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Early ALT response to corticosteroid treatment distinguishes idiosyncratic drug-induced liver injury from autoimmune hepatitis

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Abstract

Background: Drug-induced liver injury (DILI) and idiopathic autoimmune hepatitis (AIH) are competing diagnoses in patients with acute liver injury (ALI) and drug intake. In absence of unequivocal markers, scores like RUCAM and AIH are used to distinguish both entities. However, in some cases the diagnosis remains ambiguous. Our aim was to identify a simple parameter to discriminate DILI and AIH shortly after starting corticosteroid treatment.

Methods: For the current analysis, 44 patients with ALI who took at least one drug and who received corticosteroids were included and comprised 22 DILI and 22 AIH cases. Scores of AIH and RUCAM were calculated at initial presentation, the final diagnosis was made from analysing the course of disease. Changes in the serum alanine aminotransferase (ALT) concentrations after starting corticosteroid treatment were determined and compared between the DILI and AIH groups.

Results: Fifty-nine per cent of patients (n = 26) were correctly classified at presentation by AIH score and RUCAM respectively. However, in one-third (n = 13) of the 44 patients, results were inconclusive and five other patients were misclassified. The decrease in ALT levels 1 week after the initiation of steroid therapy was significantly more pronounced in patients with the final diagnosis of DILI than in AIH patients (accuracy 77%). This difference was also observed in the 18 initially misclassified or inconclusive cases (accuracy 83%).

Conclusion: Short-term response of ALT to corticosteroid therapy helps to differentiate DILI and AIH. This finding may be helpful in treatment decision for patients with inconclusive diagnostic scores.

KEYWORDS
acute liver injury, alanine aminotransferase, DILI, medication, toxicity

Abbreviations: AIH, autoimmune hepatitis; ALF, acute liver failure; ALI, acute liver injury; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, anti-mitochondrial antibodies; ANA, antinuclear antibodies; DILI, drug-induced liver injury; HLA, human leukocyte antigen; IgG, immunoglobulin G; LKM, liver kidney microsome; MH, monocyte-derived hepatocyte-like; RUCAM, Roussel Uclaf Causality Assessment Method; SLA, soluble liver antigen; SMA, smooth muscle antibodies; TB, total bilirubin; ULN, upper limit of normal.

Sabine Weber and Andreas Benesic contributed equally to the work.

1 | INTRODUCTION

Drug-induced liver injury (DILI) is a rare adverse drug reaction that is the leading cause of acute liver failure (ALF) in the US and Europe.[1-4] The reported incidence ranges from 14 to 19 cases per 100 000 individuals per year, but might be higher since only a few population-based studies exist.[5,6] Currently, DILI is a diagnosis of exclusion; other, more common causes of liver injury must be ruled out to establish the diagnosis. In absence of a reliable laboratory test, DILI diagnosis can be a challenge, particularly when features of autoimmune hepatitis (AIH) are present.[7] Since immunologic mechanisms play a major role in pathogenesis of idiosyncratic DILI[8] a range of drugs has been associated with injury patterns that may be indistinguishable from idiopathic AIH.[9-11]

Moreover, histological patterns typically associated with AIH have also been observed in patients with DILI, further complicating the differentiation between the two entities.[12-14] The finding of cirrhosis at presentation and subsequent signs of chronicity favour the diagnosis of AIH. Yet, since only 20%-30% of patients with acute liver injury (ALI) due to AIH will show signs of cirrhosis,[11,15,16] the absence of cirrhotic liver tissue cannot be used as a diagnostic criterion for DILI.

At initial presentation, autoimmune features cannot reliably discriminate between DILI and aggravated pre-existing or new-onset AIH.[11,17] In these unclear cases, international treatment guidelines recommend treatment with corticosteroids and close monitoring of the patients upon reduction in immunosuppressive therapy.[18-20] Although some patients with DILI and autoimmune features will recover spontaneously upon discontinuation of the causative agent, immunosuppressive drugs are often used if liver injury persists.[17] DILI with autoimmune features usually responds well to corticosteroid therapy and the tapering and ultimate withdrawal of immunosuppressive therapy is rarely accompanied by a relapse.[11,17,21] In contrast, most patients suffering from AIH present with a flare up of hepatitis when immunosuppressive therapy is reduced or withdrawn after remission, and in most cases, patients require long-term, possibly lifelong immunosuppression.[22,23] Therefore, once corticosteroid treatment has been initiated in DILI patients – either because of the suspicion of AIH or because of persistent liver injury despite withdrawal of medication – the exclusion of AIH can only reliably be confirmed by a more favourable outcome under long-term immunosuppression. Taking into the account the more beneficial response towards corticosteroids seen in DILI patients, it seems plausible that discontinuation could be conducted more rapidly in those patients and therefore unnecessary long-term immunosuppression with a broad spectrum of side effects might be avoided. However, data about the early response towards immunosuppressive therapy in DILI and AIH patients are scarce. Without being able to reliably differentiate DILI and AIH, rapid reduction or discontinuation of immunosuppressive therapy harbours the potentially life-threatening risk of disease recurrence in the case the underlying cause of liver injury was idiopathic AIH.

Therefore, in the specific subset of patients with an inconclusive diagnosis, reliable diagnostic criteria are needed to differentiate DILI and AIH early in the disease course to provide the optimal therapy for each individual patient.

Key points

- This study shows that the decrease in alanine aminotransferase serum concentrations after 1 week of corticosteroid treatment helps to separate drug-induced liver injury (DILI) from autoimmune hepatitis patients.
- This may prevent unnecessary long-term treatment with immunosuppressive drugs in DILI patients.

Considering the above-mentioned different features DILI and AIH during long-term immunosuppression, we hypothesized that the early response towards corticosteroid treatment might be more marked in patients with DILI than in AIH patients. To this end, we investigated 44 patients (22 patients with DILI and 22 patients with AIH as final diagnosis) from our prospective study on idiosyncratic DILI.[24] They either presented with autoimmune features and/or did not show any improvement of liver injury despite the withdrawal of the causative agent and thus received corticosteroids during the course of the disease. We aimed to identify a simple parameter such as ALT for the differentiation of DILI and AIH early after treatment initiation and compared the diagnostic value of AIH score, RUCAM, histology and outcome during short-term immunosuppression.

