Serious liver injury induced by Nimesulide: an international collaboration study reporting 57 cases

Fernando Bessone[1], Nelia Hernandez[2], Manuel Mendizabal[3], Ezequiel Ridruejo[4], Gisela Gualano[5], Eduardo Fassio[5], Mirta Peralta[6], Hugo Fainboim[6], Margarita Anders[7], Hugo Tanno[1], Federico Tanno[1], Raymundo Parana[8], Inmaculada Medina-Caliz[9], Mercedes Robles-Diaz[9], Ismael Alvarez-Alvarez[9], Hao Niu[9], Camilla Stephens[9], Luis Colombato[10], Marco Arrese[11], M Virginia Reggiardo[1], Suzane Kioko Ono[12], Flair Carrilho[12], M Isabel Lucena[9≠], Raul J Andrade[9≠]

[1] Hospital Provincial del Centenario, University of Rosario School of Medicine, Rosario, Argentina.

[2] Hospital de Clínicas, Facultad de Medicina, Montevideo, Uruguay.

[3] Hospital Universitario Austral, Buenos Aires, Argentina.

[4] Centro de Educación Médica e Investigaciones Clínicas, Buenos Aires , Argentina.

[5] Hospital Alejandro Posadas, Buenos Aires , Argentina.

[6] Hospital Muñiz, Buenos Aires , Argentina.

[7] Hospital Alemán, Buenos Aires, Argentina.

[8] Facultad de Medicina, Universidad Nacional de Bahia, Salvador de Bahia, Brazil.

[9] Servicios de Aparato Digestivo y Farmacología Clínica, Hospital Universitario Virgen de la Victoria, Instituto de Investigación Biomédica de Málaga-IBIMA, Universidad de Málaga, Málaga, Spain. CIBERehd, Madrid, Spain

[10] Hospital Británico, Buenos Aires, Argentina.

[11] Pontificia Universidad Catolica de Chile, Santiago de Chile, Chile

[12] Hospital Das Clinicas, Sao Paulo, Brazil

[≠] share senior authorship

Corresponding Author

Prof. Fernando Bessone

Department of Gastroenterology and Hepatology

Hospital Provincial del Centenario

University of Rosario School of Medicine

Urquiza 3101, 200, Rosario, Argentina

bessonefernando@gmail.com

Prof. M. Isabel Lucena

Department of Clinical Pharmacology
School of Medicine
University of Malaga
Boulevard Louis Pasteur 32, 29071, Malaga, Spain
lucena@uma.es

ABSTRACT

Nimesulide is a non-steroidal anti-inflammatory drug still marketed in many countries. We aim to analyze the clinical phenotype, outcome, and histological features of nimesulide-induced liver injury (nimesulide-DILI). We analyzed 57 cases recruited from the Spanish and LATIN DILI registries. Causality was assessed by the RUCAM scale. Mean age of the whole case series was 59 years (86% women) with a median time to onset of 40 days. A total of 46 patients (81%) were jaundiced. Nimesulide-DILI pattern was hepatocellular in 38 (67%), mixed in 12 (21%), and cholestatic in 7 (12%) cases. Transaminases were elevated with a mean of nearly 20-fold the upper limit of normality (ULN), while alkaline phosphatase showed a 2-fold mean elevation above ULN. Total bilirubin showed a mean elevation of 13-fold the ULN. Liver histology was obtained in 14 cases (25%), most of them with a hepatocellular pattern. Median time to recovery was 60 days. Overall, 12 patients (21%) developed acute liver failure (ALF), five (8.8%) died, three underwent liver transplantation (5.3%), and the remaining four resolved. Latency was ≤15 days in 12 patients (21%) and one patient developed ALF within seven days from treatment initiation. Increased total bilirubin and aspartate transaminase levels were independently associated with the development of ALF. In summary, nimesulide-DILI affects mainly women and presents typically with a hepatocellular pattern. It is associated with ALF and death in a high proportion of patients. Shorter (≤15 days) duration of therapy does not prevent serious nimesulide hepatotoxicity, making its risk/benefit ratio clearly unfavorable.

Keywords: Nimesulide; NSAID, Hepatotoxicity; Acute Liver Failure; Hepatitis; Cholestasis

INTRODUCTION

Nimesulide is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, anti-inflammatory and antipyretic effects, due to its potent inhibitory activity on cyclooxygenase-2 (COX-2) enzyme. However, the mechanism of action of nimesulide probably involves as well its sulphonanilide moiety (Bennett 2001).

Nimesulide was marketed initially in Italy back in 1985 and it was not until 1997 that its potential to induce liver injury (nimesulide-DILI) was recognized (Bessone et al. 1997). In the initial report, nimesulide was associated with a variety of DILI patterns such as hepatitis, cholestatic jaundice, and acute liver failure (ALF). Several years later, another report from the same group again drew attention to the increased number of ALF cases associated with nimesulide (Bessone et al. 2001). Thereafter, other studies followed further describing a wide spectrum of nimesulide-DILI phenotypes (Agúndez et al. 2011; Andrade et al. 2000; Bessone and Tanno 2000; Bhattacharya et al. 2005; Castañeda Hernández and Barragán Padilla 2004; Chatterjee et al. 2008; Dumortier et al. 2002; Gallego Rojo et al. 2002; Grignola et al. 1998; Lewin 2002; Lukić et al. 2009; McCormick et al. 1999; Montesinos et al. 2001; Ozgür et al. 2003; Papaioannides et al. 2003; Pérez-Moreno et al. 2000; Polimeni et al. 2006; Quadranti 2004; Rodrigo et al. 2002; Romero Gómez et al. 1999; Schattner et al. 2000;Stadlmann et al. 2002; Tan et al. 2007; Tejos et al. 2000; Van Steenbergen et al. 1998). Nimesulide-DILI cases have been continuously reported over recent years in many countries where nimesulide is still marketed (Kwon et al. 2019).

This high proportion of severe forms of DILI commonly associated with fatal outcome led national health authorities in several countries around the world to suspend nimesulide marketing authorization. Thus, the agent was withdrawn from the market in Spain (2002) and in Argentina in 2009 (Administración Nacional de Medicamentos Alimentos y Tecnología 2009; Agencia Española de Medicamentos y Productos Sanitarios 2002). In May 2007, the Irish Medicines Board announced the withdrawal of oral nimesulide-containing products from the market due to elevated number of cases of ALF requiring liver transplantation associated with nimesulide use (Health Products Regulatory Authority 2007).

The European Medicines Agency (EMA) adopted regulatory measures that started in 2004 with a restriction of nimesulide prescriptions, as well as a maximum daily dose of 100 mg twice a day (European Medicines Agency 2012a). Recommendations by EMA to limit nimesulide use to less than 15 days, arguing that severe and fulminant nimesulide-DILI is highly unlikely to develop before this period, resulted in a further safety appraisal in 2012.

Despite health authorities consider that the benefit/risk balance of short-term nimesulide use is positive, based on a drug-gastrointestinal safety profile compensating for the hepatotoxicity risk (European Medicines Agency 2012b), recently published data consistently demonstrate a higher risk of severe liver damage associated with nimesulide compared to other NSAIDs (Kwon et al. 2019; Donati et al. 2016). Yet, nimesulide is

currently marketed in more than 50 countries in Latin America, Eastern European and Asia, both for adult and pediatric populations. However, it has never been available in the United States.

In the present collaborative study including cases from Argentina and Spain, we aimed to analyze the clinical phenotype, histological features, and outcome of nimesulide-DILI in the largest series reported to date, specifically assessing the impact of duration of therapy. Our series encompasses 57 patients suffering nimesulide-DILI, and highlights the severity of this reaction, frequently resulting in ALF and death and the fact that a shorter duration of therapy is not associated with a less serious outcome.

MATERIAL AND METHODS

Cohort features

Fifty-seven nimesulide-DILI cases identified at the Hepatology Department of Hospital Provincial del Centenario (University of Rosario Medical School) from 1988 to 2009 and cases submitted to the Spanish DILI Registry from 1994 to 2002 were analyzed.

Following our first observed case of nimesulide-DILI in 1988 (Bessone et al. 1997), we built a database containing detailed demographic, clinical, laboratory, imaging, and histological information. It also included a close follow up of these patients from the detection of liver toxicity up to normalization of liver tests. In the prospective Spanish DILI Registry, all cases included were evaluated by a clinician and submitted to the coordinating center, where they were re-evaluated by a panel of DILI experts before being accepted into the database. The time elapsed from the initial intake of the medication to onset of symptoms or liver biochemical alteration (latency), as well as that between discontinuation of the suspected agent and recovery, was analyzed. Serology and specific biochemistry were assessed to rule out viral and autoimmune hepatitis, alcoholic liver disease and other potential etiologies of acute liver injury, such as Wilson disease, ischemic hepatitis, as indicated. Appropriate imaging tests were performed to exclude biliary disease. All cases met the DILI biochemical criteria proposed by Aithal *et al.* (2011). The CIOMS/RUCAM scale was used for additional causality assessment. Acute liver failure was defined in this study as patients developing hepatic encephalopathy and coagulopathy (International Normalized Ratio [INR] >1.5) as reported by Møller et *al.* (2007).

