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Use of Hy's Law and a New Composite Algorithm to Predict Acute Liver Failure in Patients With Drug-Induced Liver Injury

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BACKGROUND & AIMS: Hy's Law, which states that hepatocellular drug-induced liver injury (DILI) with jaundice indicates a serious reaction, is used widely to determine risk for acute liver failure (ALF). We aimed to optimize the definition of Hy's Law and to develop a model for predicting ALF in patients with DILI. **METHODS:** We collected data from 771 patients with DILI (805 episodes) from the Spanish DILI registry, from April 1994 through August 2012. We analyzed data collected at DILI recognition and at the time of peak levels of alanine aminotransferase (ALT) and total bilirubin (TBL). **RESULTS:** Of the 771 patients with DILI, 32 developed ALF. Hepatocellular injury, female sex, high levels of TBL, and a high ratio of aspartate aminotransferase (AST):ALT were independent risk factors for ALF. We compared 3 ways to use Hy's Law to predict which patients would develop ALF; all included TBL greater than 2-fold the upper limit of normal (×ULN) and either ALT level greater than 3 × ULN, a ratio (R) value (ALT × ULN/ alkaline phosphatase × ULN) of 5 or greater, or a new ratio (nR) value (ALT or AST, whichever produced the highest ×ULN/ alkaline phosphatase × ULN value) of 5 or greater. At recognition of DILI, the R- and nR-based models identified patients who developed ALF with 67% and 63% specificity, respectively, whereas use of only ALT level identified them with 44% specificity. However, the level of ALT and the nR model each identified patients who developed ALF with 90% sensitivity, whereas the R criteria identified them with 83% sensitivity. An equal number of patients who did and did not develop ALF had alkaline phosphatase levels greater than 2 × ULN. An algorithm based on AST level greater than 17.3 × ULN, TBL greater than 6.6 × ULN, and AST:ALT greater than 1.5 identified patients who developed ALF with 82% specificity and 80% sensitivity. **CONCLUSIONS:** When applied at DILI recognition, the nR criteria for Hy's Law provides the best balance of sensitivity and specificity whereas our new composite algorithm provides additional specificity in predicting the ultimate development of ALF.

Keywords: Idiosyncratic Hepatotoxicity; Prognostic Risk Factor; Prediction; Progression.

Drug-induced liver injury (DILI) is a challenge for clinicians, the pharmaceutical industry, and regulatory agencies.[1] Furthermore, acute DILI has been reported to occur in 5%–10% of patients hospitalized for jaundice,[2] and more

Abbreviations used in this paper: **ALF, acute liver failure; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under receiver operating characteristic curve; DILI, drug-induced liver injury; INR, international normalized ratio; LR, likelihood ratio; nR, new ratio; OLT, orthotopic liver transplantation; R, ratio; ROC, receiver operating characteristic; TBL, total bilirubin; ULN, upper limit of normal.**

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often evolves to fulminant hepatic failure than other causes of acute hepatic injury in Western countries.[3,4] Idiosyncratic hepatotoxicity was the presumptive cause in 13%–15% of cases of acute liver failure in reports from the United States and Sweden.[3,4] The spontaneous survival for patients with acute liver failure owing to idiosyncratic drug reactions is usually poor, with 60%–80% mortality without liver transplantation.[5,6]

In the late 1960s, Hyman Zimmerman,[7] the pioneer of modern hepatotoxicology, observed that the combination of jaundice and drug-induced hepatocellular injury was associated with a 10%–50% fatality rate from liver failure (before liver transplantations were performed), provided that other causes for increased bilirubin levels were excluded (hemolysis, Gilbert syndrome, and a major component of cholestasis). Analyses of large numbers of patients with suspected DILI from independent groups have validated these observations, showing 11.7% mortality/liver transplantation in patients with hepatocellular DILI and jaundice in the Spanish DILI Registry[8] and 12.7% in the Swedish Adverse Drug Reactions Advisory Committee retrospective database.[9]

Zimmerman's observation that hepatocellular DILI with jaundice indicates a serious reaction has been referred to as "Hy's Law."[10] Hy's Law cases have been defined more recently as drug-induced liver injury resulting in increased alanine aminotransferase (ALT) levels greater than 3 × the upper limit of normal (ULN) and total bilirubin (TBL) levels greater than 2 × the ULN after excluding other potential causes. This definition has been used by the US Food and Drug Administration over the years to identify drugs potentially capable of causing severe liver injury in the setting of clinical drug development.[1] To exclude cholestatic or mixed cases, the guidance for clinical trials states that for a Hy's Law case the liver injury should not have a significant alkaline phosphatase (ALP) increase reflecting a cholestatic component.[1] However, the definition of a significant cholestatic component is not precisely outlined.[11] An alternative approach to the definition of Hy's Law is to apply the ratio (R) value (ALT × ULN/ALP × ULN) to select hepatocellular cases rather than focusing only on ALT increases because it is assumed that there is much less risk of acute liver failure in cholestatic cases.

Although Hy's Law is based on Zimmerman's[7] clinical observations, its main use today is as a hepatotoxicity indicator in drug development. Nevertheless, Hy's Law also can provide a risk estimation of ALF progression in DILI cases induced by already marketed drugs. The use in this setting, however, is limited by its low specificity. The presence of a more specific algorithm/scale for physicians to predict potential ALF outcomes in established DILI cases could improve the clinical care for these patients.

In the present study we used the Spanish DILI Registry database in search of the best way to identify DILI patients who ultimately progressed to ALF and therefore can be viewed as true Hy's law cases because they developed ALF. We aimed to analyze whether the R value as well as a new ratio (nR) value, in conjunction with total TBL, provide a better way of identifying Hy's Law cases for ALF prediction than the widely used definition of Hy's Law based on ALT increases and whether ALP increases greater than 2-fold indicate cholestatic predominance sufficient to decrease the risk of acute liver failure/orthotopic liver transplantation (ALF/OLT). Once we optimized the standard approach to defining Hy's Law we recognized that there remained room for improvement in specificity. Therefore, we developed a composite statistical model with increased specificity to better predict an unfavorable outcome in patients with DILI in the clinical setting.

