

Distinct phenotype of hepatotoxicity associated with illicit use of anabolic androgenic steroids

M. Robles-Diaz^{*,†}, A. Gonzalez-Jimenez^{*}, I. Medina-Caliz^{*}, C. Stephens^{*,†}, M. García-Cortes^{*,†}, B. García-Muñoz^{*}, A. Ortega-Alonso^{*}, E. Blanco-Reina^{*}, R. Gonzalez-Grande[‡], M. Jimenez-Perez[‡], P. Rendón[§], J. M. Navarro[¶], P. Gines^{†,***}, M. Prieto^{†,††}, M. Garcia-Eliz^{††}, F. Bessone^{‡‡}, J. R. Brahm^{§§}, R. Paraná^{¶¶}, M. I. Lucena^{*,†}, R. J. Andrade^{*,†}, on behalf of the Spanish DILI Registry^a & the SLatinDILI Network^a

*Servicio de Farmacología Clínica and Unidad de Gestión Clínica (UGC) de Gastroenterología y Hepatología, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Universitario Virgen de la Victoria, Universidad de Málaga (UMA), Málaga, Spain.

†Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Barcelona, Spain.

‡UGC Enfermedades Digestivas, IBIMA, Hospital Universitario Regional Málaga, UMA, Málaga, Spain.

§UGC Enfermedades Digestivas, Hospital Universitario Puerta del Mar, Cádiz, Spain.

¶Servicio Aparato Digestivo, Hospital Costa del Sol, Málaga, Spain.

**Instituto de Enfermedades Digestivas y Metabolismo, Hospital Clinic, Barcelona, Spain.

††UGC Enfermedades Digestivas, Instituto de Investigación Sanitaria La Fe, Hospital La Fe, Valencia, Spain.

‡‡Hospital Provincial del Centenario, Universidad Nacional de Rosario, Rosario, Argentina.

§§Hospital Clínico, Universidad Católica de Chile, Santiago, Chile.

¶¶Complejo Hospitalario Professor Edgar Santos, Universidad Federal de Bahía, Salvador de Bahía, Brazil.

Correspondence to:

Dr M. I. Lucena, Departamento de Farmacología, Facultad de Medicina, Boulevard Louis Pasteur 32, Campus de Teatinos s/n, 29071 Málaga, Spain.
E-mail: lucena@uma.es

^aSee Appendix.

Publication data

Submitted 28 August 2014
First decision 15 September 2014
Resubmitted 26 September 2014
Resubmitted 20 October 2014

This article was accepted for publication after full peer-review.

SUMMARY

Background

We have observed an increase in hepatotoxicity (DILI) reporting related to the use of anabolic androgenic steroids (AAS) for bodybuilding.

Aim

To characterise phenotype presentation, outcome and severity of AAS DILI.

Methods

Data on 25 cases of AAS DILI reported to the Spanish (20) and Latin-American (5) DILI Registries were collated and compared with previously published cases.

Results

AAS DILI increased from representing less than 1% of the total cases in the Spanish DILI Registry in the period 2001–2009 to 8% in 2010–2013. Young men (mean age 32 years), requiring hospitalisation, hepatocellular injury and jaundice were predominating features among the AAS cases. AAS DILI caused significantly higher bilirubin values independent of type of damage when compared to other drug classes ($P = 0.001$). Furthermore, the cholestatic AAS cases presented significantly higher mean peak bilirubin ($P = 0.029$) and serum creatinine values ($P = 0.0002$), compared to the hepatocellular cases. In a logistic regression model, the interaction between peak bilirubin values and cholestatic damage was associated with the development of AAS-induced acute kidney impairment (AKI) [OR 1.26 (95% CI: 1.035–1.526); $P = 0.021$], with $21.5 \times \text{ULN}$ being the best bilirubin cut-off point for predicting AKI risk (AUCROC 0.92). No fatalities occurred.

Conclusions

Illicit recreational AAS use is a growing cause of reported DILI that can lead to severe hepatic and renal injury. AAS DILI is associated with a distinct phenotype, characterised by considerable bilirubin elevations independent of type of damage. Although hepatocellular injury predominates, acute kidney injury develops in cholestatic cases with pronounced jaundice.

INTRODUCTION

Anabolic androgenic steroids (AAS) are synthetic derivatives of testosterone whose medical indications are mainly male hypogonadism, breast cancer, anaemia and hereditary angioneurotic oedema. However, several AAS such as stanozolol, methyltestosterone, oxandrolone, fluoxymesterone and danazol are also used without medical supervision for performance enhancement and muscle building purposes due to their anabolic effects, stimulating protein synthesis and positive nitrogen balance.¹ When used for muscle building, AAS are mainly administered in cycles of 2–14 weeks alternated with 2–4 weeks of drug-free cycles.² The use of AAS products without a prescription from a licensed physician is illegal in most countries, including Spain. Nonetheless, illicit use of AAS products among body builders and young athletes (mainly males) appears to be growing both in the professional setting and the recreational field.³ It is believed that the illicit consumption of AAS currently exceeds the therapeutic use, although no official data are available on illicit AAS usage. Testosterone derivatives can be potentially harmful when taken unsupervised or as illegal derivatives. This led the US Food and Drug Administration to issue a public health warning on bodybuilding products marketed as containing steroids or steroid-like substances in 2009.⁴ Health hazards related to anabolic steroid use include cardiac hypertrophy,⁵ mental health problems⁶ and a variety of liver disorders including intrahepatic cholestasis,⁷ hepatitis,⁸ adenoma,⁹ hepatocellular carcinoma¹⁰ and hepatic peliosis, a rare form of sinusoidal dilatation.¹¹

To date, only isolated case reports and smaller case series of AAS-induced liver injury (AAS DILI) have been reported. As we have observed an increase in the reporting rate of AAS DILI cases to the Spanish DILI Registry, we aimed to analyse the reported frequency of AAS hepatotoxicity over the years in this database and the recently set up Spanish–Latin-American DILI Network as well as to characterise the clinical signature and severity of this form of hepatotoxicity.

