The discovery of better biomarkers of drug induced liver injury (DILI) needs to use other endpoints than ALT. A pilot proof of concept study

Hugo Perazzo[1*], Michael Merz[2], Thierry Poynard[1] and the SAFE-T consortium.

(1) UPMC APHP Liver Center (Pierre et Marie Curie University, Public Assistance Hospitals of Paris); *INSERM 938 Unit, Paris (2) Novartis, Bonn, Germany

SAFE-T

Abstract

Background
Severe acute DILI are identified and classified using increase of ALT. The most popular criteria are Temple´s corollary (3xULN-ALT) and Hy´s law criteria (3xULN-ALT and bilirubin>2xULN (34 µmol/L).
To assess the performance of better DILI biomarkers than ALT, it is mandatory to use another endpoints than ALT, without too low prevalence. The aim was to check the utility of biological and clinical endpoints "ALT-free" in a prospective pilot study before starting a validation study.
Methods
During and episode of DILI biochemical severity (B-sDILI) was define as an increase of total bilirubin as up to 2xN (34 micromol/L) if < 2xN at baseline; or an increase of 10 micromol/L if baseline>2xN; clinical severity (C-sDILI) was define as DILI-related death, transplantation or hospitalization. As a proof of concept we compare the predictive performance of serum apolipoprotein-A1 (ApoA1) and ActiTest (AT) as candidate DILI-biomarker versus ALT, the Standard of Care. As part of a pilot prospective study of the SAFE-T consortium we assess consecutive patients hospitalized in a tertiary referral center for DILI, using a centralized lab and an independent adjudication committee.
Results
In one year 25 patients were included, 8 females, mean 48 years of age, 8 acetaminophen alone or in combination. Seven cases (28%) of C-sDILI occurred: 1 death, 1 transplantation and specific hospitalization in other 5 cases; B-sDILI occurred in 6 (24%); 11(44%) cases had either C or B-sDILI. The repeated 3 biomarkers had significant kinetics (reateated varainace analysis P< 0.01) during 12-weeks follow-up [mean 6 samples/patient], with significant predictive values at baseline for CB-sDILI: AUROCs for AT/APOA1/ALT were 0.74 (95%CI 0.45-0.89;P=0.01), 0.69 (0.35-0.87; P=0.07), and 0.71(0.42-0.87;P=0.03), without differences between AT/ApoA1 and ALT (P=0.88/P=0.18). At 12 Weeks only 6 cases had totally normal liver function tests as well as normal fibrosis biomarkers (FibroTest/Fibroscan). Three cases with repeated cycles of chemotherapy had adaptive profiles, which deserve a specific analysis. Gilbert syndrome, atazanavir co-prescription, hemolysis, and cardiac insufficiency were factors associated with total bilirubin or GGT variability.
Conclusion
This pilot study permitted to validate an "ALT-free strategy" for planning the statistical analysis of the forthcoming validation phase of new DILI-biomarkers..

Background

- Severe acute Drug Induced Liver Injury (DILI) is identified and classified using increase of ALT.
- The most popular criteria are Temple´s corollary (3xULN*-ALT) and Hy´s law criteria (3xULN-ALT and bilirubin>2xULN (34 µmol/L).
- To assess the performance of better DILI biomarkers than ALT, it is mandatory to use another endpoints than ALT.
- *ULN= Upper limit of the normal

Aim

The aim was to check the utility of biological and clinical endpoints "ALT-free" in a prospective pilot study before starting a validation study.