The pattern of liver damage was classified based on biochemical parameters to calculate the R ratio value from the first available blood test after DILI recognition. Liver injury was defined as hepatocellular when patients presented a 5-fold or higher rise in alanine aminotransferase (ALT) alone or when the ratio of serum activity (activity is expressed as a multiple of the upper limit of normality [ULN]) of ALT to alkaline phosphatase (ALP)) was 5 or more. Liver injury was defined as cholestatic when a 2-fold or higher rise in ALP alone or when a ratio of serum activity of ALT to ALP of 2 or lower was observed. When the ratio of the serum activity of ALT to ALP was between 2 and 5, liver injury was termed mixed (Aithal et al. 2011).

The new Hy´s law was defined as DILI with hepatocellular injury (nR ≥5, nR = ALT or aspartate aminotransferase (AST), whichever the highest/ULN ÷ alkaline phosphatase/ULN value) and TBL >2 x ULN (Robles-Diaz et al. 2014). DILI was classified as mild, moderate, severe, or fatal/liver transplantation (fatal/Tx) based on the DILI severity index scale (Aithal et al. 2011). The study protocol was approved by the local Ethics Committee at the Hospital Provincial del Centenario, Rosario, Argentina and at the Virgen de la Victoria University Hospital in Málaga, Spain, and all subjects gave informed consent.

Statistical analysis

Quantitative variables were expressed as mean and standard deviation or range, or median and interquartile range (IQR). Quantitative variables were compared using the Mann-Whitney U test, or Kruskal Wallis test, as appropriate, and post hoc analysis with Bonferroni correction for multiple comparisons were performed. Differences in qualitative variables were assessed with the Chi-Squared or Fisher exact test, as appropriate.

Multivariable logistic regression analyses were performed to evaluate the contribution of independent factors to the development of ALF. Model selection was done applying the stepwise regression method and the likelihood ratio test. The goodness of fit of the selected model was evaluated by including the squared predictor and evaluating its significance if the Hosmer-Lemeshow test was not valid. All results were considered statistically significant when the p-value was lower than 0.05. All analyses were performed using R Studio 1.1.463 (R Studio team, Boston, MA).

RESULTS

The case inclusion associated with nimesulide-DILI was carried out from 1988 to 2009 in Argentina and between 1994 and 2002 in Spain, coinciding with nimesulide withdrawal from the market in each country (Administración Nacional de Medicamentos Alimentos y Tecnología 2009; Agencia Española de Medicamentos y Productos Sanitarios 2002). Our cohort of 57 patients comprised 47 cases recruited at the Hospital Provincial del Centenario, University of Rosario, Argentina, and 10 well-documented cases from the Spanish DILI Registry.

Clinical characteristics of the population

Of the 57 nimesulide-DILI cases included in our series, 49 (86%) patients were women. The mean age in the case series was 59 years (range: 9-82 years), with 34 cases (60%) being older than 60 years, and 24 (42%) older than 65 years. Application of the CIOMS/RUCAM scale resulted in 27 (48%) cases classified as highly probable, 18 (32%) as probable, and 12 (21%) as possible.

The median nimesulide dose was 200 mg/day (range 100-200), with 47 patients (83%) receiving 200 mg/day, mainly indicated for pain induced by arthritis, osteoarthritis, or trauma. The median latency period was 40 days (range: 3-238 days). A total of 47 patients

(83%) had a latency period shorter than 90 days. Interestingly, eight patients (14%) developed nimesulide-DILI after 6 months from starting the drug.

Hepatocellular damage was the most frequent pattern of liver injury in our series (38 patients, 67%), with the remaining cases presenting either mixed (12 patients, 21%) or cholestatic injury (seven patients, 12%). Demographic characteristics, clinical, and laboratory findings according to the type of liver damage are shown in Table 1.

The most common symptoms at presentation were asthenia (37%), nausea (37%), vomiting (32%), and pruritus (16%). Jaundice was present in 81% of patients, 51% required hospitalization, and 21% developed encephalopathy. Hospital admission was independent of the type of liver injury (p=0.783, Table 1).

The biochemical profile of nimesulide-DILI cases on admission was characterized by mean elevations in TBL of 13 x ULN. Serum AST and ALT were increased in all patients with a mean value of 20 x ULN and 18 x ULN, respectively. Mean ALP elevation was 2 x ULN, and the mean INR in the entire case series was 1.9. Peripheral eosinophilia was present in 16% of cases, with no evidence of rash, other immunoallergic manifestations or autoimmune features.

Patients with cholestatic or mixed pattern exhibited significantly higher frequency of pruritus (p=0.004), but lower prevalence of jaundice (p=0.031). In addition, these patients had higher mean values of ALP (p<0.001), but lower values of ALT (p<0.001) and AST (p=0.007) on admission than patients exhibiting hepatocellular pattern.

Interestingly, in 16% of cases, AST was 1.5 times higher than ALT on admission. Nimesulide-DILI cases presenting a hepatocellular liver injury were more frequently severe (9, 24%) or fatal/Tx (7, 18%), while cases with moderate severity (13, 68%) predominated in the cholestatic/mixed group (p=0.115) (Table 1). Correspondingly, the new Hy's law was fulfilled in 37 cases (65%) and performed as expected with 19% of liver-related death/liver transplant cases among those that fulfilled the new Hy's law criteria. All nimesulide-DILI cases are depicted according to ALT and total bilirubin level in Fig. 1.

Forty-nine (86%) patients were considered to have acute, self-limited damage, and the median time to recovery was 60 days (IQR: 35-90). Overall, 41 patients (72%) showed a biochemical normalization of liver parameters within 90 days. Detailed information for each of the 57 nimesulide-DILI cases is shown in Supplemental table 1.

Liver histology findings were obtained from 14 (25%) patients and linked to a wide spectrum of liver lesions, including hepatocellular (hepatitis, massive, and submassive necrosis), mixed (cholestatic hepatitis with and without eosinophilia), and pure cholestasis patterns. Acute hepatocellular damage represented by hepatitis was observed in 3 (21%) cases, while more severe liver lesions were documented in 7 (50%) cases, with lobular collapse (n=2), submassive necrosis (n=1), and massive necrosis (n=4). Mixed and cholestatic patterns were found in 3 and 1 patients, respectively.

Cholestatic hepatitis was characterized by lobular necrosis of hepatocytes and hepatocanalicular cholestasis predominantly in zone 3. Portal cellularity was mainly composed by lymphocytes and eosinophils in two patients. Pure cholestasis was observed in one case who showed marked hepatocanalicular pigment overload with abundant biliary plugs affecting zone 3 without both duct damage and inflammation. Acute hepatitis induced by nimesulide ranged from mild to moderate portal and lobular injury with different degree of inflammation. Apoptotic bodies and spotty necrosis were a common finding. Intense portal and lobular inflammation associated with liver cell necrosis involving groups of hepatocytes and scarce areas of lobular collapse were documented in one patient. Besides this latter case, other six severe forms of liver injury presented massive (n=4) and submassive (n=2) liver necrosis, characterized by different degree of drop out and loss of hepatocytes, confluent necrosis, extensive collapsed areas, and panacinar necrosis in one patient. Detailed liver biopsy findings are provided in Supplemental table 2 and Fig. 2.

Demographic and clinical features of patients who did or did not develop ALF

Twelve (21%) patients developed ALF (Table 2). All of them were women with a mean age of 55 years (range: 9-71 years), with 50% being older than 60 years and 42% older than 65 years. All patients presented jaundice and some degree of encephalopathy on admission. The youngest patient who developed ALF (case 23, Table 3) was a 9-year-old girl prescribed nimesulide drops for a prolonged history of sore throat, who successfully underwent liver transplantation.

The prescribed dose was 200 mg/day in 10 cases (83 %) indicated for pain in nine patients (75%). There was no difference in the calculated cumulative dose in patients who did or did not develop ALF (median: 10,500 mg vs 6,000 mg; p=0.133). Similarly, despite a longer latency in patients who developed ALF (60 days) compared to those who did not go into ALF (30 days), no significant difference was detected (p=0.108) (Table 2).

Hepatocellular pattern of liver injury was present in all ALF cases except for one case belonging to the mixed group (case 12, Table 3). Liver histology was obtained from six patients, showing a different degree of severe hepatocellular necrosis ranging from submassive liver injury characterized by extensive centrilobular necrosis with collapsed areas to massive necrosis. Large zones of complete multiacinar liver necrosis associated with massive dropout of hepatocytes were also documented (Table 3).