2 | METHODS

2.1 | Patients

Data from patients who were referred to the University Hospital Munich and recruited for our study on the diagnosis of idiosyncratic DILI in patients with ALI (ClinicalTrials.gov: NCT 02353455) were analysed. For this investigation, all patients presenting at our centre between March 2013 and October 2018 with ALI and the intake of at least one drug or herbal and dietary supplement were evaluated. Written informed consent was obtained from each patient. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki and was approved by the ethics committee of the Faculty of Medicine, LMU Munich (Project Number 55-13). All authors had access to the study data and reviewed and approved the final manuscript.

At enrolment, all study participants were questioned about their medical history, symptoms associated with liver injury, comorbidities, current or previous medications and the consumption of herbal and dietary supplements or over-the-counter medication. Age, gender, ethnicity, height, weight and relevant data from clinical investigations were recorded. Serological data on hepatitis A, B, C, D and E, cytomegalovirus, Epstein-Barr virus and herpes simplex virus were available from all patients enrolled. Indirect immunofluorescence testing for antinuclear antibodies (ANA) and anti-mitochondrial antibodies (AMA) is regarded positive by the standards of our hospital's Institute for Laboratory Medicine at

titre was ≥1:100. Titres for smooth muscle antibodies (SMA), soluble liver antigen (SLA) and liver kidney microsome (LKM) obtained by ELISA and/or Western Blot are not available and only positive or negative results being reported. Immunoglobulin G (IgG) levels with a normal range of 7-16 g/L were tested in every patient upon onset. Genotyping of human leukocyte antigen (HLA)-DR1 locus was additionally performed in a proportion of patients using the Genotyping Kits OneLambda RSSO1A 015_02, OneLambda RSSOH1C 06A_02, OneLambda RSSO1B 018_04 20012016, OneLambda RSSOH2B1 011_0 and OneLambda RSSO2Q 011_04 161018. Histology reports from patients in whom liver biopsy was performed as deemed necessary by the treating physicians were extracted from the medical records. The type of infiltration (portal vs interface) and of cholestasis (ductal, canalicular, hepatocellular or hepatocellular and canalicular) as well as the degree of fibrosis (mild, moderate, intermediate and severe) were recorded. The intensity of the infiltrates, cholestasis and specific inflammatory cell infiltrates was graded as mild, intermediate and severe. In addition, the diagnosis suggested in the final histology report was noted.

For the current analysis, out of 288 patients enrolled in our study until October 2018, 44 patients fulfilling the criteria for ALI (see next paragraph) who received corticosteroids during the course of their disease were analysed. The final diagnosis was DILI in 22 cases and AIH in 22 cases. Of the 116 patients with non-DILI–related ALI, 86 patients were excluded from the analysis because of alternative causes of ALI, leaving 30 AIH cases. Eight of those AIH patients were excluded because they were either lost to follow-up or follow-up was too short to verify the final diagnosis. In addition, 150 DILI patients were excluded because they did not receive corticosteroids (Figure 1). The final diagnosis of DILI or AIH was made retrospectively after reassessment of the initially suspected diagnosis based on the course of disease, response to steroid treatment and relapse after withdrawal of immunosuppression and liver histology (Figure 2). In addition, the MetaHeps test, using monocyte-derived hepatocyte-like (MH) cells to detect drug toxicity with high sensitivity and specificity,[25] was performed for every patient.

Acute liver injury was defined according to consensus criteria established by an expert group in 2011: (a) alanine aminotransferase activity (ALT) ≥ 5 × upper limit of normal (ULN); (b) alkaline phosphatase activity (ALP) ≥ 2 × ULN or (c) ALT ≥ 3 × ULN and total bilirubin (TB) ≥ 2 × ULN.[26-29] The ULN was 35 U/L (women) and 50 U/L (men) for ALT. For ALP, the ULN 105/135 U/L (women/men) and the ULN for TB was 1 mg/dL. The Roussel Uclaf Causality Assessment Method (RUCAM) was calculated for up to five drugs and the maximum score was used for diagnosis.[30] The causative agent was identified using the RUCAM and expert opinion and supported by the MH cell test results. For every patient, the pretreatment AIH score defined by the Revised Scoring System for the diagnosis of AIH was calculated.[31] The cut-off values for probable and definite AIH were 12 and 15 respectively.

Acute liver failure was defined by the criteria of the American Association for the Study of Liver Diseases: (a) the absence of pre-existing liver disease; (b) coagulopathy with an INR (international normalized ratio) ≥1.5 in the absence of oral anticoagulants; and (c) hepatic encephalopathy.[28]

All patients were closely monitored after the initiation of corticosteroid treatments, including an evaluation of the clinical development and laboratory tests to assess liver damage and function. The maximum reduction in ALT concentrations per day during week 1 and 2 was assessed for every patient. Maximum reduction in ALT was calculated using ALT values on day 5-7 for week 1 and day 11-14 for week 2. In order to avoid bias by different days of blood sampling after treatment initiation, ΔALT per day was calculated from the difference of ALT at treatment initiation and ALT on day X of treatment divided by X. ΔALT% per day was calculated as percentage of ΔALT per day of ALT at treatment initiation to minimize bias by different ALT levels at treatment initiation. Relapse was defined as an increase in ALT levels that exceeded the ULN or baseline value in case ALT levels had not previously been normalized. Although relapse of AIH is defined as an elevation of the ALT level >3 × ULN according to criteria established by the international AIH group, a relapse might also occur with a more moderate increase in ALT levels, particularly when IgG levels are also elevated again.[18] Thus, patients who presented with an increase in ALT levels ranging between 1 and 3 times the ULN or baseline value after tapering or withdrawal of immunosuppressive therapy were also regarded as having a relapse and treatment was reinitiated accordingly.

2.2 | Statistical analysis

Data were tested for normal distribution using the Kolmogorov-Smirnov test and are presented as mean ± SD or median and range respectively. Statistical analyses were performed using SPSS software (version 25.0.0.1; IBM). After testing for a normal distribution, parametric or non-parametric tests (Chi-square test, Fisher's exact test, Student's *t* test, Kruskal-Wallis test or Mann-Whitney *U* test) were applied. $P < .05$ was considered to indicate a statically significant difference. Receiver operating characteristic (ROC) curves were calculated using SPSS to evaluate the cut-off values, sensitivity and specificity of the investigated parameters.

