%)	9 (10%)	3 (3%)
Mean duration of treatment, days (95% CI)	134 (64–205)	65 (29–100)	64 (40–89)
Mean time to onset, days (95% CI)	119 (47–192)	61 (25–96)	53 (30–76)
Laboratory parameters, mean value (range)			
Total bilirubin (*mg/dL*)	7.8 (0.2–45.6)	9.7 (0.2–37)[b]	6.9 (0.3–33.1)
ALT (× ULN)	31 (2.3–203)[b]	4.8 (0.4–38.7)	7.5 (1.4–23.5)
Alkaline Phosphatase (× ULN)	1.3 (0.1–7.1)	5.2 (0.7–32.7)[b]	2.3 (0.5–6.7)
Outcome			
Recovery, mean days (95% CI)	81 (70–92) (n = 213)	104 (73–136) (n = 71)	95 (72–118) (n = 85)
Acute liver failure, n (%)	15 (6%)[a]	1 (1%)	2 (2%)
Liver transplantation, n (%)	7 (3%)	1 (1%)	0
Death, n (%)	18 (7%)	4 (5%)	2 (2%)
Chronicity, n (%)	27 (11%)	8 (10%)	11 (12%)
Positive rechallenge, n (%)	21 (8%)	1 (1%)	4 (4%)
Drug ≤ 3 y on the market, n (%)	48 (19%)[a]	4 (5%)	7 (7%)
Labelled information on hepatotoxicity, n (%)	172 (67%)	69 (78%)	71 (72%)

Total bilirubin (N < 1.0 mg/dL); ALT, alanine transaminase; AP, alkaline phosphatase.
NOTE. Values are expressed as multiples of the upper limit of normal (ULN). The ALT and AST values are those at presentation, whereas bilirubin values are the peak. Hypersensitivity features refers to the presence of fever, rash, and/or eosinophilia.
[a]Refers to the existence of significant differences among groups ($P < .05$).
[b]Kruskal–Wallis test ($P < .0001$).

sand patients are exposed to the drug, and regulatory authorities are often compelled to make decisions based on scanty, fragmentary, and incomplete epidemiologic data.[15] In addition, whereas a major challenge is to be able to identify predisposed subjects before they receive the drug, genetic and environmental factors that appear to operate in determining individual susceptibility are still poorly understood. Therefore, assembling bona fide cases is crucial to obtain reliable information that could provide new insights into epidemiology and pathogenesis of hepatotoxicity. These efforts are virtually impossible for a single hospital unit as has been recognized by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health, which has recently sponsored a cooperative agreement to develop a drug-induced liver injury network, consisting of 6 university hospitals from different states in the United States.[16]

The present study analyzes the cases of toxic liver injury prospectively collected from several Spanish tertiary and secondary care centers over a 10-year period in a network of different clinical specialists working in collaboration. It seems probable that the network has contributed to create a "pharmacoepidemiological" culture among participating physicians, increasing awareness of drug-induced hepatotoxicity. These efforts may have clear advantages over spontaneous reporting because it minimizes underreporting and selective reporting, and, in addition, the information collected is of much better quality.

Furthermore, if the registry were population based, then a crude incidence rate could also be estimated. However, because the adherence of units to the project was not the same throughout the study period, we only attempted to estimate the annual incidence of hepatotoxicity at the coordinating center during a 6-year period as referred to the population attending the hospital. A potential limitation for data interpretation is that the pediatric population, which in Spain encompasses subjects up to 14 years of age, was clearly underrepresented. This should prompt the development of strategies to accomplish the implementation of specific networks in this orphan field.[17] The incidence figures obtained are nonetheless noteworthy and higher than expected with the spontaneous reporting system and those found in a recent epidemiologic study in Catalonia (Spain),[18] al-

Table 3. Main Laboratory Findings by Drug Class in Patients With Drug-Induced Liver Injury