Patients and Methods

The study cohort encompassed all patients with idiosyncratic drug-induced liver injury entered into the Spanish DILI Registry since its foundation in April 1994 until August 2012. This prospective database contains detailed demographic, clinical, laboratory, imaging, and histologic (when available) information both at presentation and at follow-up evaluation of the patients included. Each case included in the study was evaluated by a clinician and remitted to the coordinating center where it was re-evaluated by a panel of DILI experts before being included in the database. A structured report form was used to record patient data, including details relating to: (1) the time lapse between the initial intake of the medication and the onset of liver disease and between the discontinuation of the suspected agent and improvement in, or recovery from, liver dysfunction; (2) serology and specific biochemistry to rule out viral hepatitis, autoimmune and metabolic liver disorders, appropriate imaging tests to exclude biliary disease, and any other alternative causes of liver injury; and (3) the outcome of the liver damage. Only cases considered as being drug-related (with the drug as the most likely cause) according to expert clinical judgment then were assessed using the Council for International Organizations of Medical Sciences scale, and only when the cases were classified as highly probable (42%), probable (49%), or possible (9%) were the data incorporated into the database. The criteria for DILI initially were those established by the Council for International Organizations of Medical Sciences (ALT level $>$ 2 × ULN, direct bilirubin level greater than 2 × ULN, or combined increases in ALT, aspartate aminotransferase (AST), and total bilirubin levels provided one of them is $>$2 × ULN)[12] and later restricted to the consensus criteria adopted in 2011 (ALT level $\geq$5 × ULN, ALP level $\geq$2 × ULN, or ALT level $\geq$3 ×ULN + TBL level $\geq$2 × ULN).[13] The pattern of liver injury was classified based on R values (ALT level × ULN/ALP × ULN).[13] A case was considered hepatocellular when R was 5 or greater, cholestatic when R was 2 or less, and mixed when the R value was between 2 and 5, using values from the first available blood analysis after DILI recognition.

The study cohort included 771 DILI patients. Of these, 738 experienced a single DILI episode, 32 had 2 DILI episodes, and 1 patient experienced 3 DILI episodes. Hence, a total of 805 DILI episodes were analyzed, of which 32 led to ALF/OLT. The definition of ALF used in this study was hepatic encephalopathy and coagulopathy (international normalized ratio [INR] $>$ 1.5) as reported by Møller et al.[14] The comparison of demographic parameters was based on available information from the 771 patients. Clinical and analytical parameter analyses were performed using available information corresponding to the 805 episodes at 3 different time points: DILI recognition, peak of ALT level, and peak of TBL level. All percentages were calculated based on the total number of available patient or episode data. For ALF case 31 (Supplementary Table 1), which occurred after accidental re-challenge, no laboratory information could

be retrieved. Underlying diseases analyzed included diabetes mellitus, arterial hypertension, hypothyroidism, and dyslipidemia. The definition of dyslipidemia was based on the criteria of the National Cholesterol Education Program's Adult Treatment Panel III: total cholesterol level greater than 240 mg/dL, high-density lipoprotein cholesterol level less than 40 mg/dL, low-density lipoprotein cholesterol level of 160 mg/dL or greater, or triglyceride level of 200 mg/dL or greater.[15]

An independent Latin American DILI cohort was used to compare with the findings in the Spanish cohort. This Latin American cohort contained similar case information owing to the use of identical data collection protocols and the cases were diagnosed using the same causality assessment procedure as for the Spanish cohort. We failed in our search for a more robust European validation cohort because of the absence of prospective follow-up data, not enough information to obtain R values at different time points, and/or an insufficient number of ALF/OLT cases in the already existing databases.

Statistics

Demographic, clinical, and analytical data corresponding to 771 patients experiencing 805 DILI episodes were compared between those who progressed to ALF/OLT and those who did not. Means were compared by the Student *t* test for independent samples. Analysis of variance was used for comparison of groups. If variables did not follow a normal distribution, a nonparametric Kruskal–Wallis analysis was performed. Analyses of qualitative variables were performed using chi-square tests. Variables that were associated with ALF/OLT in univariate analyses were included as potential covariates in a multiple logistic regression model.

Multivariate analyses were performed using a decision tree method. The chi-squared automatic interaction detection algorithm was used to develop a predictive algorithm for an ALF/OLT outcome in DILI cases. A receiver operating characteristic (ROC) curve based on a specific coordinate (point) in the ROC space to test each of the ALF prediction methods was performed.[16] The McNemar test was used to determine statistically significant differences in sensitivity and specificity between the 4 ALF prediction methods. All results were considered statistically significant when the *P* value was less than .05. All analyses were performed using IBM SPSS Statistics (version 20) and IBM SPSS Modeler (version 15) software package programs (IBM Corp, Chicago, IL).

Results

Demographic and Clinical Presentation of DILI Patients Who Did or Did Not Develop ALF/OLT

A total of 771 patients, who had 805 DILI episodes between April 1994 and August 2012, were included in the analysis. Thirty-two of these DILI patients (4%) developed ALF resulting in death (19; 59%), OLT (12; 38%), or recovery (1; 3%). In addition, 10 patients died within 6 months of DILI recognition of non–liver-related causes. The overall mean age was 54 years (range, 11–89 y), and 394 (51%) were men. The main causative drug group was antibiotics (36%), followed by nervous system (13%), musculoskeletal (12%), cardiovascular drugs (12%), and antineoplastic agents (8%). Jaundice was present in 66% and 52% of the episodes required hospitalization. The mean time between the first blood analysis after DILI initiation and death/liver transplantation in the ALF group was 26.6 days.

When comparing demographics and clinical presentation of the DILI patients (Table 1), we found a female preponderance in the ALF/OLT group (20, 64%). Interestingly, no patient with a diagnosis of dyslipidemia developed ALF/OLT, although there were 101 patients (14%) with dyslipidemia in the non-ALF/OLT DILI group ($P = .027$). The presence of hypersensitivity features and positive autoantibody titers (antinuclear, anti–smooth muscle, antimitochondrial, and liver kidney microsomal autoantibodies) were more frequent in the ALF/OLT group, although none of the differences were statistically significant. Of the 783 episodes with both ALT and ALP values in the first blood analysis, 63% had a hepatocellular type of liver injury ($R \geq 5$). Of these, 26 (5.3%) developed ALF/OLT, whereas only 4 (1.4%) episodes in the cholestatic/mixed group progressed to ALF/OLT. The proportion of cases with hepatocellular damage was significantly larger in the ALF/OLT group compared with the non-ALF/OLT group ($P = .021$).