Patients and Methods

Included patients

- Patients were recruited in tertiary center (Pitié –Salpêtrière Hospital, Paris) from July 2011 to June 2012
- N=25 patients with Drug Induced Liver Injury (DILI) defined as:
  - ALT > 3 x ULN or 2-fold baseline ALT
  - History of drug intake
- RUCAM-score was calculated at inclusion

Patients and Methods *continued*

Exclusion criteria

- Acute viral hepatitis (A, B, C, E, CMV, EBV and Herpes)
- Chronic viral hepatitis
- Autoimmune hepatitis
- Primary biliary cirrhosis
- Primary sclerosing cholangitis
- Extrahepatic cholestasis
- Ischemic liver damage
- Liver metastasis

End-points:

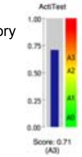
- Biochemical severity DILI (B-sDILI):
  - Increase of total bilirubin (TB) > 2xULN (34 µmol/l) if TB < 2xULN at baseline
  - Increase of 10 µmol/l in TB level if TB > 2xULN at baseline
- Clinical severity DILI (C-sDILI):
  - Liver transplantation
  - DILI-related death
  - Hospitalisation

Follow-up

- Patients were followed during 12 weeks (W)
- Visits previewed : baseline, day 1, day 3, week 1, W2, W3, W4, W8 and W12
- Mandatory visits: baseline, W1 and between W4 and W12

Biomarkers

- *Standard of care: ALT*
- *Proof of concept: Apolipoprotein-A1 (ApoA1)*
  - The principal constituent of HDL-cholesterol
- *ActiTest ® (BioPredictive, Paris, France)*
  - Panel of patented biomarkers for estimation of liver necro-inflammatory activity
  - Scores from 0 to 1.
  - Combines age, gender with total bilirubin, GGT, haptoglobin, apolipoprotein-A1, alpha 2-macroglobulin and ALT



Results

Description of 25 included patients

Patient	Gender	Birth	Baseline	Age (years)	BMI (Kg/m²)	Alcohol intake	g/day	Suspected drugs	Indication of drug	DILI type	RUCAM-score	DILI
1	M	1950	21/06/2011	62	24,2	yes	20	rituximab; fludarabine	leukemia. HBV reactivation	Hepatocellular	3	Possible
2	F	1989	19/07/2011	22	23,2	no		carbamazepine; trimethoprim sulphamethoxazole; prednisone; valacyclovir; sirolimus	lymphoproliferative disorder	Hepatocellular	5	Probable
3	M	1967	27/07/2011	44	22,7	yes	100	acetaminophen (8g)	abdominal pain	Hepatocellular	7	Probable
4	F	1980	29/08/2011	31	21,2	no		acetaminophen (30 g); tramadol; bromazepam; zolpidem; cetirizine	suicidal	Hepatocellular	11	High probable
5	M	1952	29/08/2011	59	31,8	yes	100	acetaminophen (4g/day)	abdominal pain	Hepatocellular	6	Probable
6	M	1953	06/09/2011	58	21,9	no		amiodarone	atrial flutter	Cholestatic	4	Possible
7	M	1989	06/09/2011	22	16,0	no		carbamazepine; amoxicilin/clavunilate	epilepsy; urinary tract sepsis	Hepatocellular	9	High probable
8	M	1955	27/09/2011	56	26,4	yes	10	amiodarone; ibuprofen	atrial flutter; pain	Hepatocellular	7	Probable
9	M	1948	19/10/2011	63	28,7	yes	20	daunorubicin. cytarabine	acute leukemia. HBV reactivation	Hepatocellular	2	Unlike
10	M	1959	28/10/2011	52	23,9	no		altiazide; bortezomib	cardiac amyloidosis	Hepatocellular	10	High probable
11	F	1980	22/11/2011	31	22,5	no		hydroxychloroquine; cyclophosphamide	lupus erythematosus	Hepatocellular	3	Possible
12	M	1955	29/11/2011	57	17,8	yes	15	methylprednisolone; methotrexate; leucovorin	cerebral lymphoma	Hepatocellular	9	High probable
13	M	1966	02/12/2011	46	19,9	no		acetaminophen (3g) rifampicin; thiamphenicol	vertebral surgery sepsis	Hepatocellular	4	Possible
14	F	1934	02/12/2011	77	29,8	no		vincistine; methotrexate; leucovorin	cerebral lymphoma	Hepatocellular	5	Possible
15	F	1958	05/12/2011	54	22,1	yes	5	pyrimethamine; sulfadiazine; leucovorin	cerebral toxoplasmosis AIDS	Hepatocellular	7	Probable
16	M	1956	12/12/2011	56	32,5	yes	110	fluvastatin; ezetimibe	dyslipidemia	Hepatocellular	9	High probable
17	M	1993	03/01/2012	19	20,2	no		olanzapine	acute delirium	Hepatocellular	7	Probable
18	M	1949	26/01/2012	62	25,8	yes	10	acetaminophen (35-70 g); bromazepam (18 mg)	suicidal	Hepatocellular	13	High probable
19	M	1945	01/02/2012	67	28,0	no		methylprednisolone; methotrexate; leucovorin	cerebral lymphoma	Hepatocellular	7	Probable
20	M	1970	23/02/2012	42	23,9	yes	20	disulfiram. carbamazepine	alcohol withdrawal. depression	Hepatocellular	10	High probable
21	F	1991	23/02/2012	20	21,5	no		acetaminophen (24g)	suicidal	Hepatocellular	9	High probable
22	M	1976	03/04/2012	36	19,5	yes	10	6-mercaptopurine. acetaminophen (4g/day)	crohn disease	Mixed	5	Possible
23	F	1959	07/06/2012	53	29,4	no		diclofenac. acetaminophe (3g/day); herbal preparation	abdominal pain	Hepatocellular	5	Possible
24	F	1948	11/06/2012	64	29,2	no		fenofibrate	dyslipidemia	Hepatocellular	8	Probable
25	M	1974	27/06/2012	38	24,7	no		azathioprine	myasthenia gravis	Hepatocellular	6	Probable