MATERIAL AND METHODS

Study cohort

Twenty-five cases of AAS DILI submitted to the Spanish DILI Registry and the SLatinDILI Network were analysed and compared to other published AAS DILI series^{12–14} as well as DILI cases caused by other pharmacological groups in our Registry.

The two prospective databases contain detailed demographic, clinical, laboratory, imaging and histological (when available) information both at presentation and at follow-up of patients included. Each case included in the study was evaluated by a clinician and remitted to the coordinating centre where it was re-evaluated by a panel of DILI experts before being included in the database. A structured report form was used to record patient data, including details relating to: (i) the time lapse between the initial intake of the medication and the onset of liver disease and between the discontinuation of the suspected agent and improvement in, or recovery from, liver dysfunction; (ii) serology and specific biochemistry to rule out viral hepatitis, autoimmune and metabolic liver disorders, appropriate imaging tests to exclude biliary disease and any other alternative causes of liver injury; and (iii) the outcome of the liver damage.¹⁵ Only cases considered as being drug-related according to expert clinical judgment were assessed using the Council for International Organizations of Medical Sciences (CIOMS) scale. The biochemical criteria for DILI were initially the consensus criteria reported by the CIOMS, and in 2011 adapted to those of Aithal *et al.*^{16, 17} The pattern of liver injury was classified based on liver biopsies when available or *R* ratio values from the first available blood test after DILI recognition. DILI cases were classified as mild, moderate, severe or fatal based on the DILI severity index scale.¹⁷

Renal dysfunction was defined as serum creatinine (SCr) values ≥ 1.5 mg/dL in patients with no pre-existing kidney damage. The incriminated drugs in DILI were classified according to the Anatomic Therapeutic Classification (ATC) recommended by the World Health Organization – Europe.¹⁸ The study protocol was approved by the Local Ethics Committee of the coordinating centre at the ‘Virgen de la Victoria’ University Hospital in Malaga, Spain and by the corresponding Ethical Review Boards in each of the participating centres in Latin America.

Statistical analyses

Univariate analyses were performed using the Student's *t*-test for numerical variables and the χ^2 test for categorical variables. Analysis of variances (ANOVAS) was used for comparisons of groups. Where variables did not follow a normal distribution, a nonparametric Kruskal–Wallis analysis was performed. *Post hoc* analyses used in conjunction with significant ANOVA and Kruskal–Wallis findings were the least significant difference test with Bonferroni's correction and the

Mann–Whitney *U*-test respectively. Variables that were associated with the development of renal dysfunction in univariate analyses were included in a logistic regression model to explore the predictive value of independent variables and interactions among these. The discriminative ability to rank patients according to their risk of developing renal dysfunction was assessed by receiver operating characteristic (ROC) curve analysis. All test were considered to be statistically significant when $P < 0.05$. All analyses were performed using the IBM SPSS 19.0 statistical software package (IBM Corporation, Armonk, NY, USA).

RESULTS

Increase in AAS DILI reporting

The number of AAS DILI cases submitted to the Spanish DILI Registry has increased in recent years. Only five AAS DILI cases were identified during the first 15 years, while the number of cases tripled to 15 cases in the last 4 years resulting in a significant increase from 1% to 8% of the total number of DILI cases in the Spanish DILI Registry. The number of identified herbal and dietary supplement (HDS) hepatotoxicity cases, excluding AAS cases, demonstrated a similar but weaker trend. Our case series also includes five additional Latin-American cases (Table 1). All further analyses were performed using the entire cohort of 25 AAS DILI cases.

Table 1 | Prevalence of drugs, herbal and dietary supplements (HDS) and anabolic androgenic steroids (AAS) hepatotoxicity recorded in the Spanish DILI Registry and the Spanish–Latin-American DILI (SLatinDILI) Network

Period	Drugs	HDS	
		AAS	Other HDS
Spanish DILI Registry			
1994–1997	109 (100%)	0 (0%)	0 (0%)
1998–2000	151 (97%)	0 (0%)	4 (3%)
2001–2003	178 (96%)	2 (1%)	6 (3%)
2004–2006	134 (94%)	2 (1%)	7 (5%)
2007–2009	113 (94%)	1 (1%)	6 (5%)
2010–2013	161 (87%)	15 (8%)*	9 (5%)
Total	846 (94%)	20 (2%)	32 (3%)
SLatin DILI Network			
2012	48 (91%)	0 (0%)	5 (9%)
2013	65 (90%)	5 (7%)	2 (3%)
Total	113 (91%)	5 (3%)	7 (6%)

* Chi-squared test $P < 0.001$.

Demographic characteristics, clinical and laboratory features

The 25 AAS DILI cases included in our series were all male with a mean age of 32 years (range 20–49 years). All patients were in good physical condition without underlying diseases prior to DILI initiation and the aim of AAS intake was to gain muscle mass, physical strength and enhanced appearance. None of the patients reported having taken any other medications in the last 3 months preceding the DILI episode. The most frequent AAS causative agent was stanozolol either alone or in combination with other androgenic steroids (nandrolone, metenolone, oxymetholone and prasterone) followed by methylephithiostanol and methasterone. During the first 15 years, three of five AAS cases were reported to have been administered intramuscularly and two orally, while in the cases identified from 2010 and onwards, only three of the 15 cases reported intramuscular administration. Stanozolol was the only compound administered intramuscularly. The total mean daily dose was 54 mg (range 1–150 mg). In cases where the treatment was not administered on a daily basis, the daily dose was calculated by dividing the total dose by the number of days covered.