3 | RESULTS

3.1 | Characteristics of the patients at presentation

3.1.1 | General features

Fourty-four patients were included in this study, 22 with DILI and 22 with AIH as final diagnosis. Patient characteristics are summarized in Table 1. No significant differences were observed in age, gender distribution, ethnicity or body mass index. The distribution of the severity score and the incidence of ALF were also comparable in both groups. Analysis of HLA genes was performed in 16 patients (10 DILI and 6 AIH patients, respectively) with similar rates of HLA-DRB1*03 or HLA-DRB1*04 polymorphisms in DILI and AIH patients (70.0% and 66.7% respectively; $P = .639$). The causative agents of DILI episodes were

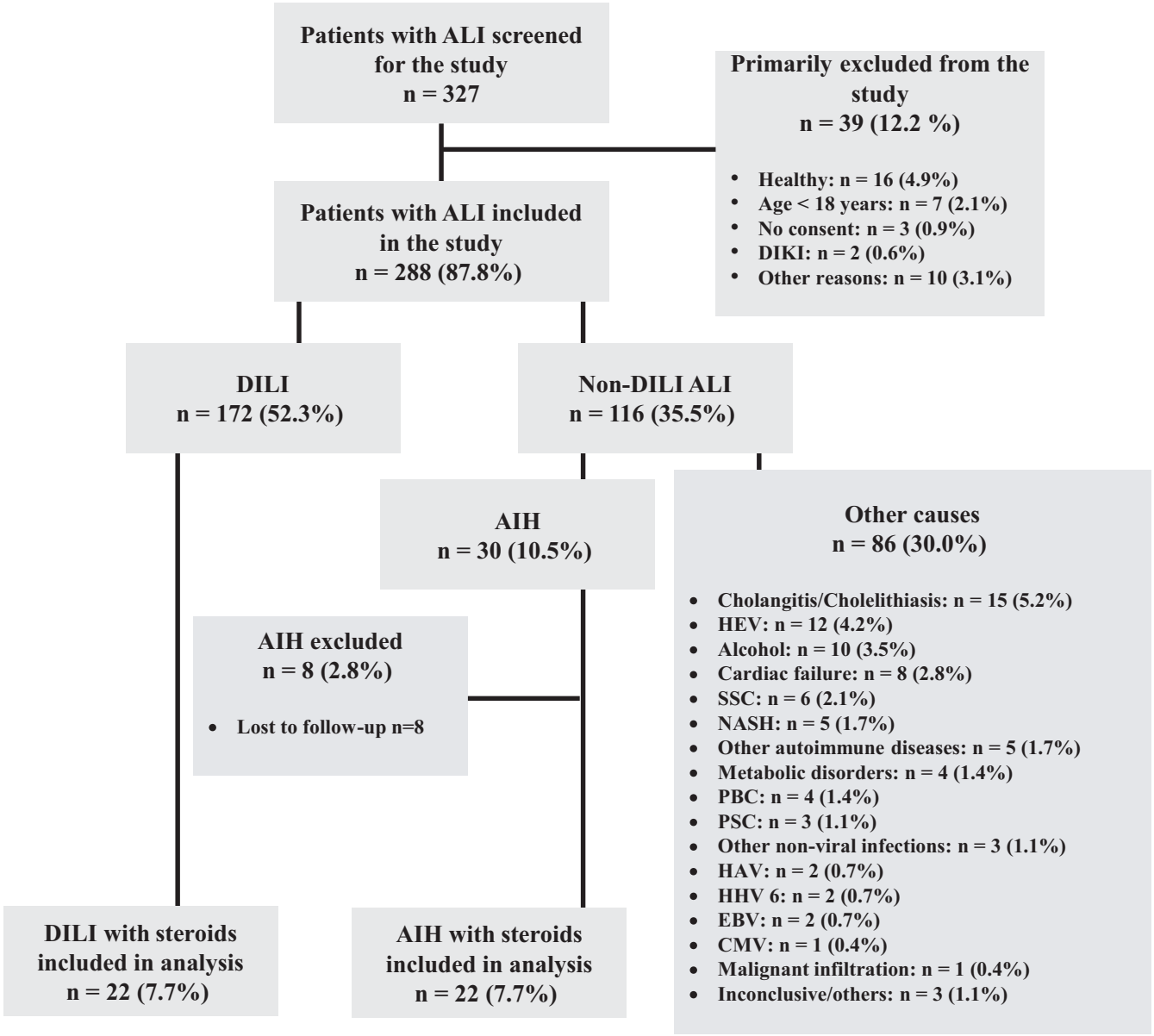

FIGURE 1 For the current retrospective analysis, patients were selected from the study on the diagnosis of idiosyncratic DILI in patients with acute liver injury: 22 DILI patients who received corticosteroids and 22 AIH patients. Number and reasons of primary exclusion as well as exclusion from the current analysis are demonstrated. Percentages of secondary exclusion were calculated from the baseline of 288 patients included in the study. AIH, autoimmune hepatitis; ALI, acute liver failure; CMV, cardiomegaly virus; DIKI, drug-induced kidney injury; DILI, drug-induced liver injury; EBV, Epstein-Barr-Virus; HAV, hepatitis A virus, HEV, hepatitis E virus; HHV 6, human herpesvirus 6; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; SSC, secondary sclerosing cholangitis. Metabolic disorders included Wilson's disease and hemochromatosis, other non-viral infections included liver abscesses and echinococcosis

non-steroidal anti-inflammatory drugs (NSAIDs) in six cases (metamizole in three and diclofenac in three cases), lipid-lowering agents in two cases (atorvastatin/ezetimibe and atorvastatin respectively), new oral anticoagulants in two cases (rivaroxaban and dabigatran), an herbal medication in one case (celandine) and other drugs in eight cases (amitriptyline, imatinib, infliximab, interferon beta, methocarbamol, methylprednisolone, minocycline and pembrolizumab). In three cases, two different drugs were equally possible causative agents (metamizole or umckaloabo, simvastatin or carbimazole and diclofenac or medroxyprogesterone, respectively). For a detailed listing of all drugs taken by DILI patients please refer to Table S1. For the concomitant medication of the individual AIH patients refer to Table S2.