Comparison of Laboratory Parameters in DILI Episodes That Did or Did Not Progress to ALF/OLT

We evaluated TBL, ALT, AST, ALP levels, and the AST/ALT ratio at different time points: DILI recognition (first laboratory values), peak of ALT, and peak of TBL (Supplementary Table 2). At DILI recognition and peak of ALT median values of TBL, the AST, ALT, and AST/ALT values were significantly higher in the ALF/OLT group compared with DILI patients with a favorable outcome ($P < .001$). At peak of TBL values, the median values of TBL, AST, and AST/ALT, but not ALT, were also significantly higher in the ALF/OLT group ($P < .001$). There were no significant differences in ALP values between the 2 groups at any of the time points.

Logistic Regression Analysis of Demographic, Clinical, and Laboratory Parameters Associated With ALF/OLT

We then used a logistic regression model to determine which parameters were predictive with respect to ALF/OLT development at the different time points. We found that the TBL level and the AST/ALT ratio at the 3 time points, hepatocellular injury at DILI recognition and TBL peak, and female sex at DILI recognition and ALT peak were significant (Table 2).

Detailed demographic, clinical, and laboratory parameters corresponding to the 31 patients who developed ALF resulting in death or OLT are shown in Supplementary Tables 1, 3, and 4.

Improving Hy's Law definition for ALF Prediction

In search of the best way to define Hy's Law for predicting an ALF outcome we compared 3 sets of inclusion criteria, using either the standard TBL level greater than 2 × ULN and ALT level greater than 3 × ULN alone or with

Table 1. Comparison of Demographic Characteristics and Clinical Presentations in 771 Drug-Induced Liver Injury Patients (805 Episodes) Who Did (31) or Did Not (774) Go on to Develop ALF Resulting in Death or OLT

	No ALF/OLT	ALF/OLT	*P* value
Mean age (range), *y*	54 (11–89)	54 (14–83)	.841
>60 y, n (%)	318 (43%)	13 (43%)	.990
Male sex, n (%)	383 (52%)	11 (36%)	.076
BMI, kg/cm^2, mean (range)	26 (16-42)	27 (19-36)	.472
Alcohol use, >40 g/day, n (%)	40 (14%)	2 (15%)	.859
Diabetes mellitus, n (%)	82 (11%)	1 (3.0%)	.167
Arterial hypertension, n (%)	147 (20%)	3 (10%)	.160
Dyslipidemia, n (%)	101 (14%)	0	.027
Hypothyroidism, n (%)	13 (1.8%)	0	.457
Clinical information			
Duration of treatment, median, *days* (range)	29 (1–8559)	37 (4–445)	.840
Time to onset, median, *days* (range)	22 (0–2425)	40 (3–557)	
Time of exposure to drug after onset of symptoms, median, *days* (range) [n]	2 (1–361) [151]	6 (1–47) [7]	.750
Inadvertent re-challenge, n (%)	51 (6.6%)	2 (6.5%)	.634
Type of liver injury (R)			.023
Hepatocellular, n (%)	467 (62%)	26 (87%)	
Cholestatic, n (%)	156 (21%)	2 (6.7%)	
Mixed, n (%)	130 (17%)	2 (6.7%)	
Hypersensitivity features, n (%)[a]	278 (36%)	16 (52%)	.075
Rash	12 (5.0%)	1 (6.0%)	.753
Eosinophilia	105 (14%)	4 (15%)	.868
Positive autoantibodies, n (%)			
ANA	80 (14%)	5 (24%)	.192
ASMA	58 (11%)	3 (16%)	.476
AMA	10 (1.8%)	1 (5.3%)	.291
LKM-1	5 (1.1%)	1 (5.9%)	.087

NOTE. The percentages shown were calculated based on the total number of episodes with available information.
AMA, antimitochondrial autoantibody; ANA, antinuclear autoantibody; ASMA, antismooth muscle autoantibody; BMI, body mass index; LKM-1, liver kidney microsomal autoantibody.
[a]Hypersensitivity features included rash, eosinophilia, lymphopenia, arthralgia, fever, and positive autoantibody titers.

the inclusion of R of 5 or greater or nR of 5 or greater (nR, ALT or AST, whichever was highest × ULN/ALP × ULN). The rationale for assessing nR was based on the finding that AST level was found to be an important predictive value for ALF/OLT development at all time points. The performance of the 3 definitions then was compared at DILI recognition, peak of ALT level, and peak of TBL level (Table 3).

ALT criteria. Almost half of all the cases in our registry fulfilled the ALT criteria at all time points. At peak ALT level, 28 of the 336 cases fulfilling the criteria developed ALF, resulting in death or OLT (93% sensitivity and 43% specificity; positive likelihood ratio [LR] 1.63; negative LR, 0.16). This time point showed the best sensitivity, however, it showed very low specificity (area under ROC curve [AUROC], 0.67).

Table 2. Logistic Regression Analysis of Statistically Significant Demographic, Clinical, and Laboratory Parameters Associated With ALF/OLT

		TBL	Hepatocellular injury	AST/ALT	Female sex
DILI recognition	OR	1.1	3.7	2.0	2.4
	95% CI	1.05–1.14	1.11–12.17	1.18–3.46	1.04–5.73
	P value	<.001	.033	.010	.040
ALT peak	OR	1.1		2.0	3.2
	95% CI	1.06–1.16		1.16–3.40	1.26–8.22
	P value	.001		.013	.015
TBL peak	OR	1.1	3.7	1.4	
	95% CI	1.10–1.19	1.09–12.60	1.02–2.01	
	P value	.001	.035	.040	

CI, confidence interval; OR, odds ratio.