Hepatocellular damage was the most frequent type of liver injury (15 patients, 60%), and the remaining cases presented with cholestatic injury. Demographic characteristics, clinical and laboratory findings, according to type of liver injury are shown in Table 2. The mean time to onset (from the beginning of treatment to the development of symptoms) did not differ among the groups. Jaundice was seen in all cholestatic cases and 13 of the 15 hepatocellular cases. The cholestatic cases had significantly higher peak TBL values as compared to the hepatocellular cases [32.5 vs. 21.4 times the upper limit of normal (\times ULN), $P = 0.029$]. Most patients required hospitalisation independent of the type of liver injury. Severe cases were found more frequently among cholestatic patients ($P = 0.017$). Furthermore, this group had significantly higher peak values of serum creatinine ($P = 0.0002$) with a mean value of 2.35 mg/dL. Six patients (cases 3, 10, 11, 17, 18 and 19, Table S1) developed renal dysfunction with serum creatinine values reaching up to 8.50 mg/dL. Two patients (cases 11 and 12) developed coagulopathy with prothrombin activity of 36% and 45% respectively. None of the patients went on to develop fulminant hepatic failure. Four patients recovered from injury within 3 months for hepatocellular type (1 case) and 6 months for cholestatic type (3 cases) and were considered to have acute self-limited damage.¹⁷ Ten

	Hepatocellular (n = 15)	Cholestatic (n = 10)	P value
Age, mean years (range)	32 (20–42)	32 (20–49)	0.80
Compound, n (%)			0.48
Stanozolol	12 (80)	5 (50)	
Methylepitiostanol	3 (20)	4 (40)	
Methasterone	–	1 (10)	
Administration route, n (%)			0.88
Oral	11 (73)	8 (80)	
Intramuscular	4 (27)	2 (20)	
Clinical information			
Daily dose, mean mg (range)	71 (32–150)	38 (1–54)	0.53
Duration of therapy, mean days (range)	81 (23–338)	53 (16–111)	0.65
Time to onset, mean days (range)	84 (13–338)	54 (7–123)	0.92
Jaundice, n (%)	13 (87)	10 (100)	0.42
Hospital admission, n (%)	9 (60)	9 (90)	0.12
Severity			0.017
Mild	2 (13%)	–	
Moderate	13 (87%)	3 (30%)	
Severe	–	7 (70%)	
Laboratory parameters, mean \times ULN (range)			
DILI recognition			
TBL	8.6 (1.8–20)	17.3 (5.7–28)	0.012
CBL	7.8 (0.6–17)	16.1 (4.2–25)	0.008
ALT	19.1 (2–142)	3.9 (1.2–14)	0.053
ALP	1.2 (0.5–3.5)	1.2 (0.1–2.1)	0.57
Peak			
TBL	21.4 (2–37)	32.5 (17–53)	0.029
CBL	16.8 (0.6–32)	28.2 (16–46)	0.006
SCr, mean mg/dL (range)	1.06 (0.9–1.3)	2.35 (1.2–8.5)	0.0002
Outcome			
Time to resolution, mean days (range)*	149 (76–218)	153 (110–180)	0.90
Resolution data, n (%)	6 (40)	3 (10)	

TBL, serum total bilirubin; CBL, conjugated bilirubin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; SCr, serum creatinine.

* Mean values were calculated based on the total number of episodes with available information. Resolution: Complete normalisation of the analytical liver profile. Severity criteria: Mild: ALT \geq 5 \times ULN or ALP \geq 2 \times ULN, and TBL $<$ 2 \times ULN; Moderate: ALT \geq 5 \times ULN or ALP \geq 2 \times ULN, and TBL \geq 2 \times ULN; Severe: ALT \geq 5 \times ULN or ALP \geq 2 \times ULN, and TBL \geq 2 \times ULN and one of the following: (i) international normalised ratio \geq 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.

Table 2 | Comparison of demographic characteristics, clinical and laboratory parameters in 25 androgenic anabolic steroid-induced liver injury (AAS DILI) cases according to type of liver damage

patients (all hepatocellular injuries but one) met the criteria for persistence [evidence of continued liver injury $>$ 3 months but $<$ 1 year (hepatocellular) or $>$ 6 months but $<$ 1 year (cholestatic)]. Five of them ultimately resolved before 1 year and only patient 7 became chronic, whereas in four patients there was insufficient follow-up to ascertain long-term outcome (Table S1). No follow-up data were available for 11 patients and their ultimate outcome remains unknown.

Comparison of the biochemical profile of AAS DILI with those of other drug classes involved in DILI

Based on the causal agents, 759 Spanish and Latin-American DILI cases were compared to the AAS DILI cohort after stratification into drug classes and liver injury types (hepatocellular vs. cholestatic/mixed). Patients with AAS DILI exhibited higher TBL values ($P < 0.001$) than any of the other drug classes independent of type of injury. Interestingly, patients with

AAS-induced hepatocellular injury presented significantly lower peak ALT values ($P = 0.012$) than the anti-infectives (excluding amoxicillin–clavulanate) ($P = 0.037$), HDS ($P = 0.002$) and nervous system drug groups ($P = 0.036$). In addition, cholestatic cases were found to have significantly lower peak ALP values compared to cardiovascular system drugs ($P = 0.035$), anti-neoplastic and immunomodulating agents ($P = 0.009$) and amoxicillin-clavulanate ($P = 0.049$; Table 3).