3.2 | Comparison of RUCAM and AIH scores

The median RUCAM of patients with DILI as final diagnosis was 6 (3-10) (median, range), and thus was significantly higher than the score of the patients with AIH ($P < .01$), who presented with a median

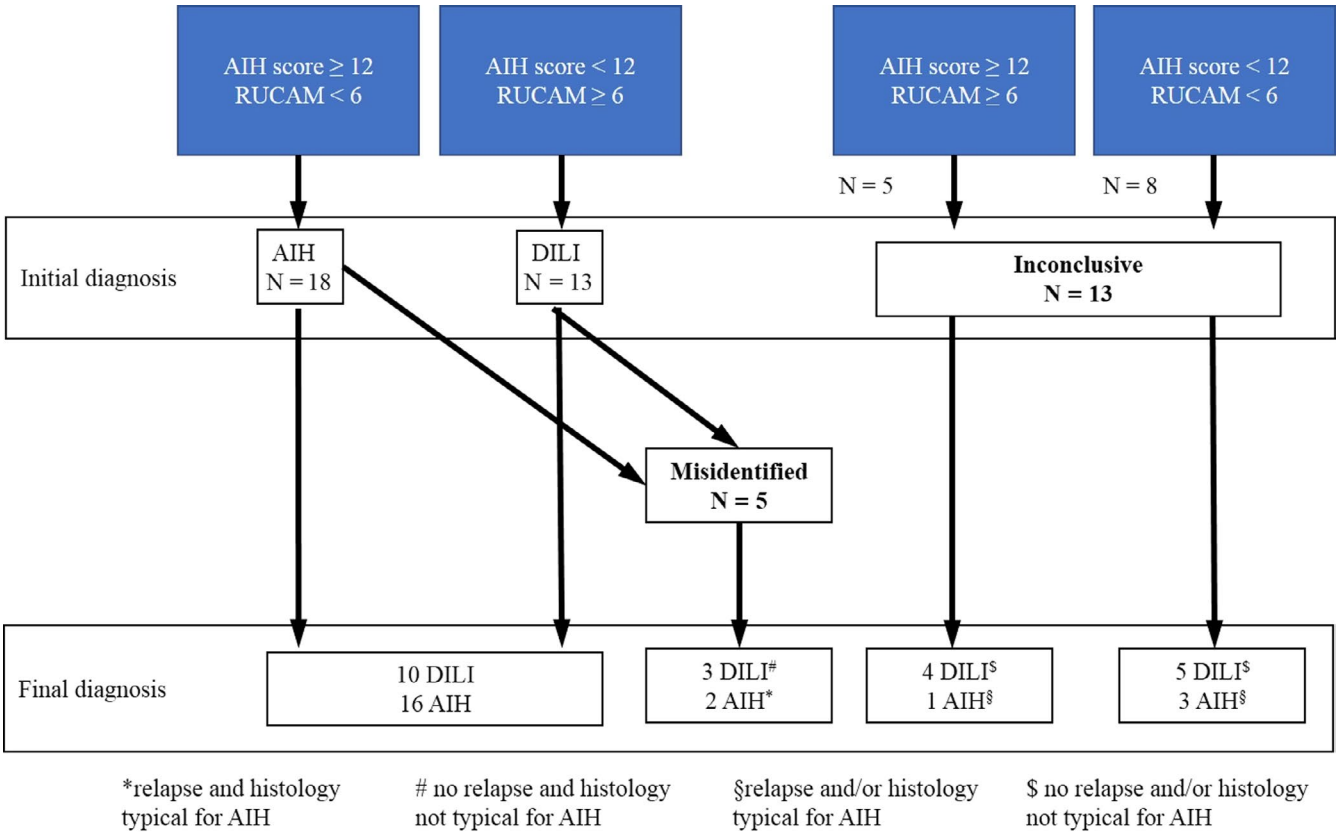

FIGURE 2 The RUCAM and AIH scores were conclusive in 31 cases, however five of those were misdiagnosed by the scoring systems. Thus, 10 DILI and 16 AIH patients were correctly diagnosed by AIH score and RUCAM. In 13 cases, AIH score and RUCAM were inconclusive: five patients had an AIH score ≥12 and RUCAM <6; eight patients had an AIH score <12 and RUCAM <6. AIH, autoimmune-hepatitis; DILI, drug-induced liver injury; RUCAM, Roussel Uclaf Causality Assessment Method

score of 3.5 (0-7). The sensitivity of the RUCAM with a cut-off of 6 was 32% and the specificity was 91%.

The AIH score was significantly higher in patients with AIH as final diagnosis than in patients with DILI, who presented median scores of 13 (9-18) and 9.5 (4-14) respectively (P = .01). The sensitivity of the pretreatment AIH score with a cut-off of ≥12 in our cohort was 59% and the specificity was 82%. When the simplified AIH score[32] was calculated, no statistical difference was found between DILI and AIH patients with a median of 4 (2-6) in DILI vs 5 (1-7) in AIH patients respectively (P = .385).

Antinuclear antibodies and elevated IgG serum levels are considered as markers pointing towards the diagnosis of AIH.[31,32] Interestingly, the rate of patients tested positive for ANA did not differ significantly between DILI and AIH (AIH: 86.4% positive, DILI 77.3% positive). In some patients, other antibodies related to autoimmune liver diseases were detected as well with the most prevalent being extractable nuclear antigens (ENA) and AMA in the AIH group (n = 3 for ENA and AMA respectively, 13.6%) and SMA (n = 3, 13.6%) and AMA-M2 (n = 2, 9.1%) in the DILI group. For an overview of all autoantibodies found in DILI and AIH patients, please also refer to Tables S1 and S2. Elevated IgG levels were more common in AIH patients (59.1% vs 36.4% in AIH and DILI patients respectively), however this difference was not statistically significant (P = .087). Diagnostic performance was similar to the AIH score, encompassing both IgG and ANA (data not shown).