Table 3. Comparison Between Sensitivity and Specificity to Determine ALF/OLT in Cases With ALT Levels Greater Than 3 × ULN + TBL Levels Greater Than 2 × ULN Versus Cases With R of 5 or Greater + TBL Level Greater Than 2 × ULN and Cases With nR* of 5 or Greater + TBL Levels Greater Than 2 × ULN at Different Time Points in the Study Population (805 Episodes)

	ALT > 3, TBL > 2		R ≥ 5, TBL > 2		nR[a] ≥ 5, TBL > 2	
	No ALF/OLT	ALF/OLT	No ALF/OLT	ALF/OLT	No ALF/OLT	ALF/OLT
At DILI recognition	318	27	250	25	255	27
Sensitivity	90%		83%		90%	
Specificity	44%		67%		63%	
LR+	1.6		2.51		2.43	
LR-	0.23		0.25		0.16	
At peak ALT level	308	28	252	23	255	25
Sensitivity	93%		79%		89%	
Specificity	43%		61%		62%	
LR+	1.63		2.02		2.34	
LR-	0.16		0.34		0.18	
At peak TBL level	367	23	231	18	248	18
Sensitivity	77%		72%		72%	
Specificity	49%		65%		65%	
LR+	1.5		2.05		2.05	
LR-	0.46		0.43		0.43	

NOTE. The percentages shown were calculated based on the total number of episodes with available information.
[a]nR was calculated with ALT or AST level, whichever was highest.

R criteria. In comparison, 275 cases fulfilled the R criteria at the time of first available laboratory values (referred to as DILI recognition), including 25 ALF cases resulting in death or OLT (83% sensitivity and 67% specificity; positive LR 2.51, negative LR 0.25). This time point showed the highest specificity and sensitivity for the R criteria (AUROC, 0.74).

nR criteria. A total of 282 cases fulfilled the nR criteria, including 27 ALF cases resulting in death or OLT at DILI recognition (90% sensitivity and 63% specificity; LR+ 2.43, LR-0.16; AUROC, 0.77). This means that a patient who is going to develop ALF is about 2.43 times more likely to have a positive test (or fulfill nR criteria) than a person who will never progress to ALF. Furthermore, the probability of having a negative test result for individuals who will develop ALF is 0.16 times or approximately one sixth of that of those who will never develop ALF. Three ALF cases were not identified with the nR criteria. Of these, 1 hepatocellular case did not fulfill TBL level greater than 2 × ULN at enrollment and 2 cases had a cholestatic/mixed type of injury. One was first seen very late (51 days after onset of symptoms) so the DILI peak was probably missed, and the other was a complex case with basal cholestasis of unknown cause in a co-infected human immunodeficiency virus and HCV patient on antituberculosis treatment (Supplementary Table 1). The nR criteria were found to be most predictive at DILI recognition compared with the other time points (Table 3). The decrease in sensitivity at peak ALT and peak TBL levels with the R and the nR criteria paralleled the evolution of injury pattern, which tended to transition toward cholestatic/mixed as the condition progressed (Figure 1).

Effect of ALP Level Greater Than Two Times the ULN on ALF Development

By using the same time points, we also examined whether an ALP level greater than 2 × ULN indicates cholestatic predominance and therefore decreases the risk of ALF/OLT. The percentage of patients with an ALP level greater than 2 × ULN was not significantly different between episodes, who did or did not go on to develop ALF/OLT, suggesting that an ALP level greater than 2 × ULN does not have a protective role in ALF development (Table 4). Hence, ALP levels greater than 2 × ULN does not exclude Hy's Law because it was observed in a quarter of the ALF cases, but the ALP value was rarely seen to be 4 or more × ULN in these cases (Supplementary Figure 1).

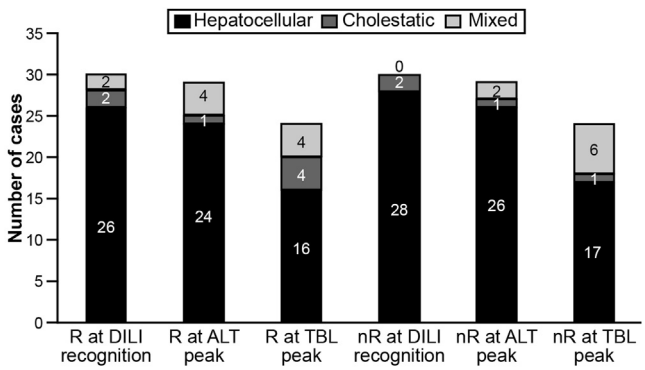

Figure 1. The evolution of the liver injury pattern in acute liver failure/orthotopic liver transplantation cases.

Table 4. Number of ALF/OLT and Non-ALF/OLT Cases With ALP Level Greater Than 2 × ULN at Different Time Points

	ALP >2 × ULN		
	ALF	No ALF	*P* value
At DILI recognition	7 (23%)	256 (34%)	.227
At peak of ALT	9 (31%)	258 (35%)	.663
At peak of TBL	4 (16%)	247 (34%)	.064

NOTE. The percentages shown were calculated based on the total number of episodes with available information.

New Prognostic Algorithm for ALF Prediction in Established DILI Episodes in Clinical Practice

Although Hy's Law serves the purpose of identifying or excluding the risk of ALF, the majority of the cases who met any of the 3 criteria did not develop ALF. Therefore, to increase the specificity, we attempted to develop a prognostic algorithm by which the true risk of a fatal outcome in DILI could be evaluated at DILI recognition by physicians in clinical practice. Here, we used a statistical approach/decision tree technique encompassing demographic, clinical, and biochemical variables at DILI recognition. Hence, this algorithm considers more parameters than Hy's Law (Figure 2). Several biochemical parameters (AST, AST/ALT, and TBL) differed between the ALF/OLT and non-ALF/OLT cases. Examining AST values, we found 17.3 × ULN to be the most appropriate cut-off point ($P < .001$). Subsequently, we divided the population into those with AST levels greater than 17.3 × ULN and those with levels of 17.3 or less × ULN. In the former group, which was found to have a higher risk of ALF/OLT, we found that a new subdivision based on the TBL level ($P = .009$) could enhance the predictive value for ALF/OLT further. Episodes with AST levels greater than 17.3 × ULN and TBL levels greater than 6.6 × ULN were found to have a higher risk of ALT/OLT progression than those with AST levels greater than 17.3 × ULN and TBL levels of 6.6 or less × ULN. The predictive value for episodes with an AST level of 17.3 or less × ULN could also be enhanced further based on their AST/ALT ratio ($P < .001$), whereby having an AST/ALT ratio of greater than 1.5 further increased the risk of ALF/OLT in this group. By using this algorithm we identified 163 (20%) of the 804 episodes as having a higher risk of ALF/OLT. Of these 163 episodes, 24 (15%) cases did in fact develop ALF, resulting in death or OLT, showing 82% specificity and 80% sensitivity (AUROC, 0.8). Although only a small number of ALF cases presented with an AST level of 17.3 or less × ULN, the predictive value in this group was enhanced based on the AST/ALT ratio ($P < .001$), independent of the absolute AST or bilirubin level; cases with an AST/ALT ratio greater than 1.5 showed a greater risk (3 incidences of ALF in 35 cases, 9%) compared with AST/ALT less than 1.5 (3 incidences of ALF in 531 cases, 0.6%).