Literature review

In search for other AAS hepatotoxicity data, we found three published USA case series including a total of 10 AAS DILI cases.^{12–14} The demographic features of the 10 AAS DILI cases were similar to those of our case series as outlined in Table 4. All cases involved young males having taken AAS products unsupervised in an attempt to increase muscle mass. The main causative drug in the published series was methasterone, while stanozolol was the most frequent culprit agent in our case series.

The external cases reported a median time to DILI onset of 36 days, which is similar to that found in our cohort (49 days). Jaundice was a prominent feature in all series. No patients developed fulminant hepatic failure in any of the case series. Renal dysfunction was limited to six cases in our cohort, all of them presenting

with cholestatic damage and high levels of TBL. Similarly, five of the previously published cases that developed renal failure presented with cholestatic damage and high TBL peak values (35–54 \times ULN). The published series had a mean recovery time of 82 days (range 56–121), while the mean recovery time in our cohort was longer, 151 (range 76–218) days. All results are shown in Tables 4 and S1.

Characterisation of risk factors for acute renal dysfunction

Six patients in our cohort and five of the published cases develop renal dysfunction during the AAS DILI episode with full recovery in all cases. These 11 patients had significantly higher peak TBL values than those who did not develop renal impairment ($P < 0.001$). Furthermore, renal dysfunction was exclusively associated with cholestatic damage. In a logistic regression model, only the interaction of peak TBL values and cholestatic damage was associated with the development of AAS-induced acute renal dysfunction [AKI; OR 1.26 (95% CI: 1.035–1.526; $P = 0.021$, coefficient 0.229, constant -6.372)]. Hence, for each unit (\times ULN) of TBL elevation in cholestatic patients, the risk of developing renal dysfunction increases by 26%. Using a ROC analysis, we estimated the best TBL cut-off point for predicting risk of renal

Table 3 | Biochemical profiles of androgenic anabolic steroid (AAS)-induced hepatotoxicity compared to those induced by other drug classes according to the type of liver damage

Drug class	<i>n</i>	Mean peak TBL (\times ULN)	Mean peak ALT (\times ULN)	Mean peak ALP (\times ULN)
Hepatocellular				
Androgenic anabolic steroids	15	21.4*	19.4†	2.0
NSAIDs	61	7.5	25.3	1.7
Anti-infectives¶	101	8.5	29.9	1.3
Amoxicillin–clavulanate	83	8.4	23.6	1.8
Cardiovascular drugs	56	5.4	24.6	1.5
Anti-neoplastic agents	46	8.4	22.6	1.3
Nervous system drugs (CNS)	98	6.8	34.8	1.5
Herbal/dietary supplements (HDS)	28	11.0	42.0	1.5
Cholestatic				
Androgenic anabolic steroids	10	32.5‡	4.6	2.2§
NSAIDs	36	10.0	5.3	3.4
Anti-infectives¶	42	9.0	6.8	3.2
Amoxicillin–clavulanate	115	10.4	10.3	3.2
Cardiovascular drugs	42	7.8	8.3	5.1
Anti-neoplastic agents	22	7.1	7.1	5.3
Nervous system drugs (CNS)	29	6.2	5.9	3.1

TBL, serum total bilirubin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; NSAIDs, nonsteroidal anti-inflammatory drugs.

¶ Anti-infectives excluding amoxicillin–clavulanate; Kruskal–Wallis test: * $P < 0.001$ AAS vs. rest of drug classes, † $P = 0.012$ AAS vs. anti-infectives, CNS and HDS, ‡ $P < 0.001$ AAS vs. rest of drug classes, § $P = 0.01$ AAS vs. amoxicillin–clavulanate, anti-neoplastic and cardiovascular drugs.

Table 4 | Demographic, clinical and laboratory parameters of anabolic androgenic steroid-induced liver injury (AAS DILI) cases in the Spanish–Latin-American DILI Registry compared to other published AAS DILI series

	Spanish–Latin-American DILI Registry (2013) (n = 25)	Kafrouni et al. ¹³ (n = 2)	Shah et al. ¹⁴ (n = 5)	Krishnan et al. ¹² (n = 3)
Demographics				
Age, mean years (range)	32 (20–49)	35 (31–40)	25 (20–33)	30 (21–38)
Male, n	25	2	5	3
Compound, n (%)				
Stanozolol	17 (68)	–	–	–
Methasterone	1 (4)	2 (100)	5 (100)	1 (33)
Methylepithiostanol	7 (28)	–	–	–
Dehydroepiandrosterona	–	–	–	1 (33)
Desoxymethyltestosterone	–	–	–	1 (33)
Clinical information				
Jaundice, n (%)	23 (92)	2 (100)	5 (100)	3 (100)
Hospital admission, n (%)	17 (68)	1 (50)	–	3 (100)
Renal dysfunction, n (%)	6 (24)	1 (50)	2 (40)	2 (66)
Duration of therapy, median days (range)	49 (16–338)	35 (28–42)	30 (15–120)	42 (28–56)
Time to onset, median days (range)	49 (14–338)	45 (21–70)	30 (15–90)	56 (28–98)
Type of liver injury, n (%)				
Hepatocellular	15 (60)	–	1 (20)	1 (33)
Cholestatic	10 (40)	2 (100)	4 (80)	2 (67)
Severity, n (%)				
Mild	2 (8)	–	–	–
Moderate	16 (64)	1 (50)	3 (60)	2 (67)
Severe	7 (28)	1 (50)	2 (40)	2 (66)
Laboratory parameters at DILI recognition, mean ×ULN (range)				
TBL	12 (1.8–28)	36 (31–42)	16 (5.8–32)	9.3 (8–10)
AST	6.3 (1.3–53)	4.8 (1.5–3.3)	2.2 (1.1–4.9)	–
ALT	14 (1.2–142)	4.5 (1.5–7.5)	2.4 (1.4–3.8)	–
ALP	1.3 (0.1–2.3)	3.3 (3.1–3.5)	1.5 (0.9–1.8)	–
Outcome				
Resolution, mean days (range)*	151 (76–218)	56	87 (59–121)	85 (75–90)
Cases with resolution data, n (%)	9 (36)	1 (50)	5 (100)	3 (100)