Drug Class	N	Mean value		
		Total Bilirubin	ALT (× ULN)	AP (× ULN)
Drugs for peptic ulcer	31	12.3	35.3	1.7
Antithrombotic agents	17	6.8	16.8	4.4
Antiarrhythmics	5	3.2	30.3	2
ACE inhibitors	8	10.3	7.2	4.1
Angiotensin II inhibitors	6	5.5	44.6	2.5
Statins	11	6.1	15.8	2.8
Fibrates	4	2.4	7.4	3.3
Penicillin with extended spectrum	7	9.3	15.8	2.4
Penicillin with betalactamase inhibitors	59	8.6	13.7	2.7
Cephalosporins and related substances	4	6.4	5.2	1.8
Macrolides	12	5.5	17.3	4.4
Quinolone antibacterials	10	8.6	27.1	2.4
Drugs for the treatment of tuberculosis	31	6.1	24.5	1.7
Antineoplastic agents	10	9.7	8.6	4.4
Antiinflammatory and antirheumatic products, non-steroids	52	8.6	19.6	2.7
Antigout preparations	3	5.8	14.4	2.3
Other analgesics and antipyretics	7	11.5	18.8	1.6
Antiepileptics	18	6.3	21.4	2.7
Antipsychotics	7	7.4	4.9	2
Anxiolytics	8	4.2	26.4	0.9
Hypnotics and sedatives	5	8.8	16.7	2.1
Antidepressants	23	5.0	20	1.7

ALT, alanine transaminase; AP, alkaline phosphatase; ACE, angiotensin converting enzyme.
NOTE. Values are expressed as multiples of the upper limit of normal (ULN).

though they are still lower than the rate of 139 per 10^6 inhabitants per year reported in the French population-based study by Sgro et al.[3] Indeed, the cases here presented are probably far from the total of incidences of toxic liver injury actually occurring during the study period. This is because the network included only hospital units, which were probably unaware of many patients with milder disease that could have been managed by general practitioners or by out-of-hospital specialists. In addition, because of the lack of a centralized alarm-signal mechanism (eg, laboratory signals), it is possible that, even within participant hospitals, some patients with hepatotoxicity admitted to other medical or surgical departments went unrecognized. Nevertheless, our estimation of incidence of toxic liver disease should be taken with caution because we do not know the number of prescriptions written for each drug. The incidence rates of drug-induced liver injury of a drug are calculated by dividing the number of cases of hepatotoxicity by the corresponding number of patients exposed or prescriptions (as a surrogate marker of person-time of exposure). Because this is not a cohort study or a population-based, case control study, our methodologic approach cannot give us real estimates of incidence for each drug or class.

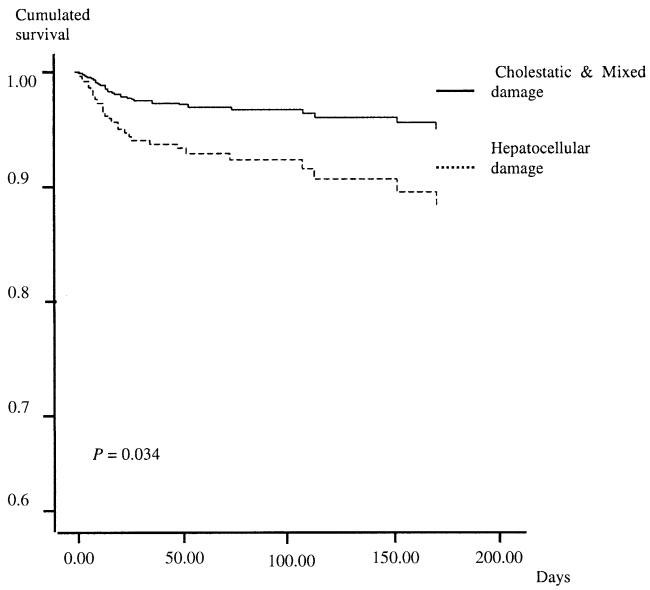

Figure 2. Cumulated survival curves of hepatocellular and cholestatic/mixed cases of drug-induced liver injury.