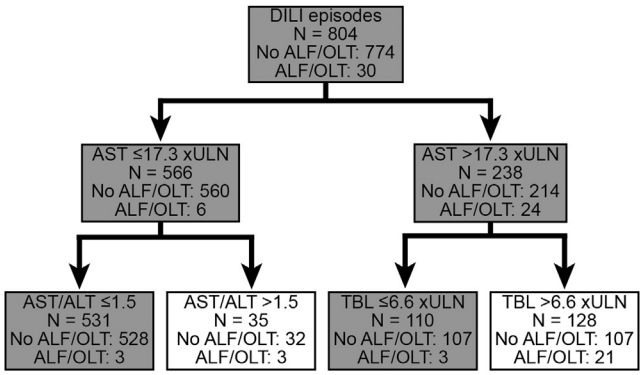

Figure 2. Prognostic algorithm for ALF/OLT to apply at DILI recognition. Cases with a higher risk of ALF/OLT are indicated in *white squares* (specificity, 82%; sensitivity, 80%; positive likelihood ratio, 4.4; negative likelihood ratio, 0.24).

Comparison of the Four Prognostic Methods for ALF Prediction at DILI Recognition

We then determined the ROC areas (AUROC) as follows: 0.67 (ALT criteria), 0.74 (R criteria), 0.77 (nR criteria), and 0.80 (prognostic algorithm) (Figure 3). Furthermore, we compared the 4 methods using the McNemar test to determine statistically significant differences in sensitivity and specificity. Here, we found differences in specificity: ALT vs R, $P < .001$; ALT vs nR, $P < .001$; ALT vs algorithm, $P < .001$; R vs nR, $P = .004$; R vs algorithm, $P < .001$; nR vs algorithm, $P < .001$, but not in sensitivity, which may be owing to the small size of the ALF cohort.

Application of New Hy's Law Definitions and a New ALF Algorithm in an Independent Latin American DILI Cohort

To further examine the new definitions of Hy's Law and the newly developed ALF algorithm we applied them to an independent cohort consisting of Latin American DILI cases. This cohort from the recently established Latin American

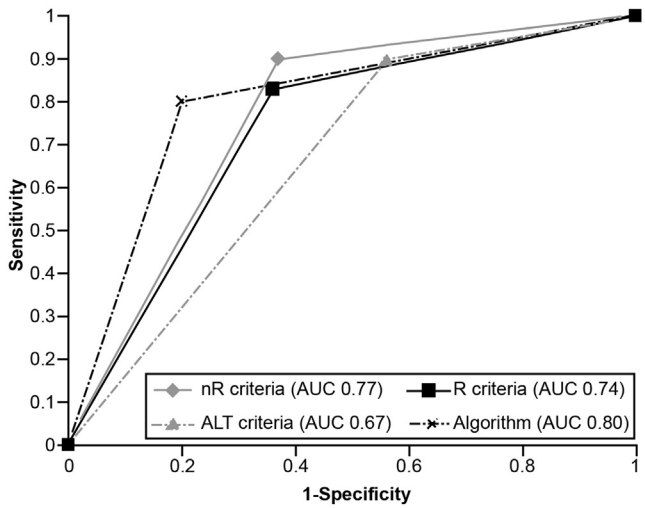

Figure 3. Comparison between the AUROC for ALT, R, and nR criteria and the prognostic algorithm.

DILI network[17] similarly contained DILI cases caused by postmarketing drugs. There were 97 DILI cases, including 5 ALF/OLT cases. When applying the ALT criteria to define Hy's Law for ALF prediction 55 cases fulfilled these criteria, including all those who developed ALF, showing 100% sensitivity and 45.7% specificity. When applying the R criteria, 30 cases were found. These included 4 of the 5 ALF/OLT cases, showing 80% sensitivity and 72% specificity. By using the nR criteria 33 cases fulfilled these criteria, of which 4 developed ALF/OLT. This translates to 80% sensitivity and 68% specificity. We then applied the new ALF algorithm, which resulted in the prediction of 20 cases with increased risk of developing ALF/OLT, including 4 of the 5 cases that in fact had an ALF/OLT outcome. Hence, the ALF algorithm showed 80% sensitivity and 82% specificity. These results are very similar to those obtained with the Spanish DILI Registry cohort, especially using the ALF algorithm.

Discussion

Prediction of severe outcome at DILI recognition remains a challenge in clinical practice. Prompt discontinuation of the offending drug is a crucial step in DILI management, but it is not enough by itself to prevent further damage and even a fulminant outcome in some cases. Assessment of DILI severity is based on derangement in coagulation parameters and the development of encephalopathy.[13,18] However, these are delayed features and at this time point liver parenchymal mass is lost extensively and liver function is affected severely and irreversibly in most patients. Acute liver failure related to idiosyncratic drug reactions is typically delayed,[19] with encephalopathy ensuing up to 26 weeks after jaundice, which makes it particularly important from a clinical perspective to be able to predict who is at risk for ALF at the initial clinical assessment. Furthermore, identifying more precisely those at higher risk of ALF would potentially provoke more close observation, hospitalization, or immediate referral to a transplant center. Alternatively, immediate identification of higher risk might lead to alternative therapeutic interventions or selection for clinical trials of new treatments. Because of the lack of specific biomarkers to predict progression of liver necrosis at early stages of acute DILI, it is necessary to rely on commonly used liver tests for this purpose. In the present study we have used the prospective Spanish DILI Registry database to test the relevance of liver tests at different time points in the prediction of an unfavorable outcome in a large cohort of well-phenotyped DILI patients. The drugs causing ALF/OLT in our series are well-recognized causes of ALF.[20]