TBL, serum total bilirubin; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

* Mean values were calculated based on the total number of episodes with available information. Resolution: Complete normalisation of the analytical liver profile. Severity criteria: Mild: ALT $\geq 5 \times$ ULN or ALP $\geq 2 \times$ ULN, and TBL $< 2 \times$ ULN; Moderate: ALT $\geq 5 \times$ ULN or ALP $\geq 2 \times$ ULN, and TBL $\geq 2 \times$ ULN; Severe: ALT $\geq 5 \times$ ULN or ALP $\geq 2 \times$ ULN, and TBL $\geq 2 \times$ ULN and one of the following: (i) international normalised 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.

dysfunction to be 21.5 mg/dL (Figure 1). At this point, the sensitivity was 100%, specificity 50% and AUROC 0.92. TBL and SCr alterations were seen to increase and decrease in parallel (Table S2).

DISCUSSION

The present case series is, to the best of our knowledge, the largest cohort of AAS DILI cases to date. We have noted a distinct increase in DILI cases caused by illicit use of AAS in Spain in the last years. The reasons behind this apparent increase are unknown. It can be assumed that the rise in AAS DILI goes parallel with an increase in the number of AAS consumers,

although exposure data are impossible to get in a setting of illegal distribution and nontherapeutic use associated with these compounds. A recent meta-analysis of 187 studies found a lifetime prevalence rate of 6.4% for males and 1.6% for females, corresponding to a global lifetime prevalence rate of 3.3%.¹⁹ Several circumstances could have contributed to the rising trend of illicit AAS usage. They may include the switch in administration route of some of these products, such as stanozolol that currently can be taken orally instead of intramuscularly. This is mainly achieved through modifications in the 17- α carbon molecule and could attract more users.²⁰ The facility by which these illicit

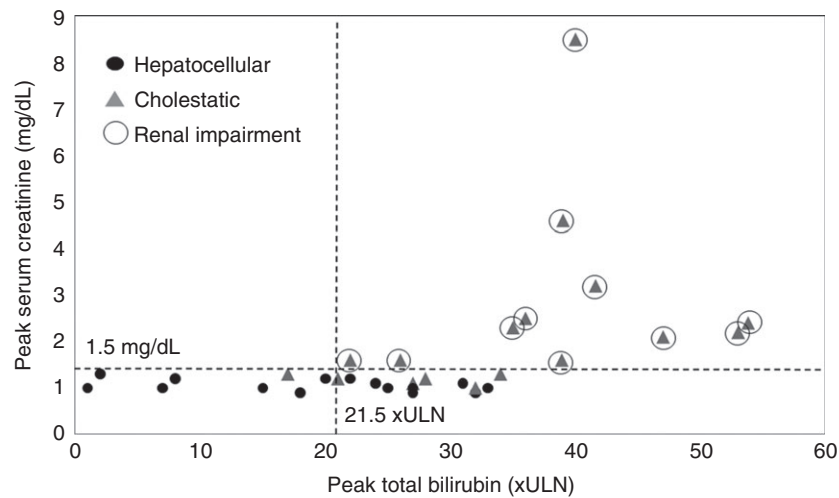


Figure 1 | Peak values of total bilirubin and serum creatinine in anabolic androgenic steroid-induced liver injury (AAS DILI). The graph demonstrates total bilirubin levels in folds of the upper limit of normal hospital range (\times ULN) and serum creatinine (mg/dL) at the time of peak elevation in 33 patients (with peak total bilirubin and serum creatinine values available) diagnosed with AAS DILI, 24 patients from the current study and nine previously published external cases. The 33 cases are classified by type of liver injury, hepatocellular or cholestatic. The indicated horizontal line represents a serum creatinine value of 1.5 mg/dL. The indicated vertical line represents a total bilirubin value of 21.5 \times ULN, the best cut-off point found for predicting acute kidney impairment. All cases that developed kidney impairment (serum creatinine $>$ 1.5 mg/dL) are encircled.

AAS products now can be purchased online can be a further cause of the rising user trend and subsequent AAS DILI recognitions. The extended AAS product range and accessibility are probable reasons behind the apparent increase in AAS DILI. However, the current awareness of AAS product hepatotoxicity potentials cannot be ignored as a contributor to the increase in AAS DILI detection, as clinicians are now more receptive to the idea of AAS products being potential DILI causative agents.

In a comparison of our case series to those of 10 previously reported AAS DILI cases, we found jaundice with high levels of TBL to be a common denominator among all the cases, which often required hospitalisation, indicating that this type of DILI can be severe and health resource consuming. While stanozolol was the most frequent causative agent in our series, methasterone dominated in the other case series. This difference may stem from availability in the corresponding countries as well as temporal differences, as the trend of illicit AAS products is constantly changing.