3.3 | Establishing the final diagnosis

3.3.1 | RUCAM and AIH scores

According to the pretreatment AIH scores and RUCAM, 18 patients were initially diagnosed with AIH and 13 with DILI as they presented with an AIH score ≥12 and a RUCAM <6 or an AIH score <12 and a RUCAM ≥6 respectively. However, the RUCAM and AIH scores were misleading in five of those 31 patients: three patients who were initially thought to suffer from AIH were finally diagnosed with DILI, while two patients who were initially thought to have DILI were diagnosed with AIH during the follow-up period (Figure 2). Furthermore, RUCAM and AIH scores were inconclusive in 13 cases (30%) with both scores either suggesting DILI and AIH as the probable diagnosis or both score pointing towards DILI and AIH being unlikely: In four DILI patients and one AIH patient, AIH score was ≥12 and RUCAM score was ≥6, while in five DILI and three AIH patients, AIH score was <12 and RUCAM score was <6. For those patients, the final diagnosis could only be established in

TABLE 1 Patient characteristics of AIH-patients and DILI-patients

Patient characteristics	DILI (n = 22)	AIH (n = 22)	*P*-value
Age, years	50 (22-76)	53 (22-76)	.60
Female sex, n (%)	14 (63.6%)	14 (63.6%)	.62
Ethnicity: Caucasian, n (%)	22 (100%)	22 (100%)	na
BMI, kg/m^2[a]	24.2 (16.8-42.5)	24.2 (19.2-34.6)	.87
RUCAM score max., points[a]	6 (3-10)	3.5 (0-7)	<.001[b]
≥6 points, n (%)	14 (63.6%)	3 (13.6%)	
AIH score, points[a]	9.5 (4-14)	13 (9-18)	.001[b]
≥12 points, n (%)	7 (31.8%)	17 (77.3%)	
Revised AIH score, points[a]	4 (2-6)	5 (1-7)	.39
Latency, d[a]	81 (12-1589)	40.5 (0-1470)	.03[b]
Time interval until the initiation of corticosteroids, d	19.5 (7-195)	16 (7-88)	.39
ANA titre[a]	1:300 (0-1:25 600)	1:400 (0-1:12 800)	.33
ANA positive, n (%)	17 (77.3%)	19 (86.4%)	.43
IgG serum levels, g/L[a]	11.0 (7.5-26.9)	17.3 (6.6-31.7)	.09
IgG > 16 g/L, n (%)	8 (36.4%)	13 (59.1%)	.13
HLA-DRB3 or 4, n of all (%)	7/10 (70.0%)	4/6 (66.7%)	.64
ALT at onset, (× ULN)[a]	27.1 (3.3-66.7)	27.8 (13.4-58.8)	.94
ALT at treatment initiation (× ULN)[a]	28.9 (6.7-88.3)	25.1 (4.3-46.7)	.20
R ratio at onset[a]	16.6 (2.9-36.7)	18 (2.8-59.2)	.73
R ratio PTBL[a]	15.8 (1.8-74.7)	16.4 (2.9-47.4)	.71
Pattern of liver injury at onset			
Hepatocellular	21 (95.5%)	20 (90.9%)	.55
Mixed	1 (4.5%)	2 (9.1%)	
ALF, n (%)	8 (36.4%)	6 (27.3%)	.52
Severity score[a]	2 (1-5)	2 (1-5)	.68
Corticosteroid of choice			.31
Prednisolone, n (%)	21 (95.5%)	22 (100%)	
Methylprednisolone, n (%)	1 (4.5 5)	0	
Maximum dosage of corticosteroid, shown as hydrocortisone dose equivalent, mg[a]	240 (160-5000)	240 (160-400)	.12

Abbreviations: AIH, autoimmune hepatitis; ALF, acute liver failure; ALT, alanine aminotransferase; ANA, anti-nuclear antibodies; BMI, body mass index; DILI, drug-induced liver injury: PTBL, peak total bilirubin level; RUCAM, Roussel Uclaf Causality Assessment Method; RUCAM max, highest RUCAM in the given patient, if several drugs were involved; ULN, upper limit of normal.
[a]Medians and lower and upper limits are shown.
*Statistically significant difference ($P < .05$).

the further course of the disease considering the response towards immunosuppressive treatment.

3.3.2 | Histological patterns

Histology was available for all 22 AIH patients and 15 of the 22 DILI patients. There were no significant differences regarding the intensity of inflammatory infiltrates or cholestasis, the type of the predominant inflammatory cells or the grade of fibrosis (Figure 3). The only discriminating feature in our cohort was the finding of an interface infiltrate, which was significantly more frequent in AIH patients (20 AIH vs 8 DILI patients, $P = .01$). In addition, a tendency towards a stronger eosinophile infiltrate was observed in AIH patients ($P = .113$). In the majority of AIH cases (n = 16/22; 72.7%) the histological pattern was compatible with AIH, while in 73.3% of the DILI patients (n = 11/15) histological features were pointing towards a possible or probable diagnosis of DILI. Yet, in five AIH patients (22.7%) and three DILI patients (20.0%), the diagnosis of DILI and AIH was equally likely according to the histological features (Tables S3 and S4). The sensitivity and specificity of the histology regarding diagnosis of DILI therefore were 73%.

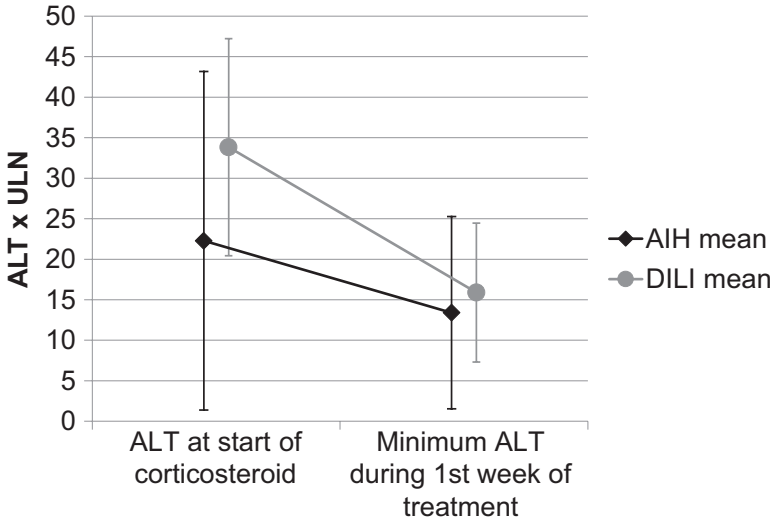

FIGURE 3 The graph shows the mean ALT serum concentrations × ULN along with the standard deviation (SD) measured at the time of initiation of corticosteroid treatment and the minimal ALT reached in treatment week 1 in patients with AIH (black line) and DILI (grey line). AIH, autoimmune-hepatitis; ALT, alanine aminotransferase; DILI, drug-induced liver injury

3.3.3 | MH cell test

The MH cell test, which was performed in every patient included in this study, showed negative results for all AIH patients (n = 22, 100%) but identified one causative drug in 18 (81.8%) and two possible causatives drugs in four (18.2%) patients with DILI as the final diagnosis (Table S3).