When comparing demographics and clinical presentation of patients who did and did not develop ALF (Table 2), patients who developed ALF had higher mean values of TBL ($p<0.001$), AST ($p<0.001$) and INR ($p<0.001$). Most patients who developed ALF showed AST higher than ALT and serum ALP $\leq$ 4 x ULN. Half of the patients with ALF showed an AST/ALT ratio over 1.5 compared to 6.7% of patients who did not develop ALF (p=0.002)

Out of the 12 patients who developed ALF, five patients died due to multiorgan failure (cases 12, 22, 31, 52 and 56), three of them while waiting for liver transplantation. Three

patients (cases 23, 35 and 39) were successfully transplanted whereas four patients recovered spontaneously after 45-150 days (median 60 days; IQR: 53-105) (Table 3).

Nine patients (75%) were receiving concomitant drugs either on a long-term basis (cases 22, 28, 32, 33, 35, 52 and 56) or with an incompatible temporal relationship (cases 12 and 45) (Table 3). Case 45 took ibuprofen 400 mg occasionally during the seven months before nimesulide-DILI, but no intake was documented within 18 days before starting symptoms of liver toxicity. Similarly, diclofenac was occasionally taken by case 12 during the previous nine months of nimesulide-DILI but no intake was documented within 16 days prior to the development of hepatotoxicity.

Logistic regression analysis showed that TBL and AST values at DILI recognition were independently associated with the development of ALF. An increase in TBL by 1 unit, increased the risk of ALF by 13% (adjusted odds ratio [aOR] 1.13; 95% CI 1.02-1.24). Likewise, a rise in AST was associated with an increased risk of ALF (aOR 1.16; 95% CI 1.03-1.30).

Clinical presentation and outcome according to latency

Most of the patients (79%) developed nimesulide hepatotoxicity after 15 days from therapy initiation. Jaundice was present in 84%, whereas 58% required hospitalization. Remarkably, hospitalization rate was significantly higher in patients with latency over 15 days) compared to those with shorter latency period (8 to 15 days; p=0.024). Interestingly, four patients (one mild, one moderate, one severe and one fatal/Tx) presented hepatotoxicity within 7 days from treatment initiation, whereas mild-to-moderate liver injury was observed in eight patients who were exposed to nimesulide between 8 and 15 days. Patients with a longer latency presented worse outcomes (20% were severe cases, and 16% were fatal/Tx) (Table 4). However, ALF was documented in a 57-year old woman treated for osteoarthritis with nimesulide 200 mg/day who developed jaundice 3 days after nimesulide intake. She developed fulminant hepatitis linked to severe coagulopathy (INR=3.9) and died due to multiorgan failure while she was waiting for liver transplantation (Table 3).

Cholestatic/mixed pattern was documented in 50% of patients who developed nimesulide-DILI within 7 and those within 15 days, while hepatocellular injury predominated in patients who developed nimesulide-DILI after 15 days (71%) (Table 4). Notably, patients with longer latency (>15 days) showed higher AST and ALT levels when compared to patients with shorter latency between 8 and 15 days (p=0.003 and p=0.002, respectively).

DISCUSSION

The global incidence of NSAID-induced hepatotoxicity ranges from 1 to 9 cases per 100,000 persons exposed (Bessone 2010). We here present a comprehensive analysis of the largest series of nimesulide-related hepatotoxicity published to date comprising 57 patients. Our analysis highlights the potential of nimesulide to induce serious liver injury, including ALF. Higher female prevalence has been demonstrated for NSAID hepatotoxicity in general (Zoubek et al. 2017), and the majority of cases of nimesulide-

associated hepatotoxicity published so far including our series, occurred in elderly women (Kwon et al. 2019). Thus, an overrepresentation of females (86%) was observed in our cohort, where 61% were women older than 60 years, and 43% were over 65 years. Interestingly, however, our series also included a pediatric case that developed severe hepatotoxicity and required liver transplantation.

Recent data taken from 33 cases of nimesulide-DILI compiled from the literature by Kwon *et al.* (2019), which were retrieved from 12 countries, including Israel, Belgium, France, Greece, Italy, Ireland, Iceland, Spain, Switzerland, Serbia, Singapore, and South Korea, showed that 85% were women with a median age of 57 years and that an age ≥55 years was a risk factor for nimesulide-DILI (Kwon et al. 2019).

It is important to highlight that 83% of our patients developed nimesulide-DILI within 90 days after initiation of therapy and 14% showed liver toxicity after 6 months of drug treatment. These data mirror those published by Kwon *et al.* (2019), who found 28 out of 33 (84%) patients developing nimesulide-DILI within 90 days, and 6% after 6 months (Kwon et al. 2019). It is possible that unknown host or environmental factors in conjunction with nimesulide led to prolonged latency in these cases. Hence, nimesulide hepatotoxicity appears to be most common within 90 days from treatment initiation but may require longer drug exposure to develop hepatotoxicity in some individuals.

Enhanced awareness of severe nimesulide-DILI is important considering that 18 (32%) of our patients developed severe forms of liver disease and 12 developed ALF. All patients who developed ALF were women and half of them were ≥60 years at presentation. These results parallel those presented by Robles-Díaz *et al.* (2014), where 13 (42%) of patients who developed ALF presented an age higher than 60 years (Robles-Diaz et al 2014).

In agreement with our results, the study of Kwon *et al.* also showed a high association of nimesulide-DILI with fatal outcome. Almost half of the 33 patients analyzed by these authors required liver transplantation or died as a result of ALF (Kwon et al. 2019). This meta-analysis included 6 studies, based on pharmacovigilance databases, and the authors highlighted that the use of nimesulide was associated with a significantly higher proportion of hepatic adverse events, compared with other NSAIDs (random-effects reporting odds ratio [ROR] 3.99, 95% CI 2.86–5.57) (Kwon et al. 2019). The severity of hepatotoxicity associated with nimesulide is, indeed, a distinctive feature from other NSAIDs based on reports from prospective Registries worldwide (Zoubek et al. 2017; Schmeltzer et al. 2016).

Comparable data have also been recently reported based on 179 cases and 1770 matched controls by Donati and coworkers (Donati et al. 2016), who analyzed nimesulide-, ibuprofen-, and ketoprofen-induced hepatotoxicity. They found that 30 cases exposed to nimesulide (aOR 2.10; 95% CI 1.28–3.47) had increased DILI risk according to the length of exposure (aOR >30 days 12.55; 95% CI 1.73-90.88) and higher doses (aOR 10.69; 95% CI 4.02-28.44). These authors concluded that, while nimesulide was associated with the highest risk of DILI, ibuprofen, and high doses of ketoprofen were linked to a modest

increase in DILI risk. No well-designed prospective studies have been carried out so far to assess the real incidence of DILI due to nimesulide and the epidemiological information comes from retrospective analyses undertaken on exposed populations comparing nimesulide-DILI with other NSAIDs (Traversa et al. 2003).

Of note, our analysis revealed that 21% of the entire study cohort showed liver damage within 15 or less days of nimesulide administration. Interestingly, 15 days is the current maximum duration of treatment approved by the EMA for this agent (European Medicines Agency 2012b). This approval is based on an alleged lower risk of nimesulide to induce DILI before this time. Hence, our observations challenge the last warning from EMA, as our data demonstrate that 15 days or less of nimesulide therapy is not a liver safe practice.

Nearly one-third of our case series developed severe or fatal hepatotoxicity. Patients who developed ALF had a longer latency period, though the difference did not reach statistical significance, probably due to the low number of cases and data dispersion. Independent risk factors identified for the development of ALF were high levels of TBL and AST values confirming previous findings (Andrade et al. 2005; Björnsson 2005; Reuben et al. 2010; Robles-Diaz et al. 2014). These findings further underscore the relevance of AST rather than ALT as a predictor of the worst outcome. Indeed, the nR Hy's Law that uses AST or ALT whichever is highest, is a more sensitive predictor of ALF than traditional Hy's law, as demonstrated by the Spanish DILI Registry group (Robles-Diaz et al. 2014), and independently validated by the DILIN group (Hayashi et al. 2017).

Interestingly, a broad spectrum of lesions was found on liver histology in 25% of our case series. A predominance of hepatocellular necrosis was observed in 64% of patients, followed by mixed injury in 27% and pure cholestasis in 9 %. Our results are in agreement with those published by Bjarnason and co-workers (2005). They observed hepatocellular necrosis in 65% of cases, mixed cholestatic hepatitis in 25%, and pure cholestasis in the remaining 10% (Bjarnason et al. 2005). The first description of pure cholestasis induced by nimesulide-DILI was reported by Van Steenbergen in two patients in a series consisting of six cases published in 1998 (Van Steenbergen et al. 1998).

