

Validation and Early Clinical Evaluation of Mechanistic Protein Biomarkers for Drug-Induced Liver Injury (DILI)

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ABSTRACT

Drug-induced liver injury (DILI) is a major cause of clinical trial failure and post-marketing drug withdrawal. A key challenge in clinical practice is the lack of specific biomarkers for accurate diagnosis and prognosis. The international Translational Safety Biomarker Pipeline (TransBioLine) project is investigating mechanistic protein biomarkers to improve DILI characterization alongside established markers such as albumin and transaminases.

The biomarkers macrophage colony-stimulating factor 1 receptor (MCSF1R), osteopontin (OPN), high mobility group protein B1 (HMGB1), glutamate dehydrogenase (GLDH), keratin 18 (K18), and caspase-cleaved keratin 18 (ccK18) reflect apoptotic, necrotic, and immunological processes involved in DILI pathogenesis.

Sex-stratified reference data were generated from healthy volunteers sampled at a single time point (n = 44 males, n = 46 females), establishing reference ranges for all biomarkers except HMGB1 in females. In a separate healthy cohort (n = 48), fasting and feeding effects were assessed, showing significant fasting-related variation in two biomarkers.

Using validated assays, learning-phase data were obtained from single time-point samples of patients with suspected DILI (n = 100), nonalcoholic or alcoholic fatty liver disease (n = 53), psoriasis or rheumatoid arthritis (n = 29), and healthy volunteers (n = 50). Preliminary analyses show markedly higher median biomarker concentrations in suspected DILI cases than in healthy and non-DILI controls, supporting their potential for improved DILI characterization.