We confirm in the current analysis the influence of hepatocellular damage and high values of TBL in the risk of developing ALF/OLT, as previously reported.[8,9,21] Overall, women were not over-represented in the Spanish database, but they represented a higher-risk phenotype. Hence, female sex was a predictor of fulminant outcome, further confirming the results of previous analyses of 603 DILI cases in our cohort[22] and a series of 133 cases of idiosyncratic drug-induced ALF reported by the Acute Liver Failure Study group,[21] in which 70% of the fulminant cases were women. Whether differences in adaptation to injury, hepatic defense mechanisms, or other features influences the predominance of females with liver failure outcomes is unknown and remains to be elucidated. Contrary to the first analysis of the DILI Network group,[23] diabetes was not associated with a worse DILI outcome. Of note, in the DILI Network cohort the majority of the deaths were not liver related, which may account for the difference. Dyslipidemia was less frequent in ALF/OLT cases than in patients with a favorable outcome. The reasons for the protective effect of a history of dyslipidemia are unclear but could be related to the more frequent use of statins by the latter group (8.7% vs 3.2%). Statins exert several potentially beneficial effects on the liver in experimental models, enhancing nitric oxide production, targeting cholesterol at different levels in the mevalonate pathway, and inhibiting drug-induced expression of genes involved in oxidative stress response, drug transport, DNA repair, cell-cycle progression, and cell death, thus providing protection from ischemia/reperfusion,[24] hepatotoxicity,[25] and drug-induced acute liver failure.[26] Statins also improve aminotransferase levels[27] in patients with hepatitis C and have an additive effect, when combined with nonselective β-blockers, in reducing portal pressure in patients with cirrhosis.[28] The finding of reduced dyslipidemia and subsequent statin intake in ALF/OLT patients is at this stage a novel observation, but requires further confirmatory studies.

Interestingly, the degree of aminotransferase increase was found to be associated with a more severe outcome, although the traditional concept in acute liver damage is that irrespective of the etiology, aminotransferase values are not associated with prognosis. Nevertheless, some studies have found that both fulminant hepatic failure[9,20] and chronic liver damage[29] in idiosyncratic DILI are associated with high levels of aminotransferases. The present study also points toward the value of AST level, as well as AST/ALT ratio, for prediction of fatal outcome at any time point. These findings are in agreement with previous studies conducted in DILI[9,30] and viral hepatitis patients,[31] and challenge the dogma that the degree of aminotransferase increase does not have a prognostic value in acute liver injury. Of note, in our cohort, AST but not ALT values remained significantly increased in the ALF/OLT group at peak of TBL, which occurs at a later phase of liver damage. The AST half-life (mostly cytoplasmic) is shorter (about 8 hours) than that of ALT (about 21 hours).[32] An ongoing increase of the AST component exceeding ALT levels might reflect unrelenting hepatocyte damage, release of mitochondrial AST, or possibly some zonal difference in AST vs ALT.

Our initial aim was to attempt to improve the definition of Hy's Law in the prediction of ALF. Thus, using the nR criteria with the first blood test available after presentation provides a good balance between sensitivity and specificity. The predictive value of AST in our new definition of

Hy's Law based on nR criteria may explain the greater sensitivity when compared with the traditional R using ALT values. Furthermore, this nR classifies more cases as hepatocellular. However, delayed presentation may diminish the reliability of predicting ALF risk because there is clearly a tendency for the liver injury pattern to shift to cholestatic/mixed status. This tendency has been observed previously.[18]

Our results do not support the use of absolute ALP level alone in the definition of Hy's Law. In our analysis, the percentage of cases with ALP greater than 2 × ULN was similar between those with and those without ALF/OLT outcomes, suggesting that an ALP level greater than 2 × ULN does not predict a lower risk of ALF/OLT. Nevertheless, the ALP increases seen in the ALF/OLT cases were mostly moderate, with only 2 patients having ALP levels greater than 4 × ULN at DILI recognition. Therefore, we believe it is better to identify Hy's Law cases as hepatocellular injury (R or nR, $\geq$ 5) accompanied by hyperbilirubinemia, without excluding cases based on ALP level.

Hy's Law is used mainly as a signal of serious hepatotoxicity during drug development. Clinical trial settings, in which patients undergo sequential liver test monitoring, typically identify cases at an earlier stage of evolution of injury, which is dampened by prompt drug withdrawal. Because the nR criteria have been developed in the postmarketing setting and focused on a specific outcome, ALF/OLT, we recommend that the modified Hy's Law should be tested by regulators, especially if they are able to combine large clinical trial data sets to generate sufficient number of ALF events.

Although the modified Hy's Law performs particularly well in predicting who will not develop ALF (ie, absence of meeting the nR criteria at DILI recognition), most of the cases who meet the criteria do not develop ALF. Therefore, we extended our analyses beyond the Hy's Law criteria in an attempt to improve the accuracy for predicting ALF when patients are first seen.

Thus, we developed a prognostic algorithm that may be used by physicians in clinical practice to evaluate DILI cases at recognition regardless of the type of liver injury. It gives a special weight to the degree of AST increase and to the AST/ALT ratio, which were found to be independent risk factors for ALF/OLT at all time points in our analysis. Apart from being highly specific while preserving sensitivity, the decision tree also allows the prediction of ALF before total bilirubin values are increased substantially. The choice between using the nR criteria or the prognostic algorithm depends on the physician's necessity for major sensitivity or specificity in the ALF prognosis. Thus, the nR criteria will capture most of the ALF cases, while the proposed algorithm will provide a heightened alert for a more serious outcome with less chance of a false-positive diagnosis.

The nR criteria for Hy's Law as well as the new algorithm were also shown to be predictive of ALF in an independent and ethnically different cohort of idiosyncratic DILI cases. However, the reduced number of ALF cases in this cohort prevents genuine validation. This may have contributed to our inability to find significant differences in this cohort. Hence, a larger prospective DILI cohort including a substantial number of ALF cases is required to properly validate performance of the modified Hy's Law definition and the new ALF algorithm.