3.4 | Corticosteroid treatment

3.4.1 | Indication for immunosuppression

The majority of patients with the final diagnosis of DILI presented with autoimmune features (18 of 22; 81.8%), including elevated autoantibody titres and IgG concentrations or an AIH score of 12 or higher, initially suggesting that AIH was a probable diagnosis.

Overall, ALT concentrations tended to be higher in the DILI group at the time of initiation of treatment when compared to the AIH patients, although this difference did not reach statistical significance (Figure 4).

The indication for the initiation of treatment with corticosteroids in 17 of the 18 DILI patients with autoimmune features was persistent or progressive ALT elevation despite the withdrawal of the causative drug in 12 cases and development of ALF with coagulopathy in five cases. One patient had developed liver injury because of a programmed cell death protein 1 (PD-1) inhibitor, a drug known to cause immune-mediated liver injury that responds well to corticosteroids[33] and thus received immunosuppressive therapy.

Regarding the four patients without autoimmune features, one patient suffered from ALI because of minocycline, which has been described to cause DILI with autoimmune features resembling AIH that responds well to corticosteroid treatment[11,34] and immunosuppressive treatment was initiated as a result of persistently elevated ALT levels. The remaining three patients presented with persistency of ALT elevation in spite of the discontinuation of the suspected drug and thus with seronegative AIH being a possible diagnosis received immunosuppression at the judgement of the treating physician.

All patients with AIH (n = 22; 100%) and most of the patients with DILI (n = 21; 95.5%) received prednisolone. One patient with DILI had clinical signs of concomitant relapsing-remitting multiple sclerosis, and thus was treated with methylprednisolone with the initial steroid dose determined based on the MS relapse (5000 mg). The median maximum equivalent cortisol dosage was 240 mg for both patients with DILI and AIH, with a range of 160-5000 mg and 160-400 mg respectively (Table 1 and Tables S1 and S2).

3.4.2 | ALT concentrations early after starting corticosteroid treatment

Notably, significant differences in the absolute and relative reductions in ALT levels per day during the first week of corticosteroid treatment were observed between the patients with DILI or AIH as final diagnosis: 3.5 × ULN per day (0.2-7.8) for patients with DILI vs 1.9 × ULN per day (0-5.7; $P = .02$) for patients with AIH or in relative terms, 10.8% (1.7%-20.0%) per day for patients with DILI vs 7.5% per day (0%-12.5%; $P < .01$) for patients with AIH.

The sensitivity and specificity of the relative reduction in ALT levels per day were 77% respectively, using 9% decrease per day during the first week as cut-off value. Thus, a 9% or greater reduction in ALT levels per day after the initiation of steroid therapy suggested a diagnosis of DILI and showed a higher sensitivity than the RUCAM and pretreatment AIH score with only a mildly reduced specificity.

During week 2, there was also a tendency towards a higher maximum reduction in ALT per day, although this difference was non-significant: 1.8 × ULN (0.1-5.0) or 6.6% (1.4%-9.1%) per day in DILI patients vs 1.4 × ULN (0.1-23.3) or 5.7% (2.1%-10.9%) per day in AIH patients ($P = .367$ and .350 respectively).

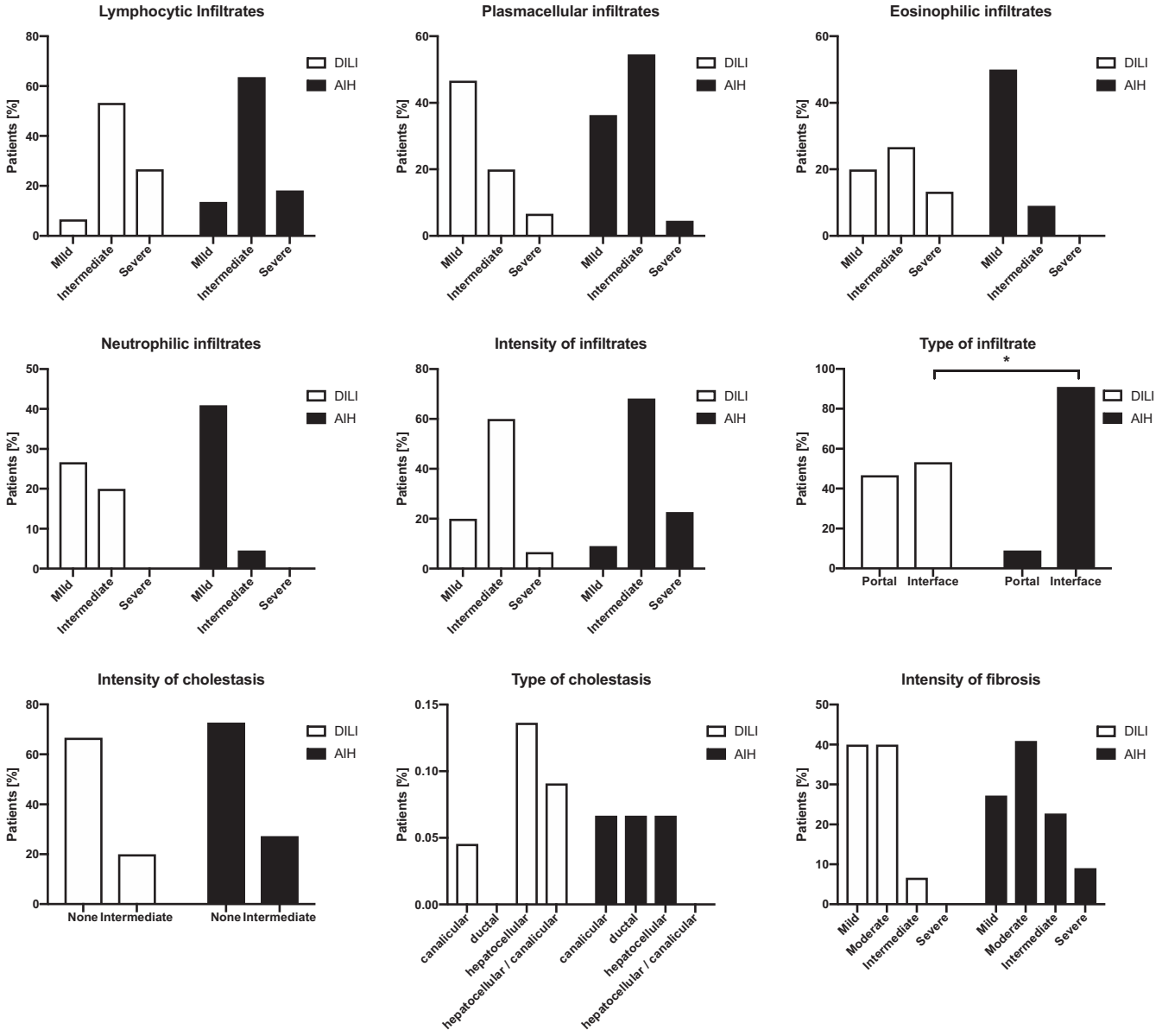

FIGURE 4 Percentage was given as number of patients with the specific features relative to the total number of samples available. Statistical significance is indicated by * ($P < .05$). AIH, autoimmune hepatitis; DILI, drug-induced liver injury