In addition to the difference in type of AAS causative agents between our cohort and the previously reported case series, we found a notable difference in the type of liver injury. In our cohort, hepatocellular injury predominated. The external cholestatic cases may have been

selected for publication due to the severity associated with renal dysfunction rather than to demonstrate typical clinical characteristics. Hence, the number of AAS case reports with hepatocellular type of damage could be underrepresented in the literature. As we retrieved all cases reported to our Registry, this study more accurately reflects the entire clinical spectrum of AAS DILI. Moreover, a recent study has demonstrated that 17α -ethinyl estradiol inhibits bile salt export pump activity *in vitro*.²¹ One may speculate that testosterone derivatives, due to similarities in the chemical structure, could likewise have potential inhibitory effects, which may lead to retention of bile acids in the hepatocyte and a biochemical appearance of hepatocellular damage. Furthermore, the distinct high levels of bilirubin observed in AAS DILI independent of the type of liver injury strongly suggests an effect of these hormones on the expression and function of canalicular ABC transporters resulting in an impaired excretion of conjugated bilirubin. Whether genetic variations in *ABCC2* and/or other canalicular transporters, similarly to what characterises Dubin–Johnson syndrome,²² determine the individual susceptibility to develop hepatotoxicity and its severity upon exposure to anabolic steroids remains to be elucidated.

A common complication associated with AAS DILI appears to be acute kidney injury. Although the

magnitude of serum creatinine increase that constitutes acute renal injury is debatable, we have used a quite restrictive definition. This does not exclude the presence of renal dysfunction in certain patients with serum creatinine levels below 1.5 mg/dL. Hence, our criteria may have led to an underestimation of the actual frequency of AKI in our cohort. Jaundice with high levels of TBL was a common denominator among all the AAS DILI cases. Interestingly, the patients who developed AKI had significantly higher TBL values than those who did not. Furthermore, there exists a close time-dependent relationship between TBL concentrations and kidney function, suggesting a crucial role for bilirubin in renal injury development in these cases. The mechanism by which this damage occurs remains unclear. Renal dysfunction has been reported in a variety of clinical settings characterised by high levels of total bilirubin such as obstructive jaundice or haemolysis.^{23, 24} Furthermore, in jaundiced patients with extrahepatic biliary obstruction, high bilirubin levels may sensitise the kidney enhancing the incidence of aminoglycoside nephrotoxicity.²⁵ In obstructive jaundice, it is thought that sulphated bile acids may interfere with Na⁺/H⁺ antiporter activity in the brush border of tubular cells and subsequently lead to membrane damage and kidney injury.²⁶ It has also been suggested that bilirubin at high concentrations could be an endogenous nephrotoxin.²³

Despite the fact that the predominating phenotype was the combination of hepatocellular injury with elevated total bilirubin concentration, no patient developed fulminant hepatic failure. This unexpected favourable outcome somehow contradicts the 'Hy's Law paradigm',²⁷ but is coincident with previous observations.^{12–14} Hence, this 0% mortality rate could be a specific drug feature similarly to that shown for erythromycin.²⁸

We also found that the biochemical profile of AAS DILI differs from that of DILI induced by other causal agents. The main difference is the prolonged and relatively severe bilirubin elevations that typically accompany AAS DILI, independent of the type of liver injury. Hepatocellular injury predominated in our AAS DILI cohort. This is similar to DILI induced by other HDS, though this type of DILI is generally seen to reach significantly higher peak ALT values, but significantly lower bilirubin values. With regard to the other types of hepatocellular DILI outlined in Table 3, the difference in ALT elevations is less obvious compared to AAS DILI, despite the fact that the AAS DILI demonstrated significantly lower peak ALT than anti-infectives and nervous system drugs. In fact, the degree of ALT elevation is less likely to be of

use in diagnosing AAS DILI in clinical practice, while the distinct bilirubin elevation should alert clinicians to enquire about potential AAS intake. Similarly, the limited ALP elevations in the cholestatic AAS DILI cases, though being significantly lower than the corresponding peak values in the amoxicillin–clavulanate, anti-neoplastic and cardiovascular drug-induced DILI cases, are less distinguishable in clinical practice. Nevertheless, the type of liver injury appear to be important in AAS DILI as only cholestatic injury was seen to be associated with acute kidney injury in our study. The awareness of this specific phenotypic presentation could refine causality assessment in the setting of AAS intake.

This study highlights the need for enhanced public awareness of the risks involved in unsupervised intake of illicit AAS products. Due to the recent increase in methylepithiostanol hepatotoxicity cases detected in the Spanish DILI Registry and reported to the Spanish Medicines Agency (Agencia Española Del Medicamento y Productos Sanitarios, AEMPS), dietary supplement products found to contain methylepithiostanol were withdrawn from the Spanish market in September 2013.²⁹

In conclusion, illicit recreational AAS use is a rapidly growing cause of DILI that can lead to severe hepatic and renal injury and represents a major health concern. AAS-induced liver injury is associated with a distinct phenotype, presenting significantly higher bilirubin values regardless the type of damage when compared to other drug classes involved in DILI. Governments should take appropriate actions to control illicit AAS access and to inform health professionals and the public on the severe risks associated with recreational use of these products.

AUTHORSHIP

Guarantor of the article: MI Lucena.

Author contributions: MRD, AGJ, IMC, CS, MIL, RJA: study concept and design. MRD, MGC, BGM, AOA, EBR, RGG, MJP, PR, JMN, PG, MP, MGE, FB, JRB, RP: acquisition of data. MRD, AGJ, IMC, CS, MIL, RJA: analysis and interpretation of data. MRD, AGJ, IMC, CS, MIL, RJA: drafting of the manuscript. MRD, MIL, RJA: critical revision of the manuscript for important intellectual content.