In summary, in the absence of more sensitive and specific biomarkers for DILI severity and progression, we have shown that there is room for improvement with the combined use of aminotransferase and total bilirubin levels that enables both a further refinement of Hy's law and a composite algorithm when the patient is first seen to estimate the risk of a fulminant course. These new tools should be validated prospectively in well-phenotyped patients with DILI.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2014.03.050.

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Supplementary Table 1. Characteristics of Hepatotoxicity Patients Who Developed Acute Liver Failure Resulting in Death or Orthotopic Liver Transplantation

Case	Age, *y*/sex	Responsible drug	BMI	Days of treatment	Indication	Associated diseases	Associated medications
1	83/M	Flutamide	25.4	445	Prostate cancer	Upper gastrointestinal bleeding	Leuprorelin, cimetidine
2	38/F	Isoniazid/rifampicin/pyrazinamide		27	Tuberculosis		
3	50/F	Isoniazid/rifampicin/pyrazinamide		53	Tuberculosis	WPW syndrome, irritable bowel syndrome	
4	29/M	Isoniazid/rifampicin/pyrazinamide		9	Tuberculosis	HIV, HCV, endocarditis, drug addiction	Trimethoprim sulfamethoxazole, fluconazole, piperacillin/ tazobactam
5	37/M	Isoniazid/rifampicin/pyrazinamide		11	Tuberculosis	HIV, HCV, drug addiction	Fluconazole, phenytoin, ceftriaxone
6	73/F	Nefazodone		50	Depression	Drug allergy	Lorazepam, iron-sorbitol-citric acid complex, almagate
7	14/F	Flutamide	21.2	89	Hirsutism, acne vulgaris		
8	70/M	Flutamide		114	Prostate cancer	Tuberculosis	Leuprorelin
9	73/F	Ebrotidine		54	Gastroesophageal reflux	Hiatal hernia, goiter	Almagate, bromazepam
10	76/F	Mianserin	26.9	64	Depression	High blood pressure	Bentazepam, citalopram, enalapril
11	66/F	Nimesulide		252	Connective tissue disease	Drug allergy	Dihydroergocristine, acetylsalicylic acid
12	61/F	Amoxicillin-clavulanate		21	Erysipelas	Hysterectomy, cirrhosis, cardiomyopathy, tuberculosis, meningitis	Risperidone, oxazepam, tazobactam
13	44/F	Ibuprofen	35.8	12	Arthrosis	Depression, breast cancer, drug allergy	Fluoxetine, letrozole
14	37/F	Orlistat	29.3	21	Overweight		Ibuprofen
15	59/F	Ibuprofen	27.3	11	Pain	Arthrosis, peripheral vascular disease	Tribenoside
16	79/F	Clomethiazole	29.7	32	Depression	Diabetes mellitus	Thioridazine
17	63/F	Nimesulide	21.6	261	Arthrosis		
18	56/F	Carbamazepine		29	Epilepsy	Non-Hodgkin lymphoma	
19	42/F	Calcium carbimide/ venlafaxine		65/79	Alcoholism	Alcoholism	Alprazolam
20	78/M	Flutamide/losartan		75/75	Prostate cancer/ hypertension	Gastric cancer	Triptorelin
21	25/M	Retinol (vitamin A)	24.7	25	Psoriasis	Perianal fistula, atopic dermatitis, allergic rhinitis	Amoxicillin
22	32/F	Isoniazid/rifampicin/pyrazinamide		34	Tuberculosis		Acetaminophen
23	68/F	Amoxicillin-clavulanate/moxifloxacin	25.7	11/3	Respiratory infection	Tuberculosis	Ibuprofen
24	63/M	Levofloxacin/doxycycline	28.4	4/6	Pneumonia	Arthrosis, AV block, laryngeal cancer	Naproxen, dalteparin
25	37/F	Amoxicillin-clavulanate	19.5	6	Dental abscess		
26	73/M	Bicalutamide		367	Prostate cancer	Drug allergy, alcoholic liver disease	Triptorelin
27	43/M	Clopidogrel	23.4	345	Coronary artery disease	Cardiomyopathy	Acetylsalicylic acid, atorvastatin
28	42/M	Orlistat	31.4	67	Obesity	High blood pressure	
29	57/F	Disulfiram		39	Alcoholism		
30	66/M	Amoxicillin-clavulanate		21	Open fracture	Hyperuricemia	Omeprazole, metamizol, others
31	43/F	Amineptine			Depression		Lorazepam, cyproterone, clorazepate dipotassium

AV block, atrioventricular block; BMI, body mass index; HIV, human immunodeficiency virus; M/F, male/female; WPW syndrome, Wolff Parkinson White syndrome.

Supplementary Table 2. Comparison of Laboratory Parameters in Drug-Induced Liver Injury Episodes, Which Did (31) or Did Not (774) Lead to ALF or OLT at Different Time Points

Laboratory parameters (×ULN), median (range)	No ALF/OLT	ALF/OLT	*P* value
At DILI recognition			
TBL	4.5 (0.1–45)	14 (1–32)	<.001
ALT	9.2 (0.5–203)	28 (1.7–104)	<.001
AST	6.3 (0.2–529)	36 (2.9–167)	<.001
AST/ALT	0.7 (0.01–9.3)	1.2 (0.5–4.9)	<.001
ALP	1.5 (0.15–22)	1.3 (0.3–7.1)	>.05
At peak ALT level			
TBL	4.5 (0.14–45)	14 (2.3–56)	<.001
ALT	10 (0.5–203)	29 (2.8–142)	<.001
AST	6.6 (0.03–528)	44 (41–167)	<.001
AST/ALT	0.7 (0–9.3)	1.2 (0.6–4.9)	<.001
ALP	1.5 (0.2–33)	1.5 (0.3–7.1)	>.05
At peak TBL level			
TBL	5.1 (0.08–54)	26 (9–56)	<.001
ALT	7.2 (0.2–177)	8.6 (1.1–143)	>.05
AST	4.7 (0.2–145)	14 (2.3–167)	<.001
AST/ALT	0.8 (0.01–12)	1.3 (0.4–7.1)	<.001
ALP	1.4 (0.15–33)	1.2 (0.4–7.5)	>.05

NOTE. The percentages shown were calculated based on the total number of episodes with available information.