The mechanisms of nimesulide-DILI remain unknown. Recent GWAs have demonstrated the participation of the adaptive immune system in DILI pathogenesis as indicated by the association of several HLA alleles with DILI risk related to specific drugs (Andrade et al. 2019). No genetic association study has been undertaken so far in patients with nimesulide hepatotoxicity. However, due to the scarce evidence of hypersensitivity features, some authors have suggested a metabolic idiosyncratic mechanism of hepatic injury (Boelsterli 2002). Thus, the reductive bio-activation of the nitroaromatic group of the nimesulide molecule by hepatic nitroreductase activity, can lead to the formation of an aromatic amine. These nitroreductases induce oxidative stress by producing highly reactive nitro anion radicals (Boelsterli 2002). Mitochondria is thought to be a major target of nimesulide toxicity since high amounts of the drug enter the mitochondria, and its bioreductive metabolism there would provoke oxidative stress-induced opening of mitochondrial permeability transition pore (Boelsterli 2002; Lucena et al. 2010). Indeed,

the predominance of AST observed as a marker of severity in these series could be explained by an important fraction of hepatocyte mitochondrial source (Robles-Diaz et al. 2014).

The metabolic idiosyncrasy hypothesis rather than immunological mechanism as responsible for nimesulide-DILI is also supported by the low frequency of peripheral eosinophilia observed in our series, in just nine of 57 cases (16%). Similarly, Kwon *et al.* (2019) found this feature in only two patients (6%). No patients developed neither dermatological signs of hypersensitivity nor nimesulide-induced autoimmune liver disease (Kwon et al. 2019). In keeping with this concept, we found only two patients in our series and two cases described by Kwon *et al.* (2019) who had eosinophilic infiltrate in liver histology (Kwon et al. 2019).

In summary, over the last decades, nimesulide has been associated with a wide spectrum of liver damage, including a variety of patterns, such as hepatitis, cholestasis, mixed forms, and ALF. Although many countries have withdrawn this agent from the market due to severe liver damage, nimesulide continues to be marketed in more than 50 countries worldwide. Our study highlights that nimesulide-DILI mainly affects females inducing a hepatocellular pattern, which is associated with ALF and death in a high proportion of patients. Furthermore, our analysis indicates that the current mitigation strategies endorsed by EMA (a maximum daily dose of 100 mg bid and the indication of short-term use (i.e. maximum 15 days of treatment)) fail to prevent nimesulide hepatotoxicity and, hence, the risk/benefit ratio of nimesulide is clearly unfavorable.

Declarations

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Conflict of interests

The authors declare that they have no conflict of interest.

Ethical standards

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

Authorship statement

Guarantor of the article: Fernando Bessone, M Isabel Lucena

Specific author contributions: FB, RJA and MIL wrote the manuscript; FB, RJA and MIL designed the study; FB, NH, MM, ER, GG, EF, MP, HF, MA, HT, FT, RP, IM-C, MR-D, CS,LC,MA,MVR,SKO and FC performed the research; FB, IA-A, HN, CS, MR-D, IM-C and MIL analyzed the data.

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Clinical collaborating centers of the Latin American DILI Registry

ARGENTINA

Hospital Provincial del Centenario, Rosario. F Bessone, H Tanno, V Reggiardo, S Ferretti, F Tanno, L Arribillaga, M Amateis, Y Zambello, A Ferretti, J Vorobioff, A Galimberti , V Trevizan, M Chiaraviglio, P Caballini, J Montero, J Ortiz, A Rodil, M La Placa, L Zitelli, F Jaureguizahar, A Ferrari, N Tamagnone, S Bulaty, J Pacual, M Tanno, G Carbonetti, G Piñero, L Muñoz, G Carnevale, Y Zambello, M Amateis, C Guerrina, A Wulfson, ML Arribillaga

Hospital Privado de Rosario. A Ruf, M Dirchwolf

Hospital de Córdoba, Ciudad de Córdoba. A Zerega

Hospital Universitario Austral. M Mendizábal, M Silva

Hospital Nacional Alejandro Posadas. G Gualano, E Fassio

Centro de Educación Médica e Investigaciones Clínicas (CEMIC), Buenos Aires. E Ridruejo

Hospital Italiano de Buenos Aires. N Sobenko, J Pizzala, L Haddad, A Villamil, A Gadano

Hospital Británico, Buenos Aires. J Benavidez, N Fernandez, L Colombato

Hospital Eva Perón, Buenos Aires. C Guma

Clínica de Nefrología, Santa Fe. L Gaite

Sanatorio de niños, Rosario. A Costaguta, A Pais

Hospital Alemán, CABA. M Anders

Hospital de infecciosas F.J. Muñiz, CABA. M Peralta, S Campuzano, S Paz, H Famboin

Hospital Italiano de La Plata, La Plata. F Gruz

Hospital Universitario Fundación Favaloro. V Descalzi

Hospital General de Agudos Dr. Cosme Argerich. G Tsariktsian, A Bruno, B Frider

Hospital Santojanni. NE Libaak

Hospital San Bernardo. C Facundo Zarbá

Hospital Aeronáutico Central. P Testa

Hospital Internacional General de Agudos. E Giraudo

Hospital Marcial Quiroga. R Romo

Nuevo Hospital Río Cuarto, Córdoba. C Mendoza

Centro de Hepatología La Plata. S Borzi

Hospital Español, Mendoza. O Galdame, M Paez

Hospital El Cruce, Buenos Aires. F Villamil

Hospital JM Penna. M Mesquida

Hospital Bonorino Udaondo, Buenos Aires. M Cartier

Hospital Presidente Perón de Avellaneda, Buenos Aires. S Chao

Sanatorio San Carlos, Bariloche. C Garcia Dans

BRAZIL

ICHC FMUSP Universidad de Sao Paulo. G Belchior, F Carrilho, SK Ono, N Lopes, G Dagostino, F Roberto, V Alves

CHILE

Pontificia Universidad Católica de Chile. M Arrese, A Ruíz, R Zapata, RM Mellado.

Participating clinical centers of the Spanish DILI Registry

Hospital Universitario Virgen de la Victoria, Málaga (coordinating center): RJ Andrade, MI Lucena, C Stephens, M García Cortés, M Robles-Díaz, A Ortega-Alonso, J Pinazo, B García Muñoz, R Alcántara, A Hernández, MD García Escaño, E del Campo, I Medina-Cáliz, J Sanabria-Cabrera, A González-Jiménez, R Sanjuán-Jiménez, A Cueto, I Álvarez-Álvarez, E Bonilla, D Di Zeo, H Niu, M Villanueva, A Papineau;

Hospital Torrecárdenas, Almería: MC Fernández, G Peláez, A Porcel, M Casado, M González Sánchez;

Hospital Universitario Virgen del Rocío, Sevilla: M Romero-Gómez, R Millán-Domínguez, B Fombuena, R Gallego, J Ampuero, JA del Campo, R Calle-Sanz, L Rojas, A Rojas, A Gil Gómez, E Vilar;

Hospital Central de Asturias, Oviedo: R Pérez-Álvarez, L Rodrigo-Sáez.

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Table 1. Demographics, clinical and biochemical findings according to hepatocellular and cholestatic/mixed type of liver injury in 57 patients with nimesulide-induced liver injury.

	All cases N=57	**Hepatocellular N=38 (67%)**	**Cholestatic/Mixed N=19 (33%)**	***p* value**
Age y, mean (range)	59 (9-82)	59 (9-82)	60 (32-82)	0.666
Female sex, n (%)	49 (86)	33 (87)	16 (84)	1.000
Associated diseases, n (%)				
Diabetes	6 (11)	3 (7.9)	3 (16)	0.389
Dyslipidemia	9 (16)	4 (11)	5 (26)	0.143
Hypertension	11 (19)	7 (18)	4 (21)	1.000
Clinical presentation, n (%)				
Jaundice	46 (81)	34 (89)	12 (63)	0.031
Pruritus	9 (16)	2 (5.3)	7 (37)	0.004
Hospitalization	29 (51)	20 (53)	9 (47)	0.783
Eosinophilia, (>500/µL)	9 (16)	7 (18)	2 (11)	0.703
Latency, median, *d* (IQR 25-75)	40 (20-90)	45 (25-90)	30 (14-45)	0.084
Cumulative dose, median *mg* (IQR 25-75)	6,000 (4,000-18,000)	8,200 (5,000- 18,000)	4,000 (2,800-9,000)	0.073
Laboratory parameters on admission x ULN, mean±SD				
TBL	13±11	16±12	8.2±6.6	0.026
AST	20±26	26±30	9.1±6.9	0.007
ALT	18±20	23±23	9.3±4.6	<0.001
ALP	2.0±1.5	1.4±1.1	3.3±1.2	<0.001
AST/ALT >1.5, n (%)	9 (16)	7 (18)	2 (11)	0.703
INR	1.9±1.4	2.1±1.6	1.4±0.7	0.137
New Hy's Law, n (%)	37 (65)	34 (89)	3 (16)	<0.001
Severity, n (%)				
Mild	8 (14)	4 (11)	4 (21)	0.115
Moderate	31 (54)	18 (47)	13 (68)	
Severe	10 (18)	9 (24)	1(5.3)	
Fatal	8 (14)	7 (18)	1 (5.3)	
Outcome				
Resolution time, median, *d* (IQR 25-75)	60 (35-90)	55 (33-73)	61 (38-124)	0.206
ALF, n (%)	12 (21)	11 (29)	1 (5.3)	0.045
Death, n (%)	5 (8.8)	4 (11)	1 (5.3)	0.655
Tx, n (%)	3 (5.3)	3 (7.9)	0 (0)	0.544