ACKNOWLEDGEMENTS

Declaration of personal interests: None.

Declaration of financial interests: This study was supported by the research grant PI12-00620, AC-0073-2013 and Agencia Española Del Medicamento y Productos

Sanitarios (AEMPS). CIBERehd is funded by Instituto de Salud Carlos III.

The funding sources had no involvement in the study design; in the collection, analysis, and interpretation of data; in the writing of the report or in the decision to submit the manuscript for publication.

We are grateful to Ramon Hidalgo at the Servicio Central de Informatica de la Universidad de Málaga for his invaluable help in the statistical analyses.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Demographic and clinical parameters in our case series of androgenic anabolic steroid-induced DILI cases and those from previously published reports.

Table S2. Evolution of serum creatinine (SCr) and total bilirubin (TBL) over time in anabolic androgenic steroid-induced liver injury cases with renal dysfunction included in the Spanish DILI Registry.

REFERENCES

- Smith DA, Perry PJ. The efficacy of ergogenic agents in athletic competition. Part I: androgenic-anabolic steroids. *Ann Pharmacother* 1992; **26**: 520–8.
- Evans NA. Current concepts in anabolic-androgenic steroids. *Am J Sports Med* 2004; **32**: 534–42.
- Kanayama G, Hudson JL, Pope HG Jr. Illicit anabolic-androgenic steroid use. *Horm Behav* 2010; **58**: 111–21.
- FDA warning on body building products marketed a containing steroids or steroid-like substances. Available at: <http://www.fda.gov/forconsumers/consumerupdates/ucm173739.htm> (accessed March 13, 2014).
- Far HR, Agren G, Thiblin I. Cardiac hypertrophy in deceased users of anabolic androgenic steroids: an investigation of autopsy findings. *Cardiovasc Pathol* 2012; **21**: 312–6.
- Lindqvist AS, Moberg T, Eriksson BO, et al. A retrospective 30-year follow-up study of former Swedish-elite male athletes in power sports with a past anabolic androgenic steroids use: a focus on mental health. *Br J Sports Med* 2013; **47**: 965–9.
- Sánchez-Osario M, Duarte-Rojo A, Martínez-Benítez B, et al. Anabolic-androgenic steroids and liver injury. *Liver Int* 2008; **28**: 278–82.
- Stimac D, Milic S, Dintinjana RD, et al. Androgenic/anabolic steroid-induced toxic hepatitis. *J Clin Gastroenterol* 2002; **35**: 350–2.
- Socas L, Zumbado M, Pérez-Luzardo O, et al. Hepatocellular adenomas associated with anabolic androgenic steroid abuse in bodybuilders: a report of two cases and a review of the literature. *Br J Sports Med* 2005; **39**: e27.
- Hardt A, Stippel D, Odenthal M, et al. Development of hepatocellular carcinoma associated with anabolic androgenic steroid abuse in a young bodybuilder: a case report. *Case Rep Pathol* 2012; **2012**: 195607.
- Cabasso A. Peliosis hepatitis in a young bodybuilder. *Med Sci Sports Exerc* 1994; **26**: 2–4.
- Krishnan PV, Feng ZZ, Gordon SC. Prolonged intrahepatic cholestasis and renal failure secondary to anabolic androgenic steroid-enriched dietary supplements. *J Clin Gastroenterol* 2009; **43**: 672–5.
- Kafrouni MI, Anders RA, Verma S. Hepatotoxicity associated with dietary supplements containing anabolic steroids. *Clin Gastroenterol Hepatol* 2007; **5**: 809–12.
- Shah NL, Zacharias I, Khettry U, et al. Methasteron-associated cholestatic liver injury: clinicopathologic findings in 5 cases. *Clin Gastroenterol Hepatol* 2008; **6**: 255–8.
- Andrade RJ, Lucena MI, Fernández MC, et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. *Gastroenterology* 2005; **129**: 512–21.
- Benichou C. Criteria of drug-induced liver disorders. Report of an international consensus meeting. *J Hepatol* 1990; **11**: 272–6.
- Aithal GP, Watkins PB, Andrade RJ, et al. Case definition and phenotype standardization in drug-induced liver injury. *Clin Pharmacol Ther* 2011; **89**: 806–15.
- ATC/DDD Index 2014. Available at: http://www.whocc.no/atc_ddd_index/ (accessed January 28, 2014).
- Sagoe D, Molde H, Andreassen CS, et al. The global epidemiology of anabolic-androgenic steroid use: a meta-analysis and meta-regression analysis. *Ann Epidemiol* 2014; **24**: 383–98.
- Kicman AT. Pharmacology of anabolic steroids. *Br J Pharmacol* 2008; **154**: 502–21.
- Morgan RE, Trauner M, van Staden CJ, et al. Interference with bile salt export pump function is a susceptibility factor for human liver injury in drug development. *Toxicol Sci* 2010; **118**: 485–500.
- Erlinger S, Arias IM, Dhumeaux D. Inherited disorders of bilirubin transport and conjugation: new insights into molecular mechanisms and consequences. *Gastroenterology* 2014; **146**: 1625–38.
- Rafat C, Burbach M, Brocheriou I, et al. Bilirubin-associated acute tubular necrosis in a kidney transplant recipient. *Am J Kidney Dis* 2013; **61**: 782–5.
- Qian Q, Nath KA, Wu Y, et al. Hemolysis and acute kidney failure. *Am J Kidney Dis* 2010; **56**: 780–4.
- Lucena MI, Andrade RJ, Cabello MR, et al. Aminoglycoside-associated nephrotoxicity in extrahepatic obstructive jaundice. *J Hepatol* 1995; **22**: 189–96.
- Sellinger M, Haag K, Burckhardt G, et al. Sulfated bile acids inhibit Na (+)-H+ antiport in human kidney brush-border membrane vesicles. *Am J Physiol* 1990; **258**: F986–91.
- Robles-Diaz M, Lucena MI, Kaplowitz N, et al. Use of Hy's law and a new composite algorithm to predict acute liver failure in patients with drug-induced liver injury. *Gastroenterology* 2014; **147**: 109–18.
- Björnsson E, Olsson R. Outcome and prognostic markers in severe drug-induced liver disease. *Hepatology* 2005; **42**: 481–9.
- Agencia Española de Medicamentos y Productos Sanitarios (AEMPS). Available at: http://www.aemps.gob.es/informa/notasInformativas/medicamentosUsoHumano/medIlegales/2013/docs/ICM_MI_09-2013-episidrol-epistane.pdf (accessed March 13, 2014).