Supplementary Table 3. Times of Treatment, Liver Transplantation/Death, and Laboratory Parameters in ULN at DILI Recognition in ALF/OLT Patients

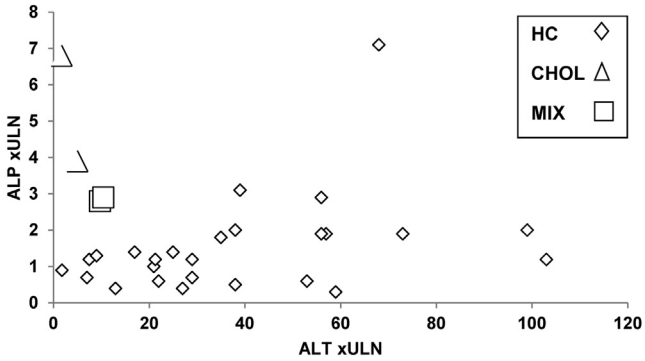
Case	Days from first blood analysis after DILI initiation until death or OLT	Days to onset of symptoms	Days of drug exposure after onset of symptoms	Days from onset of symptoms to first analysis	New ratio	AST	ALT	ALP	TBL	PT/INR
1	22 (death)	448	0	5	HC	9.7	7.5	1.2	32	31%
2	19 (death)	19	8	7	HC	35	29	0.7	13	30%
3	8 (OLT)	45	8	11	HC	38	35	1.8	3.4	58%
4	32 (death)	6	3	0	HC	47	9.7	2.8	2.3	
5	16 (death)				CHOL	3.8	1.7	6.8	2.0	89%
6	25 (death)	47	3	9	HC	44	27	0.4	17	6%
7	9 (OLT)	75	14	15	HC	46	59	0.3	7.6	29%
8	44 (death)	83	31	0	HC	38	38	0.5	16	34%/1.71
9	7 (death)	47	7	7	HC	67	57	1.9	15	22%
10	95 (death)	45	19	20	HC	22	22	0.6	26	53%
11	17 (death)	238	14	11	HC	47	25	1.4	14	35%
12	11 (death)	71	0	0	HC	2.9	1.8	0.9	27	41%
13	5 (death)	7	5	5	HC	26	10	2.9	9.6	42%
14	18 (OLT)	21	0	9	HC	28	39	3.1	9.5	38%
15	54 (OLT)	3	8	9	HC	40	68	7.1	20	63%
16	31 (death)	21	11	13	HC	62	56	1.9	16	45%
17	25 (OLT)	210	51	51	CHOL	7.7	4.6	3.9	28	
18	(death)	4	25	27	HC	68	56	2.9	11	31%
19	28 (death)	64	1	2	HC	21	17	1.4	11	
20	5 (death)	75	0	0	HC	167	99	2.0	12	27%
21	68 (OLT)	25	0	0	HC	31	38	2.0	14	50%
22	9 (OLT)	33	1	1	HC	21	21	1.0	3.2	50%
23	7 (OLT)	11	0	11	HC	8.0	13	0.4	15	INR 4
24	24 (death)	2	2	0	HC	5.9	7.0	0.7	1.0	61%
25	42 (OLT)	29	0	6	HC	45	29	1.2	10	32%
26	74 (death)				HC	22	21	1.2	8.8	
27	(OLT)	299	46	24	HC	47	103	1.2	15	67%
28	(OLT)	67	0	26	HC	71	53	0.6	20	45%
29	0 (OLT)	34	5	10	HC	69	73	1.9	14	13%
30	23 (death)	21	0	6	HC	24	9.0	1.3	21	46%/1.7

CHOL, cholestatic; HC, hepatocellular; PT, prothrombin time.

Supplementary Table 4. Laboratory Parameters in ULN at Peak ALT and Peak TBL Levels in Acute Liver Failure/Orthotopic Liver Transplantation Patients

	Peak of ALT level					Peak of TBL level				
Case	AST	ALT	ALP	TBL	PT/INR	AST	ALT	ALP	TBL	PT/INR
1	9.7	7.5	1.2	32	31%	4.9	2.5	1.2	33	17%
2	35	29	0.7	13	30%	2.3	6.0	0.7	31	20%
3	65	39	1.5	14	40%	65	39	1.5	14	40%
4	47	9.7	2.8	2.3		7.8	1.2	7.5	29	
5	14	7.3	3.6	9	44%	14	7.3	3.6	9.0	44%
6	44	27	0.4	17	6%	4.2	1.6	0.4	31	20%
7	46	59	0.3	7.6	29%	17	27		17	16%
8	38	38	0.5	16	34%/INR, 1.71	8.1	6.6	0.8	46	INR, 1.7
9	69	65		17	16%	69	65		17	16%
10	22	22	0.6	26	53%	22	22	0.6	26	53%
11	47	25	1.4	14	35%	2.6	1.8	1.0	38	
12	4.1	2.8	0.9	56	51.7%	4.1	2.8	0.9	56	51.7%
13	26	10	2.9	9.6	41.7%	64	9.0		14	35.5%
14	28	39	3.1	9.5	38%	15	11	1.1	20	17%
15	40	68	7.1	20	63%	15	15	4.1	31	46%
16	62	56	1.9	16	45%	6.2	8.0	1.4	35	10%
17	7.7	4.6	3.9	28		3.0	1.8	1.2	43	
18	68	56	2.9	11	31%	6.3	7.5	1.9	23	8%
19	21	17	1.4	11		4.1	1.1	1.3	43	
20	167	99	2.0	12	27%	129	99	2.0	13	25%
21	57	58	2.0	26	46%	57	58	2.0	26	46%
22	34	21	1.0	5.6	10%	46	18		17	10%
23	9.4	14	3.0	7.4	INR, 4	8.1	13	0.4	15	INR 4
24	40	38	1.5	4.2	17%	2.7	5.3	0.9	30	18%
25	45	29	1.2	10	31.7%	53	27	2.2	14	37%/INR, 1.8
26	22	21	1.2	8.8			3.7		23	41%
27	103	143	0.8	27	35%	103	143	0.8	27	35%
28	89	60	0.6	24	30%	17	19	0.9	26	26%
29	69	73	1.9	14	13%	69	73	1.9	14	13%
30		18	2.8			24			21	

PT, prothrombin time.



Supplementary Figure 1. ALP/ALT relationship at DILI recognition in acute liver failure/orthotopic liver transplantation cases.