Abbreviations: TBL, serum total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase. Eosinophilia defined as eosinophils >5%, SD, Standard deviation; Severity index: Mild: ALT ≥5 x ULN or ALP ≥2 x ULN, and TBL <2 x ULN; Moderate: ALT ≥5 x ULN or ALP ≥2 x ULN, and TBL ≥2 x ULN; Severe: ALT ≥5 x ULN or ALP ≥2 x ULN, and TBL ≥2 x ULN and one of the following: (i) international normalized ratio ≥1.5, (ii) ascites and/or encephalopathy, disease duration <26 weeks, and absence of underlying cirrhosis, (iii) other organ failure considered to be due to DILI; Fatal: death or transplantation due to DILI. ALF, acute liver failure; Tx, liver transplant. New Hy's Law defined as: DILI with hepatocellular injury (nR ≥5, nR = ALT or AST, whichever the highest/ULN ÷ alkaline phosphatase/ULN value) and TBL >2 x ULN.

Table 2. Comparison of demographics, clinical and biochemical parameters in 57 patients with nimesulide hepatotoxicity who did or did not develop acute liver failure (ALF).

	ALF N=12 (21%)	**Non ALF N=45 (79%)**	***p* value**
Age y, mean (range)	55 (9-71)	60 (16-82)	0.457
Female sex, n (%)	12 (100)	37 (82)	0.182
Associated diseases, n (%)			
Diabetes	2 (17)	4 (8.9)	0.596
Dyslipidemia	2 (17)	7 (16)	1.000
Hypertension	2 (17)	9 (20)	1.000
Clinical presentation, n (%)			
Jaundice	12 (100)	34 (76)	0.097
Pruritus	1 (8.3)	8 (18)	0.667
Hospitalization	12 (100)	17 (38)	<0.001
Eosinophilia (>500/µL)	0 (0)	9 (20)	0.180
Latency, median *d* (IQR 25-75)	60 (35-135)	30 (20-62)	0.108
Cumulative dose, median *mg* (IQR 25-75)	10,500 (6,000-20,900)	6,000 (3,000- 12,400)	0.133
Pattern of liver injury, n (%)			
Hepatocellular	11 (92)	27 (60)	0.045
Mixed/Cholestatic	1 (8.3)	18 (40)	
Laboratory parameters on admission x ULN, mean±SD			
TBL	25±11	10±9.2	<0.001
AST	46±41	13±15	<0.001
ALT	33±28	14±15	0.002
ALP	2.1±1.4	2.0±1.5	0.907
AST/ALT>1.5, n (%)	6 (50)	3 (6.7)	0.002
INR	3.9±1.8	1.3±0.3	<0.001
New Hy's Law, n (%)	11 (92)	26 (58)	0.041
Severity, n (%)			
Mild	0 (0)	8 (18)	<0.001
Moderate	0 (0)	31 (69)	
Severe	4(33)	6 (13)	
Fatal	8 (67)	0 (0)	
Outcome			
Resolution time, median, *d* (IQR 25-75)	60 (60-150)	60 (35-76)	0.218
Death, n (%)	5 (42)	0 (0)	<0.001
Tx, n (%)	3 (25)	0 (0)	0.008

Abbreviations: TBL, serum total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase. Eosinophilia defined as eosinophils >5%, SD, Standard deviation; Severity index: Mild: ALT ≥5 x ULN or ALP ≥2 x ULN, and TBL <2 x ULN; Moderate: ALT ≥5 x ULN or ALP ≥2 x ULN, and TBL ≥2 x ULN; Severe: ALT ≥5 x ULN or ALP ≥2 x ULN, and TBL ≥2 x ULN and one of the following: (i) international normalized ratio ≥1.5, (ii) ascites and/or encephalopathy, disease duration <26 weeks, and absence of underlying cirrhosis, (iii) other organ failure considered to be due to DILI; Fatal: death or transplantation due to DILI. ALF, acute liver failure; Tx, liver transplant. New Hy's Law defined as: DILI with hepatocellular injury (nR ≥5, nR = ALT or AST, whichever the highest/ULN ÷ alkaline phosphatase/ULN value) and TBL >2 x ULN.

Table 3. Demographics, clinical, biochemical features, and histological characteristics of the 12 patients with nimesulide-induced acute liver failure.

Case Nº	Sex / Age (yr)	Indication	Doses (mg/d)	Concomitant medication	Latency (d)	TBL (x ULN)	ALT (x ULN)	AST (x ULN)	ALP (x ULN)	INR	Pattern of liver injury / Histological findings	Outcome / Recovery (d)
12	F / 57	Osteoarthritis	200	Levothyroxine, diclofenac (occasionally)	3	7.0	12	10	3.0	3.9	Mixed	Death
22	F / 66	Osteoarthritis	200	Dihydroergocristine (1 yr)	238	34	12	10	2.0	2.7	Hepatocellular / Submassive necrosis	Death
23	F / 9	Odynophagia	200	No	30	13	76	123	5.0	3.6	Hepatocellular / Massive necrosis (explanted liver)	LTx
28	F / 54	Osteoarthritis	200	Levothyroxine, NPH insulin (5 yr)	180	25	40	43	1.0	2.9	Hepatocellular	Recovery (45)
31	F / 32	Osteoarthritis	200	No	90	32	10	16	0.3	5.8	Hepatocellular / Massive necrosis (postmortem)	Death
32	F / 71	Osteoarthritis	200	Enalapril, levothyroxine (3 yr)	90	23	20	40	4.0	3.6	Hepatocellular	Recovery (150)
33	F / 65	Osteoarthritis	200	Levothyroxine (5 yr)	60	14	25	47	1.0	2.5	Hepatocellular	Recovery (60)
35	F / 56	Arthralgia	100	Losartan, amiodarone, hydrochlorothiazide (long term)	60	22	23	33	0.6	3.1	Hepatocellular / Submassive necrosis (explanted liver)	LTx
39	F / 63	Osteoarthritis	200	No	210	27	37	58	2.0	4.5	Hepatocellular / Massive necrosis	LTx
45	F / 56	Back pain	200	Ibuprofen (occasionally)	20	47	101	133	3.0	3.1	Hepatocellular / Massive necrosis	Recovery (60)
52	F / 67	Lower back pain	200	Metformin (2 yr)	45	23	11	17	2.0	8.5	Hepatocellular	Death
56	F / 66	Lower back pain	200	Levothyroxine (7 yr)	40	28	26	22	1.0	2.4	Hepatocellular	Death