3.4.3 | Subgroup analysis of unequivocal cases and complex patients

In order to evaluate ALT decrease in patients unequivocally identified by the diagnostic scores, we performed a ROC analysis of these 24 cases (eight DILI, 16 AIH). The relative reduction in ALT during the first week showed best differentiation of DILI and AIH at 9% of pretreatment ALT per day with a sensitivity for DILI of 88% and a specificity of 75% respectively.

We also performed a subgroup analysis of the patients initially misclassified (n = 5) by AIH and RUCAM score or with an inconclusive initial diagnosis because of the AIH score and RUCAM (n = 13). Of the patients studied, five cases had an AIH score ≥12 and RUCAM ≥6 suggesting both AIH and DILI as probable diagnosis. On the other hand, AIH score <12 and RUCAM <6 was found in eight cases, indicating that both AIH and DILI were equally unlikely to have caused ALI despite a high clinical suspicion and rule-out of alternative diagnosis. Thus, a total of 18 patients (41%) were either misclassified (n = 5) or inconclusive (n = 13) at initial presentation. Strikingly, the relative reduction in ALT per day in the first week with a cut-off of ≥9% for the diagnosis of DILI differentiated DILI and AIH in every misclassified patient. Moreover, the relative reduction in ALT per day in the first week also accurately distinguished between AIH and DILI in three of four the AIH and seven of the nine DILI patients with an inconclusive initial diagnosis, reaching a sensitivity of 78% and specificity of 75% for the diagnosis of DILI. In the combined group of five misclassified patients and 13 patients with inconclusive initial scores (n = 18), relative ALT

reduction could differentiate AIH and DILI in 15 cases resulting in a sensitivity and specificity of 83%. Histological reports pointed towards the right diagnosis in only 11 of those 16 cases (sensitivity 60%, specificity 83%), while in two of those cases histology was not available. Thus, in patients with an inconclusive diagnosis upon presentation, the relative reduction in ALT shortly after initiation of steroid treatment was more accurate in distinguishing AIH and DILI than a liver biopsy. For an overview of the individual patients' diagnosis according to AIH score and RUCAM, histological report and ALT reduction under immunosuppression please also refer to Tables S3 and S4.

3.5 | Long-term follow-up

3.5.1 | Normalization of ALT levels

All patients from our study cohort were regularly seen in our liver unit with clinical and laboratory follow-up assessments. The median follow-up period was 19 months (3-54) for patients with DILI and 23 months (1-220) for patients with AIH respectively. During long-term follow-up, four patients had a fatal outcome or needed liver transplantation: One patient who had developed DILI as a result of anti-PD-1 antibody treatment with pembrolizumab for metastatic melanoma died from cancer-related causes approximately 3 months after inclusion in our study. One DILI and two AIH patients underwent high urgency liver transplantation due to progressive liver failure. After exclusion of those four patients, the minimal follow-up in AIH patients was 7 and 10 months in the DILI group.

The rates of remission under immunosuppression, defined as normalization of ALT serum levels, tended to be higher in the DILI group compared to the AIH group (95.5% vs 77.3%; $P = .08$). The median duration until serum ALT concentrations normalized was 86 days (range 14-264 days) and 84 days (range 14-362 days) for patients with DILI and AIH respectively ($P = .82$).

3.5.2 | Relapse after tapering or discontinuation of immunosuppressive therapy

The above-mentioned four patients with a fatal outcome or the need for liver transplantation were excluded from the final analysis leaving 20 DILI and 20 AIH for a final follow-up. The outcome of those remaining patients varied strongly between the two groups: Additional immunosuppression with azathioprine or mycophenolic acid during tapering of corticosteroids was significantly less frequent in DILI patients: 17 (85.0%) AIH patients received azathioprine or mycophenolic acid, while in the DILI group only four (20.0%) patients did (two patients with relapse and two patients who were initiated on azathioprine because of initial AIH diagnosis; $P < .01$ [17 von 20 vs 4 von 20]). Furthermore, immunosuppression could successfully be discontinued completely in 17 of the 20 (85.0%) DILI patients, but only in one of the 20 AIH patients (5.0%; $P < .01$). The proportion of patients showing a relapse during the long-term follow-up was significantly lower in DILI patients (15.0% vs 70.0%, $P < .01$).

4 | DISCUSSION

Autoimmune features in patients with DILI[11] or seronegative AIH[8] may pose significant difficulties in finding the right diagnosis and thus treatment. In those cases, the most reliable criterion to distinguish DILI with autoimmune features from idiopathic AIH is the absence of relapse at least 1 year after disease onset when immunosuppressive therapy has been reduced, discontinued or was never initiated.[7,11,18] However, once corticosteroids have been initiated, it is not known when and how to taper immunosuppression if DILI is suspected since no discriminating feature other than a more beneficial outcome in DILI patients under long-term immunosuppression has been described as yet. Therefore, despite AIH score and RUCAM, diagnostic criteria to discriminate DILI and AIH early after starting immunosuppression are urgently needed in to avoid unnecessary long-term immunosuppressive treatment in DILI patients.

On this background, we compared 44 patients with the final diagnosis of AIH or DILI who were treated with corticosteroids. In our cohort, elevation of liver tests at the onset of disease and the proportion of patients developing ALF were fairly similar. The majority of patients (77.3% of patients with DILI and 86.4% of patients with AIH) was positive for ANA. Some patients in each group also tested positive for other autoantibodies associated with AIH. This finding is consistent with previous studies reporting that not only patients with AIH but also a proportion of DILI patients present with autoantibodies.[35,36] Therefore, the presence of autoantibodies was not helpful to distinguish both entities.