APPENDIX COLLABORATORS

SPANISH DILI REGISTRY

Participating clinical centres

Hospital Universitario Virgen de la Victoria, Málaga (coordinating centre): RJ Andrade, MI Lucena, M García-Cortés, M Robles-Díaz, R Alcántara, B García-Muñoz, A Ortega-Alonso, C Stephens, I Medina-Cáliz, I Moreno, A González-Jiménez, A Papineau.

Hospital Torrecárdenas, Almería: MC Fernández, G Peláez, M Casado, M González-Sánchez.

Hospital Universitario Virgen de Valme, Sevilla: M Romero-Gómez, R Calle-Sanz, R Millán-Domínguez, L Rojas, B Fombuena, J Ampuero, JA del Campo, H Pastor-Ramírez, J Funuyet R Gallego.

Hospital de Mendaro, Guipúzcoa: A Castiella, EM Zapata.

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

Hospital Costa del Sol, Marbella (Málaga): JM Navarro, IM Mendez Sánchez.

Hospital Sant Pau, Barcelona: C Guarner, G Soriano, EM Román.

Hospital Morales Meseguer, Murcia: H Hallal, E García-Oltra, JC Titos-Arcos, A Pérez-Martínez, C Sánchez Cobarro, JM Egea-Caparrós.

Hospital de Donosti, San Sebastián: J Arenas, MI Gomez Osua, A Gómez-García, FJ Esandi.

Hospital de Basurto, Bilbao: S Blanco, P Martínez-Odriozola.

Hospital Alto Deba, Mondragón, Guipúzcoa: P Otazua.

Hospital Universitario San Cecilio, Granada: J Salmerón, A Gila.

Hospital Clínico, Valladolid: JM González, ML Ruiz-Rebollo, S Lorenzo.

Hospital Universitario Regional de Málaga, Málaga: M Jiménez, R González-Grande.

Hospital La Fe, Valencia: M Prieto, M García-Eliz, M Berenguer.

Hospital de Sagunto, Valencia: J Primo, JR Molés, A Garayoa.

Hospital de Laredo, Cantabria: M Carrascosa.

Hospital 12 de Octubre, Madrid: E Gómez-Domínguez, I Fernández.

Hospital Germans Trias i Pujol, Badalona, Barcelona: E Montane, A Barriocanal, R Planas.

Hospital Clínic, Barcelona: P Ginés, S Lens, JC García, I Alfaro.

Hospital Universitario de Canarias, La Laguna, Tenerife: A Aldea, M Hernandez-Guerra, M Moreno-Sanfiel.

Hospital de Alcorcon, Alcorcon, Madrid: C Fernandez, M Fernandez-Gil.

Hospital Reina Sofía, Cordoba: JL Montero, M de la Mata.

Hospital Miguel Servet, Zaragoza: J Fuentes-Olmo, EM Fernández-Bonilla.

Hospital de Albacete, Albacete: JM Moreno, P Martínez-Rodenas, M Garrido.

Hospital Virgen de las Nieves, Granada: F Nogueras.

Hospital Puerta de Hierro, Madrid: J de la Revilla, M Trapero, M Gómez.

Hospital Quirón de Marbella, Málaga: VM Aguilar, M de Sola.

Hospital Puerta del Mar, Cádiz: P Rendón.

Hospital Parc Taulí de Sabadell, Barcelona: M Vergara, J Sánchez Delgado.

Hospital de Salamanca, Salamanca: F González-San Martín.

SPANISH-LATIN-AMERICAN DRUG-INDUCED LIVER INJURY NETWORK

Participating clinical centres

Hospital Provincial del Centenario, Rosario, Argentina: F Bessone, J Montero.

Hospital de Clínicas, Montevideo, Uruguay: N Hernandez, A Sanchez, M di Pace, C Dacoll, H Cohen.

Hospital Rebagliati, Lima, Perú: M Davalos.

Hospital Clínico de la Universidad Católica de Chile, Santiago, Chile: M Arrese, A Ruiz, JR Brahm, J Arancibia.

Hospital de Clínicas, Asuncion, Paraguay: M Giral, M Gadischesky, L Ughelli.

Hospital General de México, Mexico City, Mexico: D Kershenobich, A Loeza.

Hospital Universitario de Maracaibo, Maracaibo, Venezuela: M Lizarzabal, E Mengual.

Hospital Universitario Prof. Edgard Santos, Universidad Federal da Bahia, Salvador de Bahia, Brasil: R Paraná, MI Schinoni.

Fundación Clínica Médica Sur, Mexico City, Mexico: N Méndez-Sánchez.

Instituto de Gastroenterología Boliviano Japones, La Paz, Bolivia: V Berdeja, A Rejas.