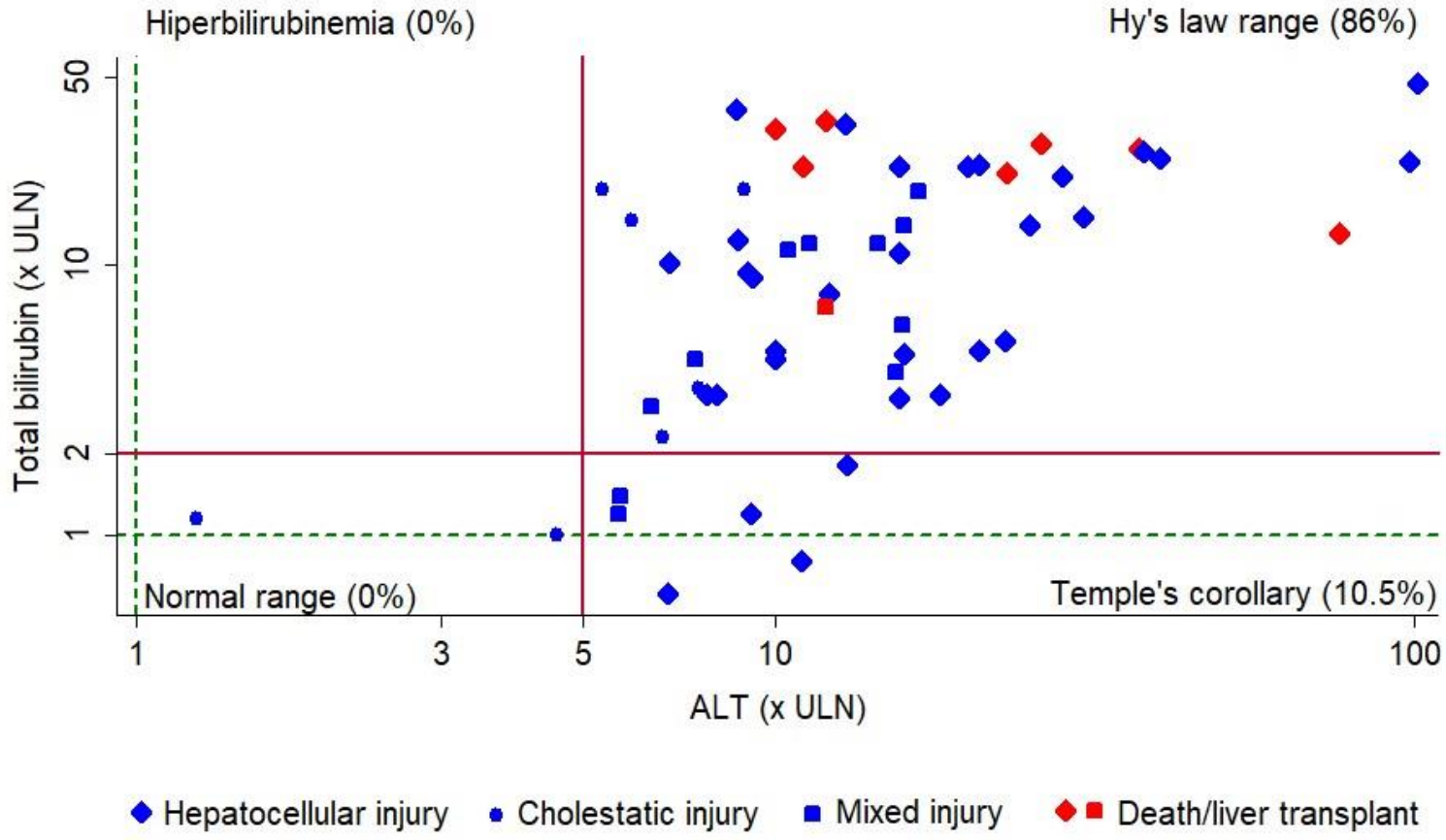
Abbreviations: yr: years; d: days; TBL, serum total bilirubin (0.1-1.2 mg/dl); ALT, alanine aminotransferase (5-35 U/L); AST, aspartate aminotransferase (4-32 U/L); ALP, alkaline phosphatase (35-105 UI/L); INR: International Normalized Ratio; LTx: liver transplantation

Table 4. Demographics, clinical and biochemical parameters in 57 nimesulide-induced liver injury according to latency period.

Whole population (N=57)	**≤7 days N=4 (7%)**	**8-15 days N=8 (14%)**	**>15 days N=45 (79%)**	***p* value**
Age y, mean (range)	48 (16-64)	59 (41-80)	60 (9-82)	0.291
Female sex, n (%)	2 (50)	7 (88)	40 (89)	0.120
Associated diseases, n (%)				
Diabetes	0 (0)	1 (13)	5 (11)	1.000
Dyslipidemia	1 (25)	2 (25)	6 (13)	0.506
Hypertension	0 (0)	0 (0)	11 (24)	0.197
Clinical presentation, n (%)				
Jaundice	3 (75)	5 (63)	38 (84)	0.252
Pruritus	1 (25)	1 (13)	7 (16)	0.808
Hospitalization	2 (50)	1 (13)	26 (58)	0.057
Eosinophilia (>500/µL)	1 (25)	1 (13)	7 (16)	0.808
Pattern of liver injury, n (%)				
Hepatocellular	2 (50)	4 (50)	32 (71)	0.303
Cholestatic/Mixed	2 (50)	4 (50)	13 (29)	
Cumulative dose, median, *mg* (IQR 25-75)	700 (550-900)	2,400 (1,500-2,800)	9,000 (6,000-18,000)	<0.001
Laboratory parameters on admission x ULN, mean±SD				
TBL	10±11	6.3±4.9	15±12	0.126
AST	16±10	5.5±4.6	23±29	0.008
ALT	18±14	7.3±2.4	20±21	0.006
ALP	3.3±1.8	1.7±1.5	2.0±1.4	0.246
AST/ALT >1.5	0 (0)	0 (0)	9 (20)	0.404
INR	2.0±1.3	1.2±0.1	2.0±1.5	0.346
New Hy's Law, n (%)	1 (25)	4 (50)	32 (71)	0.107
Severity, n (%)				
Mild	1 (25)	2 (25)	5 (11)	0.268
Moderate	1 (25)	6 (75)	24 (53)	
Severe	1 (25)	0 (0)	9 (20)	
Fatal	1 (25)	0 (0)	7 (16)	
Outcome				
Resolution time, median, *d* (IQR 25-75)	8 (6-60)	43 (33-61)	60 (37-90)	0.052
ALF, n (%)	1 (25)	0 (0)	11 (24)	0.276
Death, n (%)	1 (25)	0 (0)	4 (8.9)	0.424
Tx, n (%)	0 (0)	0 (0)	3 (6.7)	1.000

Abbreviations: TBL, serum total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase. Eosinophilia defined as eosinophils >5%, SD, Standard deviation; Severity index: Mild: ALT ≥5 x ULN or ALP ≥2 x ULN, and TBL <2 x ULN; Moderate: ALT ≥5 x ULN or ALP ≥2 x ULN, and TBL ≥2 x ULN; Severe: ALT ≥5 x ULN or ALP ≥2 x ULN, and TBL ≥2 x ULN and one of the following: (i) international normalized ratio ≥1.5, (ii) ascites and/or encephalopathy, disease duration <26 weeks, and absence of underlying cirrhosis, (iii) other organ failure considered to be due to DILI; Fatal: death or transplantation due to DILI. ALF, acute liver failure; Tx, liver transplant. New Hy's Law defined as: DILI with hepatocellular injury (nR ≥5, nR = ALT or AST, whichever the highest/ULN ÷ alkaline phosphatase/ULN value) and TBL >2 x ULN.

Fig. 1 Description of alanine aminotransferase and total bilirubin levels in fold of upper limit of normal (ULN) in nimesulide-DILI cases.



Each point represents a unique nimesulide-DILI case's alanine aminotransferase (ALT) and total bilirubin values. Axis are represented in fold of upper limit of normality (ULN), on a log scale.

Fig. 2 Liver histology findings from four nimesulide-DILI cases.