Interestingly, polymorphism of HLA-DR3 or -DR4 was equally prevalent in DILI and AIH patients (70% vs 67%). HLA-DRB1*03:01 and HL-DRB1*04:01 encoding HLA-DR3 and HLA-DR4 are alleles with a well-known association to a higher susceptibility for AIH type 1 in Europe and North America.[37,38] Regarding DILI, a variety of associations with HLA variants have been described, that is, HLA-B*57:01 in the case of flucloxacillin-induced DILI, HLA-DRB1*15:01 in the case of DILI induced by amoxicillin/clavulanic acid and HLA-A*33:01 for a variety of drugs including ticlopidine, methyldopa erythromycin and fenofibrates.[39-42] However, no association of HLA-DR3 and -DR4 with DILI has been described so far, although previous data have shown that a proportion of DILI patients and healthy controls can present with those HLA variants albeit less frequently than in AIH patients.[43,44] Our data suggest that HLA variants associated with higher susceptibility for AIH might also be present in a relevant proportion of DILI patients. However, towing to the limited number of patients for which HLA genotyping was available no statistically significant results can be concluded from the present data.

In our cohort, the pretreatment AIH score was significantly higher in AIH patients, whereas RUCAM was significantly higher in DILI patients. However, the sensitivity and specificity of the pretreatment AIH score with a cut-off of ≥12 were only 59% and 82% respectively. The sensitivity and specificity of the pretreatment AIH score were even lower for the simplified AIH score, most likely because of the observation that a high proportion of AIH patients in our cohort were seronegative and presented with a histology only

compatible but not typical for AIH. The sensitivity and specificity of the RUCAM were 32% and 91% respectively. Histological analysis of liver biopsies could not distinguish DILI and AIH either since the only significant difference was the predominance of interface infiltrates in AIH patients. In addition, there was a tendency, albeit not significant, towards a higher frequency of eosinophils in AIH patients. Both of those findings are in line with previous data.[14,45] However, regarding the type and intensity of cellular infiltrates and of cholestasis no other distinguishing feature was found that could have helped to accurately direct diagnosis towards DILI or AIH.

These findings confirm the need for methods to improve discrimination of AIH and DILI, especially if corticosteroid treatment has been initiated in inconclusive cases. However, data on the short-term outcome of DILI patients under immunosuppressive treatment and optimal tapering regimes are scarce.

Strikingly, we observed marked differences in the response to only 1 week of corticosteroid treatment between patients with AIH and DILI. The absolute and relative reductions in serum ALT levels per day during the first week of treatment were significantly higher in the DILI group than in patients with AIH.

Notably, for a cut-off value of 9% reduction in ALT levels per day during the first week of treatment, the sensitivity and specificity reached 77% respectively. Thus, decrease in ALT levels seems to be a useful tool to support the pretreatment AIH score and RUCAM, especially in complex cases: The reduction in ALT per day during the first week of corticosteroid treatment showed excellent results when differentiating DILI and AIH patients who had been misclassified by the RUCAM or AIH score with a sensitivity and specificity of both 100%. In addition, the relative ALT reduction in patients with an inconclusive score result (both RUCAM and AIH scores being either ≥6 and ≥12 or <6 and <12) exhibited a sensitivity and specificity of 78% and 75% respectively. In all patients, who were either misclassified or inconclusive at treatment initiation, a 9% reduction in ALT levels per day during the first week of treatment discriminated DILI from AIH with 83% sensitivity and specificity respectively. However, not only in complex, initially inconclusive cases, but also in the unequivocal cases in our cohort (n = 24), the relative reduction in ALT could distinguish between DILI and AIH with a sensitivity and specificity of 88% and 75% respectively.

This supports our findings for the total cohort comprising both unequivocal and inconclusive cases. In addition to the conventional causality assessment and histological evaluation, an in vitro test established in our centre was applied to all patients of the current study. This in-house test is based on drug reactivity of hepatocyte-like cells generated from monocytes of individual subjects for the diagnosis or exclusion of DILI.[24,25] Interestingly, the MH cell test identified the culprit drug or a combination of drugs in all DILI patients, while the test was negative for all drugs used by AIH patients. Thus, supporting the final diagnosis used for our analysis.

To the best of our knowledge, this study is the first to show a significant difference in the response of laboratory parameters to short-term corticosteroid therapy between patients with DILI and AIH, enabling the differentiation of the two entities early in the course of immunosuppressive therapy. Based on these findings, the dynamics of ALT levels in patients undergoing corticosteroid therapy recorded shortly after initiation are a potentially useful additional diagnostic criterion to distinguish DILI patients who have been initiated on corticosteroids because of the presence of autoimmune features or persistency of liver injury from idiopathic AIH. Notably, the median times to ALT normalization were not different between AIH and DILI patients in our cohort. This observation underscores our finding of the particular importance of the difference in Delta ALT during the first week of treatment.

We propose that a reduction in ALT levels of 9% per day during the first week of steroid treatment indicates that the patient is suffering from a DILI episode rather than idiopathic AIH and the risk of relapse upon tapering and withdrawal of immunosuppression might be minimal. The reduction of ALT should especially be regarded in patients in whom the diagnosis of DILI or AIH is inconclusive with the RUCAM and AIH score.

Our study has limitations, such as the rather small number of patients. Secondly, all of the patients included were Caucasian. Thus, there is a potential selection bias of milder DILI cases, since recent data suggest that African-Americans are more likely to develop a more severe form of DILI with higher morbidity and mortality.[46] Furthermore, the analysis was conducted in a highly selected cohort comprising patients with intake of at least one drug who received corticosteroids during the course of their disease. In addition, the analysis could only be done retrospectively, since a response to immunosuppressive therapy and the appearance of relapse must be considered as an additional diagnostic criterion. In conclusion, the dynamics of ALT levels shortly after the initiation of immunosuppressive therapy seems to represents a useful diagnostic tool to help separate DILI from AIH.

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CONFLICT OF INTEREST

SW and IR have nothing to disclose; AB and ALG are the stockholders of MetaHeps GmbH, owners of IP.

AUTHORS' CONTRIBUTIONS

SW, AB and ALG: conception and design of the study; analysis and/or interpretation of data; drafting of the manuscript; approval of the final version of the manuscript, IR: generation, collection, assembly and analysis of data; approval of the final version of the manuscript. Writing assistance: none.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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