Characteristics

Male gender	17 (68%)
Age (years)	53 (19-77)
BMI (Kg/m²)	24 (16 - 33)
Alcohol intake (g/day)	0 (0-110)
ALT (U/l)	405 (61-15896)
AST (U/l)	201 (20-30831)
GGT (U/l)	191 (23-3562)
Total bilirubin (µmol/l)	21 (2-491)
DILI type	
Hepatocellular	*23 (92%)*
Mixed	*1 (4%)*
Cholestatic	*1 (4%)*
RUCAM score	7 (2-13)
Unlike (1-2)	*1 (4%)*
Possible (3-5)	*7 (28%)*
Probable (6-8)	*9 (36%)*
High Probable (> 8)	*8 (32%)*

Summary of Endpoints

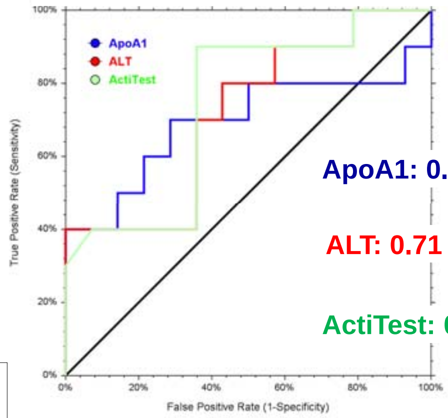
- **Biochemical severity DILI (B s-DILI):**

6 (24%) patients presented increased total bilirubin

- **Clinical severity DILI (C s-DILI):**

7 (28%) patients had complications:
- One death
- One liver transplantation
- Five specific hospitalizations

Biomarkers ROC curves to predict severe DILI



AUROC (95% CI)

ApoA1: 0.69 (0.35-0.87; P=0.07)

ALT: 0.71 (0.42-0.87; P=0.03)

ActiTest: 0.74 (0.45 0.89; P=0.01)

p=0.18 (ApoA1 vs ALT)

p=0.88 (ALT vs ActiTest)

Conclusion

This pilot study permitted to validate an "ALT-free strategy" for planning the statistical analysis of the forthcoming validation phase of new DILI-biomarkers.

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Disclosures:
TP is the inventor of FibroTest ActiTest and the founder of BioPredictive, the company that markets the FibroTest ActiTest. Patents belong to the French Public Organization APHP.

Bio Predictive