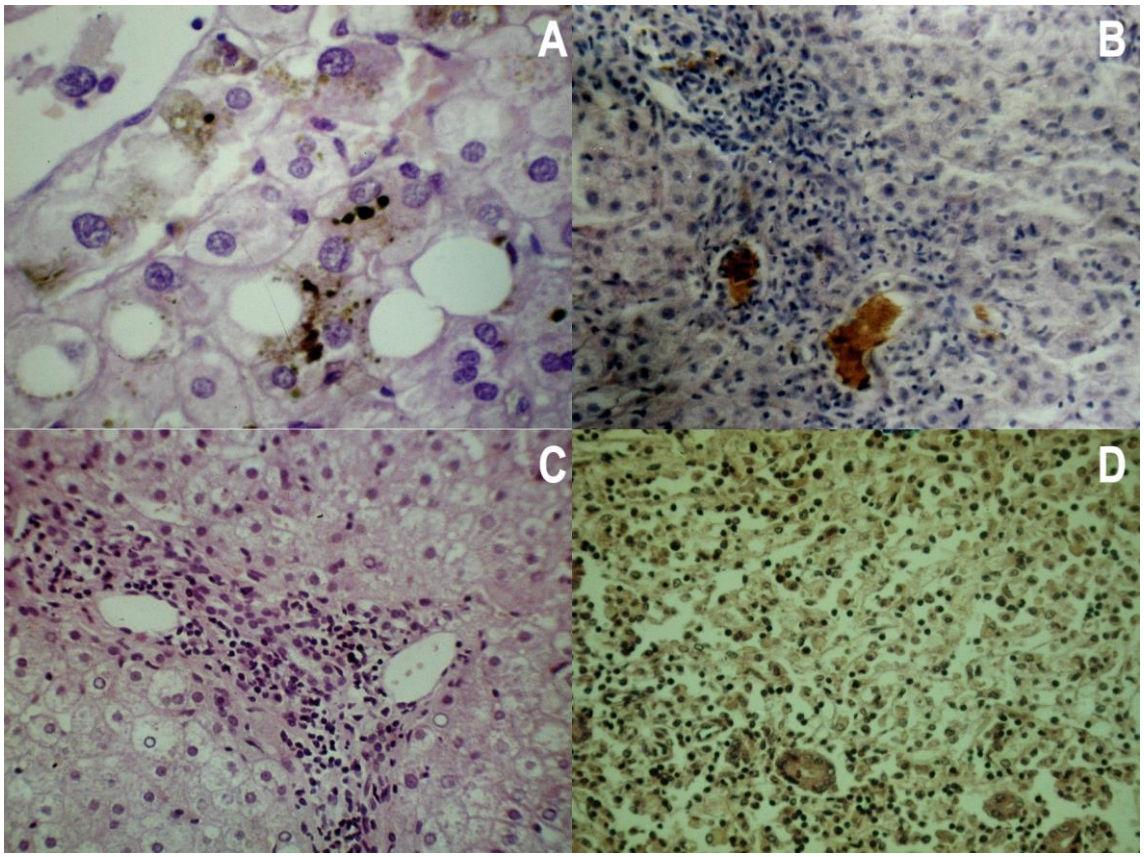


Fig. 2A Case number 9. Pure cholestasis: hepatocytes with balloonized cytoplasm an hepatocanalicular cholestasis (H&E, 40x). **Fig. 2B** Case number 14. Cholestatic hepatitis: mixed portal and parenchymal inflammatory infiltrate. Presence of cytoplasmic bile pigment and biliary plugs (H&E, 10x). **Fig. 2C** Case number 27. Acute hepatitis (hepatocellular): portal inflammatory infiltrate and ballonized hepatocytes, with images of steatosis and isolated glycogenic nuclei (H&E, 10x). **Fig. 2D** Case number 45. Fulminant hepatitis: panacinar hepatic necrosis with marked ductular proliferation. Mononucleated inflammatory cells interposed between necrotic elements (H&E, 10x).

Supplemental Table 1. Demographics, clinical and biochemical characteristics of the 57 patients with nimesulide-induced liver injury.

Case Nº	Sex / Age (yr)	Indication	Clinical presentation	Underlying disease	Doses (mg/d)	Concomitant medication	Latency (d)	Bilirubin (x ULN)	ALT (x ULN)	AST (x ULN)	ALP (x ULN)	INR	Pattern of liver injury / Histological findings	Severity	Outcome / Recovery (d)
1	F / 67	Osteoarthritis	None	None	200	No	30	1.2	9.1	6.7	0.4	1.1	Hepatocellular	Mild	Recovery (50)
2	M / 80	Osteoarthritis	None	Hypertension	200	No	45	1.2	5.7	4.6	1.3	1.0	Mixed	Mild	Recovery (31)
3	F / 51	Odynophagia	Rash	None	200	No	14	1.0	4.5	1.5	3.9	1.1	Cholestatic	Mild	Recovery (35)
4	F / 16	Pain	None	None	200	Ciprofloxacin	5	0.6	6.8	5.9	1.2	1.0	Hepatocellular	Mild	Recovery (8)
5	F / 59	Osteoarthritis	Hospitalization	Diabetes, hypertension	200	Atenolol, captopril, hydrochlorothiazide	230	1.2	1.2	1.3	5.0	ND	Cholestatic	Mild	Recovery (550)
6	F / 51	Headache	None	Dyslipidemia	200	No	30	1.8	13	10	0.4	1.0	Hepatocellular	Mild	Recovery (30)
7	F / 62	Trauma	None	None	200	Diclofenac, ibuprofen, occasionally	90	0.8	11	5.7	0.4	1.0	Hepatocellular	Mild	Recovery (90)
8	F / 54	Odynophagia	None	None	100	Paracetamol, occasionally	14	1.4	5.7	4.0	1.5	1.0	Mixed	Mild	Recovery (62)
9	F / 32	Lower back pain	None	None	200	Amiodarone, long term	30	2.3	6.6	3.1	3.8	1.1	Cholestatic / Pure cholestasis	Moderate	Recovery (38)
10	F / 53	Lower back pain	None	Dyslipidemia	100	Levothyroxine, enalapril, long term	14	3.0	6.4	2.3	1.3	1.1	Mixed	Moderate	Recovery (30)
11	F / 56	Headache	Jaundice, rash, hospitalization	Hypertension	100	No	30	11	10	4.3	2.4	1.4	Mixed / Cholestatic hepatitis	Moderate	Recovery (63)
12	F / 57	Osteoarthritis	Jaundice, hospitalization, encephalopathy	Dyslipidemia	200	Levothyroxine, diclofenac, occasionally	3	7.0	12	10	3.0	3.9	Mixed	Fatal	ALF / Death
13	M / 48	Osteoarthritis	Jaundice	Hypertension	200	No	45	3.5	7.6	5.1	3.9	1.2	Cholestatic / Cholestatic hepatitis	Moderate	Recovery (180)
14	F / 62	Osteoarthritis	Jaundice, hospitalization	None	200	No	20	19	8.9	8.7	4.6	1.5	Cholestatic / Cholestatic hepatitis	Moderate	Recovery (180)
15	F / 60	Osteoarthritis	Jaundice, hospitalization	Diabetes, dyslipidemia	200	Metformin, long term	15	15	6.0	6.9	4.3	1.4	Cholestatic	Moderate	Recovery (45)
16	F / 72	Osteoarthritis	Rash	None	100	No	20	14	16	12	3.3	1.0	Mixed	Moderate	Recovery (20)

17	F / 77	Osteoarthritis	Jaundice, rash, hospitalization	None	200	Diclofenac, occasionally	35	12	11	12	2.8	1.4	Mixed	Moderate	Recovery (120)
18	F / 82	Neck pain	Jaundice, rash, hospitalization	Diabetes, dyslipidemia	200	Metformin, long term	40	12	14	12	3.4	1.2	Mixed	Moderate	Recovery (65)
19	F / 73	Lower back pain	Jaundice, hospitalization	None	100	No	210	19	5.4	11	3.3	1.5	Cholestatic / Acute hepatitis with collapse	Moderate	Recovery (240)
20	M / 64	Trauma	Jaundice, rash	None	200	No	4	6.0	16	19	5.6	1.1	Mixed	Moderate	Recovery (60)
21	M / 55	Pain	Jaundice, hospitalization	None	200	Atenolol, long term	5	26	38	29	3.5	2.0	Hepatocellular	Severe	Recovery (6)
22	F / 66	Osteoarthritis	Jaundice, hospitalization	None	200	Dihydroergocristine, long term	238	34	12	10	2.0	2.7	Hepatocellular / Submassive necrosis	Fatal	ALF / Death
23	F / 9	Odynophagia	Jaundice, hospitalization	None	200	No	30	13	76	123	5.0	3.6	Hepatocellular / Massive necrosis (explanted liver)	Fatal	ALF / LTx
24	F / 54	Arthralgia	Jaundice	None	200	No	42	3.2	16	6.9	0.6	1.1	Hepatocellular	Moderate	Recovery (30)
25	M / 74	Trauma	Jaundice	None	200	No	56	4.5	10	9.1	0.4	1.1	Hepatocellular / Acute hepatitis	Moderate	Recovery (70)
26	F / 61	Osteoarthritis	Jaundice, hospitalization	Hypertension	200	Fosinopril, torasemide, long term	62	15	30	31	1.7	ND	Hepatocellular	Moderate	Recovery (62)
27	F / 52	Osteoarthritis	Jaundice, rash, hospitalization	None	200	Paracetamol 500 mg, occasionally	63	19	17	29	4.1	1.6	Mixed / Acute hepatitis	Severe	Recovery (124)
28	F / 54	Osteoarthritis	Jaundice, rash, hospitalization, encephalopathy	Diabetes	200	Levothyroxine, NPH insulin, long term	180	25	40	43	1.0	2.9	Hepatocellular	Severe	ALF / Recovery (45)
29	F / 66	Arthralgia	Jaundice	Hypertension	200	No	210	5.2	23	20	2.2	1.4	Hepatocellular	Moderate	Recovery (76)
30	F / 35	Arthralgia	Jaundice, hospitalization	None	200	No	90	24	21	20	0.7	1.4	Hepatocellular	Moderate	Recovery (90)
31	F / 32	Osteoarthritis	Jaundice, hospitalization	None	200	No	90	32	10	16	0.3	5.8	Hepatocellular / Massive necrosis (postmortem)	Fatal	ALF / Death
32	F / 71	Osteoarthritis	Jaundice, hospitalization, encephalopathy	None	200	Enalapril, levothyroxine, long term	90	23	20	40	4.0	3.6	Hepatocellular	Severe	ALF / Recovery (150)