Contrary to other epidemiologic data that have found a higher predominance of female sex among patients with hepatotoxicity,[3,19] in our study, there was no difference in sex distribution, whereas a higher predominance of male sex at older ages was observed as in the study of Amdal et al.[20] An important exception was that patients suffering from FHF were predominantly women. This fact has also been recognized in 2 recent studies from the United States of patients with acute liver failure.[21,22]

Table 4. Demographic Characteristics, Clinical and Laboratory Findings of the Patients Who Developed Drug-Induced Fulminant Hepatic Failure Compared With Any Other Presentation

Variables	Fulminant Hepatic Failure (n = 18)	Other Presentations (n = 428)	*P* Value
Mean age (range), y	53 (14–83)	53 (13–88)	NS
Women, n (%)	16 (89%)	201 (47%)	<.0001
Mean duration of treatment, days (± SD)	111 ± 142	105 ± 453	NS
Mean time to onset, days (± SD)	92 ± 141	93 ± 466	NS
Clinical presentation: Jaundice, n (%)	18 (100%)	297 (69%)	<.003
Hepatocellular damage, n (%)	15 (83%)	243 (57%)	<.028
Laboratory parameters, mean value ± SD			
Total bilirubin (mg/dL)	16.9 ± 10.5	7.6 ± 7.5	<.0001
ALT (× ULN)	30.4 ± 21.6	19.9 ± 23.9	NS
AP (× ULN)	2.0 ± 1.6	2.3 ± 2.8	NS
Liver transplantation, n (%)	6 (37%)	2 (0.5%)	<.001
Death, n (%)	12 (67%)	12 (3%)	<.001
Drug ≤ 3 y on the market, n (%)	3 (17%)	56 (13%)	NS
Labelled information, n (%)	8 (44%)	304 (71%)	<.032

NS, nonsignificant; total bilirubin (N < 1.0 mg/dL); ALT, alanine transaminase; AP, alkaline phosphatase.
NOTE. Values are expressed as multiples of the upper limit of normal (ULN).

The therapeutic class of drugs more frequently recorded as the cause of toxic hepatitis was similar to those found in recent studies,[3,18] and, because the registry was not restricted to specific drugs, it provides a good picture of the most frequent causes of hepatotoxicity in this geographic area. Interestingly, there was a high number of cases attributed to H2-receptor antagonist, "the mini-epidemic" of ebrotidine accounting for most of them.[6] Indeed, this type of study does mirror particular toxicity problems that episodically occur with some drugs as well as point out that the pattern of causative drugs somehow depends on the pharmaceutic policy and prescription pattern in each country. For instance, contrary to reported studies,[19,23] we could record no single case of sulindac-related hepatotoxicity, which probably highlights the low consumption of this NSAID in Spain. Also, emerging toxicity challenges, like the increase of hepatotoxicity associated with herbal remedies, have also been reflected in our registry.

The present study has singled out amoxicillin-clavulanate as the drug more frequently incriminated in hepatotoxicity, accounting for 12.8% of the series. Although an estimation of the risk of liver damage associated with this drug could not be made, our data are in agreement with those of a population-based, case control study recently published.[24] Hypersensitivity features of an immunoallergic nature were present only in a 23% of the cases. Attempts to establish the mechanisms of hepatotoxicity based on these manifestations are nonetheless rudimentary because blood and hepatic eosinophilia are late tissue responses,[25] and a more accurate classification of patients with immunoallergic hepatitis by the use of specific serum autoantibodies,[26] or in vitro lymphocyte transformation testing,[27] have been unsuccessful.

Table 5. Factors Associated With the Development of Fulminant Hepatic Failure

Independent variables	Coefficient	OR (95% CI)	*P* Value
Women	3.220	25.04 (4.14–151)	<.0001
Hepatocellular damage	2.064	7.87 (1.68–36.9)	<.009
Total bilirubin (mg/dL)	0.143	1.15 (1.09–1.22)	<.0001

CI, confidence interval; OR, odds ratio.
NOTE. Constant = −8.7

The predominant pattern of hepatic damage was hepatocellular as has consistently been shown in other large case series.[3,18,19] Interestingly, many drugs were ascribed to more than 1 pattern of liver damage, indicating that the proposed "signature" for each drug should be taken with caution.[28] The factors that determine the type of hepatic cell to be the predominant target of the toxic effects of drugs or its metabolites remain to be elucidated, but recent data support the notion that certain HLA class II alleles are important in explaining why a given drug may cause different patterns of liver damage.[29]

Our study also supports the notion that jaundiced patients with cytolytic damage are more prone to evolve to acute liver failure than patients with cholestatic/mixed damage. Actually, the figure of 11.7% of liver transplantation and/or death does validate "Hy's rule," which predicts an incidence no lower than 10% of these outcomes in drug-induced hepatocellular jaundice,[5] and suggests that these patients should probably be faced with close scrutiny in an in-hospital basis for the development of impending liver failure. Further learning in this sense could be drawn by analyzing the patients with

drug-induced hepatotoxicity that evolved to FHF. Our data show that the association of female sex, hepatocellular damage, and a baseline, high serum bilirubin level sharply increases the risk of developing this severe outcome. However, the certainty of this particular profile for predicting the risk of FHF should be further validated.