33	F / 65	Osteoarthritis	Jaundice, hospitalization, encephalopathy	Hypertension	200	Levothyroxine, long term	60	14	25	47	1.0	2.5	Hepatocellular	Severe	ALF / Recovery (60)
34	F / 70	Osteoarthritis	Jaundice, hospitalization	None	200	Prednisone, lansoprazole, long term	44	21	28	21	1.3	2.1	Hepatocellular	Severe	Recovery (37)
35	F / 56	Arthralgia	Jaundice, hospitalization, encephalopathy	Hypertension	100	Losartan, amiodarone, hydrochlorothiazide, long term	60	22	23	33	0.6	3.1	Hepatocellular / Submassive necrosis (explanted liver)	Fatal	ALF / LTx
36	F / 62	Osteoarthritis	Jaundice	None	200	No	30	24	98	93	2.5	ND	Hepatocellular	Moderate	Recovery (30)
37	F / 66	Osteoarthritis	Jaundice	None	100	No	14	7.8	12	16	1.1	1.2	Hepatocellular	Moderate	Recovery (41)
38	M / 67	Lower back pain	Jaundice, hospitalization, encephalopathy	None	200	No	60	38	8.7	8.1	0.4	2.5	Hepatocellular	Severe	Recovery (90)
39	F / 63	Osteoarthritis	Jaundice, hospitalization	None	200	No	210	27	37	58	2.0	4.5	Hepatocellular / Massive necrosis	Fatal	ALF / LTx
40	F / 80	Odynophagia	Jaundice	Hypertension	200	Propranolol, long term	25	9.0	9.2	3.3	1.4	1.1	Hepatocellular	Moderate	Recovery (35)
41	F / 82	Odynophagia	Jaundice	None	200	No	180	4.8	21	19	0.9	1.2	Hepatocellular	Moderate	Recovery (60)
42	F / 75	Lower back pain	Jaundice, rash, hospitalization	None	200	Levothyroxine, enalapril, long term	180	33	13	27	2.5	2.2	Hepatocellular	Severe	Recovery (150)
43	F / 60	Neck pain	Jaundice	Diabetes, dyslipidemia, hypertension	200	Metformin, losartan, long term	20	3.3	7.8	4.9	0.4	1.1	Hepatocellular / Acute hepatitis	Moderate	Recovery (50)
44	F / 64	Osteoarthritis	Jaundice, hospitalization	None	200	Atenolol, amiodarone, long term	45	12	8.7	3.9	0.9	1.5	Hepatocellular	Moderate	Recovery (45)
45	F / 56	Back pain	Jaundice, hospitalization	None	200	Ibuprofen, occasionally	20	47	101	133	3.0	3.1	Hepatocellular / Massive necrosis	Severe	ALF / Recovery (60)
46	F / 80	Trauma	Jaundice	None	200	Ibuprofen, occasionally	10	9.4	9.0	8.0	0.6	1.3	Hepatocellular	Moderate	Recovery (63)
47	F / 70	Fever	Jaundice	Dyslipidemia	200	No	90	4.5	7.5	9.1	1.7	1.3	Mixed	Moderate	Recovery (53)
48	F / 65	Lower back pain	Jaundice	None	200	No	15	3.3	8.1	2.1	0.5	1.4	Hepatocellular	Moderate	Recovery (28)

49	F / 75	Arthralgia	Jaundice	None	200	Enalapril, long term	165	3.3	18	24	2.1	1.1	Hepatocellular	Moderate	Recovery (20)
50	M / 50	Lower back pain	Jaundice	None	100	Losartan, long term	60	4.8	10	11	1.8	1.1	Hepatocellular	Moderate	Recovery (35)
51	F / 61	Osteoarthritis	Jaundice, hospitalization	Dyslipidemia, hypertension	200	No	120	11	16	22	1.5	1.4	Hepatocellular	Moderate	Recovery (65)
52	F / 67	Lower back pain	Jaundice, hospitalization, encephalopathy	Diabetes, dyslipidemia	200	Metformin, long term	45	23	11	17	2.0	8.5	Hepatocellular	Fatal	ALF / Death
53	M / 41	Osteoarthritis	Jaundice	None	200	No	15	10	6.8	4.0	0.5	1.2	Hepatocellular	Moderate	Recovery (60)
54	F / 33	Odynophagia	Jaundice	None	200	No	21	4.0	15	17	3.2	1.1	Mixed	Moderate	Recovery (60)
55	F / 22	Pain	Jaundice, hospitalization	None	200	No	28	4.7	16	7.5	1.1	1.0	Hepatocellular	Moderate	Recovery (6)
56	F / 66	Lower back pain	Jaundice, hospitalization	None	200	Levothyroxine, long term	40	28	26	22	1.0	2.4	Hepatocellular	Fatal	ALF / Death
57	F / 72	Trauma	Jaundice	None	200	No	25	23	16	11	0.5	1.5	Hepatocellular	Severe	Recovery (90)

M: male; F: female; yr: years; d: days; ALT: alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; ULN: upper limit of normal; INR: International Normalized Ratio; ALF: acute liver failure; ND: no data;

LTx: liver transplantation

Supplemental Table 2. Detailed liver biopsy findings available in 14 patients with nimesulide-induced liver injury.

Case Nº	Sex / Age (yr)	Liver biopsy findings
9	F / 32	*Pure cholestasis.* Hepatocanalicular cholestasis mainly affecting zone 3 without both duct damage and inflammation. Isolated canalicular bile plugs.
11	F / 56	*Cholestatic hepatitis.* Mild hepatocellular and canalicular cholestasis linked to marked lobular inflammation with swelling of hepatocytes showing foamy cytoplasm and ballooning degeneration. Portal lymphocytic infiltrate associated with mild fibrosis in two portal tracts
13	M / 48	*Cholestatic hepatitis.* Zonal necrosis associated with inflammation predominantly affecting lobular parenchyma. Inflammatory infiltrate is composed by lymphocytes, eosinophils and macrophages, associated with isolated zones of lobular disarray. Hepatocytes with swelling cytoplasmic, and both isolated apoptotic bodies and spotty necrosis.
14	F / 62	*Cholestatic hepatitis.* Intense portal and lobular inflammation mimicking acute hepatitis. Swelling of hepatocytes and infiltration of foamy macrophages in the sinusoids. Marked hepatocanalicular cholestasis in zone 3 linked to canalicular biliary plugs.
19	F / 73	*Acute hepatitis with collapse (hepatocellular).* Intense portal and lobular inflammation associated with liver cell necrosis involving groups of hepatocytes. Isolated areas of lobular collapse. Inflammatory infiltrate with lymphocytes, isolated plasma cells, and neutrophils. Both isolated foci of spotty necrosis and marked hepatocellular cholestasis.
22	F / 66	*Submassive necrosis.* Marked drop-out of and loss of hepatocytes alternating with several areas of complete multilobular necrosis. Apoptotic hepatocytes in zone 3 with mild inflammation and scarce macrophages. Isolated areas hepatic regeneration.
23	F / 9	*Massive necrosis.* Massive drop-out and loss of hepatocytes mainly in centrolobular areas. Extensive zones of bridging necrosis with inflammatory infiltrate predominantly composed by lymphocytes and neutrophils.
25	M / 74	*Acute hepatitis (hepatocellular).* Mild hepatitis with spotty cell death and regenerative features such as binucleate hepatocytes and thick cell plates. Portal and lobular infiltrate with lymphocytes, neutrophils and isolated eosinophils.
27	F / 52	*Acute hepatitis (hepatocellular).* Moderate portal and lobular hepatocellular injury and inflammation. Presence of numerous macrophages in the sinusoids and prominent sinusoidal Kupffer cells. Both isolated apoptotic bodies and spotty necrosis. Minimal and isolated macrovesicular steatosis.
31	F / 32	*Massive necrosis.* Massive nonzonal confluent necrosis with collapsed parenchyma intermingled with ductular reaction as cellular regenerative attempt. No inflammatory infiltrate was observed.
35	F / 56	*Submassive necrosis.*

		Confluent submassive necrosis showing collapsed parenchyma associated with ductular reaction of hepatocytes accompanied by no inflammatory infiltrate. Abundant hypertrophied Kupffer cells and biliary plugs.
39	F / 63	*Massive necrosis.* Extensive centrilobular necrosis with both parenchymal collapsed areas and mild inflammatory infiltrate. Intense cholestasis with frequent biliary plugs. Ductular proliferation as cellular regeneration.
43	F / 60	*Acute hepatitis.* Lobular swelling of hepatocytes associated to ballooning degeneration. Intense infiltration of foamy macrophages in the sinusoids and portal presence of lymphocytes and eosinophils. Mild hepatocanalicular cholestasis and swelling of hepatocyte in zone 3. Isolated biliary plugs.
45	F / 56	*Massive necrosis.* Panacinar massive necrosis of hepatocytes associated with liver cell dropout and collapse of the reticulin framework.

Abbreviations: F: female; M: male; yr: years