On the other hand, there are scarce data on the long-term outcome of the hepatic disease in the patients in whom the offending drug is withdrawn. Our data show that, in 10% of the patients, liver profile remained altered during a limited follow-up. Although it is generally accepted that, if the patient does not suffer from acute liver failure, complete recovery after drug withdrawal is the rule, a single published retrospective study has reported evidence of persisting damage in 13 out of 33 patients evaluated.[30] To address this important issue, a prospective 3-year study in a large cohort of patients is currently underway.

Finally, reporting of the cases submitted to the registry and thereafter to the Spanish Pharmacovigilance System has generated/amplified signals and has prompted in some instances the adoption of regulatory measures that varied between changes in the product-labeling information[7,8] and drug withdrawal.[6,31,32]

In summary, in this large series, amoxicillin-clavulanate was the most common drug associated with liver injury, and 11.7% of patients with drug-induced hepatocellular jaundice progressed to death or transplantation. This registry has proved to be an effective instrument in detecting cases of idiosyncratic liver disease and in delineating a profile of risk factors for severity and has contributed to the protection of public health. Much has to be learned in the field of hepatotoxicity; however, the current results of this network should encourage the development of similar projects. Efforts must be directed toward increasing our knowledge in this difficult aspect of liver disease by getting many health care workers engaged as well as obtaining the continuous support of health authorities to achieve the ultimate goal, which is to prevent hepatic adverse reactions to drugs.

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References

1. Bakke OM, Manocchia M, De Abajo F, Kaitin KI, Lasagna L. Drug safety discontinuations in the United Kingdom, the United States, and Spain from 1974 through 1993: a regulatory perspective. Clin Pharmacol Ther 1995;58:108–117.
2. Larrey D. Epidemiology and individual susceptibility to adverse drug reactions affecting the liver. Semin Liver Dis 2002;22:145–155.
3. Sgro C, Clinard F, Ouazir K, Chanay H, Allard C, Guilleminet C, Lenoir C, Lemoine A, Hillon P. Incidence of drug-induced hepatic injuries: a French population-based study. Hepatology 2002;36: 451–455.
4. Aithal GP, Rawlins MD, Day CP. Accuracy of hepatic adverse drug reaction reporting in one English health region. BMJ 1999;319: 1541.
5. Zimmerman HJ. Hepatotoxicity. The adverse effects of drugs and other chemicals on the liver. 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 1999.
6. Andrade RJ, Lucena MI, Martín-Vivaldi R, Fernandez MC, Nogueras F, Pelaez G, Gomez-Outes A, Garcia-Escaño MD, Bellot V, Hervás A, Cárdenas F, Bermudez F, Romero M, Salmerón J. Acute liver injury associated with the use of ebrotidine, a new H_2-receptor antagonist. J Hepatol 1999;31:641–646.
7. Andrade RJ, Lucena MI, Alcantara R, Fraile JM. Bentazepam-associated chronic liver disease. Lancet 1994;343:860.
8. Andrade RJ, Lucena MI, Rodriguez-Mendizabal M. Hepatic injury caused by acarbose. Ann Intern Med 1996;124:931.
9. Cahalan D. Quantifying alcohol consumption: patterns and problems. Circulation 1981;64:7–14.
10. Benichou C. Criteria of drug-induced liver disorders. Report of an international consensus meeting. J Hepatol 1990;11:272–276.
11. World Health Organization Collaborating Center for drugs statistics methodology. Anatomical Therapeutic Chemical (ATC) classification index including defined daily dose (DDDs) for plain substances. Oslo: World Health Organization Collaborating Center for drugs statistics methodology, 2002.
12. Kaplowitz N. Causality assessment versus guilt-by-association in drug hepatotoxicity. Hepatology 2001;33:308–310.
13. 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;46:1323–1330.
14. Lucena MI, Camargo R, Andrade RJ, Perez-Sanchez C, Sanchez De La Cuesta F. Comparison of two clinical scales for causality assessment in hepatotoxicity. Hepatology 2001;33:123–130.
15. Arnaiz JA, Carne X, Riba N, Codina C, Ribas J, Trilla A. The use of evidence in pharmacovigilance. Case reports as the reference source for drug withdrawals. Eur J Clin Pharmacol 2001;57:89–91.
16. Hoofnagle JH. Drug-induced liver injury network (DILIN). Hepatology 2004;40:773.
17. Peire MA, Lucena MI, Ruiz-Extremera A, Jara P, Romero-Gonzalez J, Andrade RJ. Toxicidad hepatica por fármacos. Donde estamos y hacia donde caminamos. An Esp Pediatr 2002;56:434–442.
18. Ibáñez L, Pérez E, Vidal X, Laporte JR, the Grup d'Estudi Multicentric d'Hepatotoxicitat Aguda de Barcelona (GEMHAB). Prospective surveillance of acute serious liver disease unrelated to infectious, obstructive, or metabolic diseases: epidemiological and clinical features, and exposure to drugs. J Hepatol 2002;37:592–600.
19. Friis H, Andreasen PB. Drug-induced hepatic injury: an analysis of 1100 cases reported to the Danish Committee on Adverse Drug Reactions between 1978 and 1987. J Intern Med 1992;232: 133–138.
20. Almdal TP, Sorensen TI. Incidence of parenchymal liver disease in Denmark, 1981 to 1985: analysis of hospitalization registry data. The Danish Association for the Study of the Liver. Hepatology 1991;13:650–655.
21. Ostapowicz G, Fontana RJ, Schiodt FV, Larson A, Davern TJ, Han SH, McCashland TM, Shakil AO, Hay JE, Hynan L, Crippin JS, Blei AT, Samuel G, Reisch J, Lee WM, and the U.S. Acute Liver Failure Study Group. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 2002;137:947–957.
22. Russo MW, Galanko JA, Shrestha R, Fried MW, Watkins P. Liver transplantation for acute liver failure from drug-induced liver injury in the United States. Liver Transpl 2004;10:1018–1023.
23. Garcia Rodriguez LA, Williams R, Derby LE, Dean AD, Jick H. Acute liver injury associated with nonsteroidal anti-inflammatory drugs and the role of risk factors. Arch Intern Med 1994;154: 311–316.
24. de Abajo FJ, Montero D, Madurga M, Garcia Rodriguez LA. Acute and clinically relevant drug-induced liver injury: a population based case-control study. Br J Clin Pharmacol 2004;58:71–80.
25. Pham B-N, Bernuau J, Durand F, Sauvanet A, Degott C, Prin L, Janin A. Eotaxin expression and eosinophil infiltrate in the liver of patients with drug-induced liver disease. J Hepatol 2001;34: 537–547.
26. Larrey D. Drug-induced liver disease. J Hepatol 2000;32:77–88.
27. Maria VA, Victorino RM. Immunological investigation in hepatic drug reactions. Clin Exp Allergy 1998;28:71–77.
28. Andrade RJ, Lucena MI. Drug-induced hepatotoxicity. N Engl J Med 2003;349:1974–1976.
29. Andrade RJ, Lucena MI, Alonso A, Garcia-Cortes M, Garcia-Ruiz E, Benitez R, Fernandez MC, Pelaez G, Romero M, Corpas R, Duran JA, Jimenez M, Rodrigo L, Nogueras F, Martin-Vivaldi R, Navarro JM, Salmeron J, de la Cuesta FS, Hidalgo R. HLA class II genotype influences the type of liver injury in drug-induced idiosyncratic liver disease. Hepatology 2004;39:1603–1612.
30. Aithal PG, Day CP. The natural history of histologically proved drug induced liver disease. Gut 1999;44:731–735.
31. Lopez-Torres E, Lucena MI, Andrade RJ, Garcia-Ruiz E, Fernandez MC, Pelaez G, Soria de la Cruz MJ, Pizarro A. Tetrabamate-induced hepatotoxicity. Report of seven cases and literature review. Gastroenterol Hepatol 2002;25:589–593.
32. Lucena MI, Andrade RJ, Gomez-Outes A, Rubio M, Cabello MR. Acute liver failure after treatment with nefazodone. Dig Dis Sci 1999;44:2577–2579.

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