NOVEL MECHANISTIC PROTEIN BIOMARKERS OF DILI

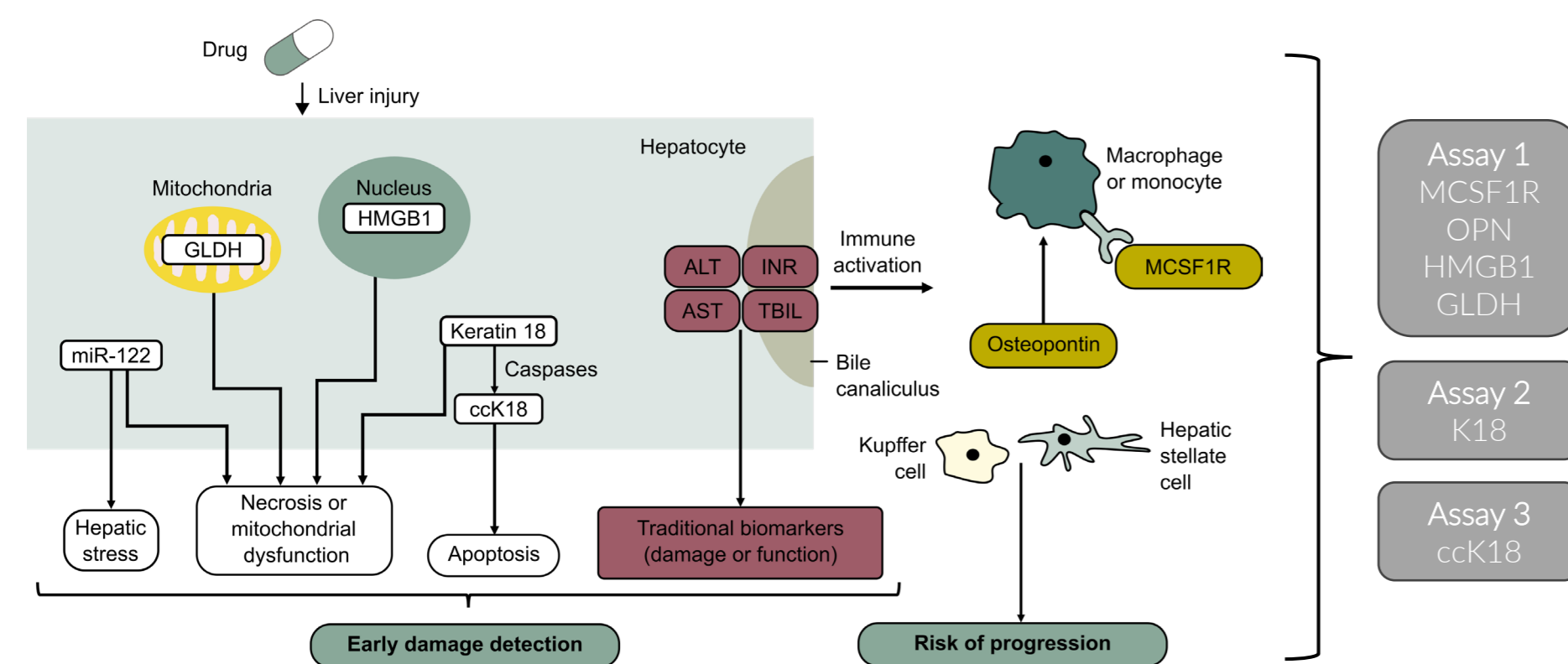


Figure 1: Assay 1 using 15 μ L EDTA plasma per replicate capable of quantifying macrophage colony-stimulating factor 1 receptor (MCSF1R), osteopontin (OPN), high mobility group protein B1 (HMGB1), and glutamate dehydrogenase (GLDH). Assay 2 using 25 μ L of EDTA plasma per replicate is capable of quantifying keratin 18 (K18). Assay 3 also using 25 μ L of EDTA plasma per replicate is capable of quantifying caspase-cleaved keratin 18 (ccK18). This biomarker panel reflects apoptotic, necrotic and immunological processes that contribute to the pathogenesis of DILI.

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REFERENCE RANGE IN NHV

Biomarker reference ranges were determined in a healthy volunteer cohort screened for liver fat using transient elastography (46 females, age 41 \pm 14 yrs; 44 males, age 43 \pm 16 yrs). Overall age ranged from 19–80 years and BMI from 18.2–31.2 kg/m² (mean 23.9). Reference ranges are shown in Table 1. HMGB1 reference intervals for females could not be established because most observations (36/46) were imputed to the LLOQ (0.57).

Table 1: Reference range in HV for exploratory DILI biomarkers.

Biomarker	Sex	Mean	SD	geoMean	CV	Median	Min	Max	RI
GLDH (ng/ml)	Female	9	7	7	79	7	2	39	(0-22)
	Male	21	30	12	147	12	2	166	(0-74)
ccK18 (U/L)	Female	89	37	82	41	82	38	185	(7-159)
	Male	100	39	94	39	90	45	219	(4-169)
HMGB1 (ng/ml)	Female	0.66	0.31	0.63	47.3	0.57	0.57	2.44	(NA-NA)
	Male	1.48	2.55	0.93	171	0.62	0.57	16.01	(0-2.88)
K18 (U/L)	Female	530	1362	204	257	135	125	6571	(0-421)
	Male	365	820	205	224	168	125	4199	(0-1600)
MCSF1R (ng/ml)	Female	264	67	256	26	251	153	466	(112-391)
	Male	270	59	264	22	265	157	450	(156-385)
OPN (ng/ml)	Female	33	10	31	30	31	15	60	(10-51)
	Male	37	9	36	26	35	13	61	(16-55)
Albumin (g/dL)	Female	4.4	0.2	4.4	0.5	4.4	3.9	4.9	(3.9-4.8)
	Male	4.5	0.3	4.5	0.7	4.5	3.9	5.1	(3.9-5.1)
TBIL (mg/dL)	Female	0.6	0.19	0.57	32.62	0.6	0.3	1.1	(0.16-0.95)
	Male	0.66	0.2	0.62	31.1	0.65	0.2	1.1	(0.24-1.08)
ALT (U/L)	Female	17	6	16	37	16	9	37	(3-28)
	Male	25	10	23	41	22	11	51	(2-46)

EFFECTS OF FASTING

A separate healthy volunteer cohort was sampled three times to assess biomarkers. The six candidate protein biomarkers showed non-normal distributions even after log transformation. Biomarker levels across fasting and feeding states are shown in Figure 3. Linear mixed-model regression indicated significant fasting effects for two biomarkers: OPN increased by 7–11% and K18 by 13–15% during fasting.

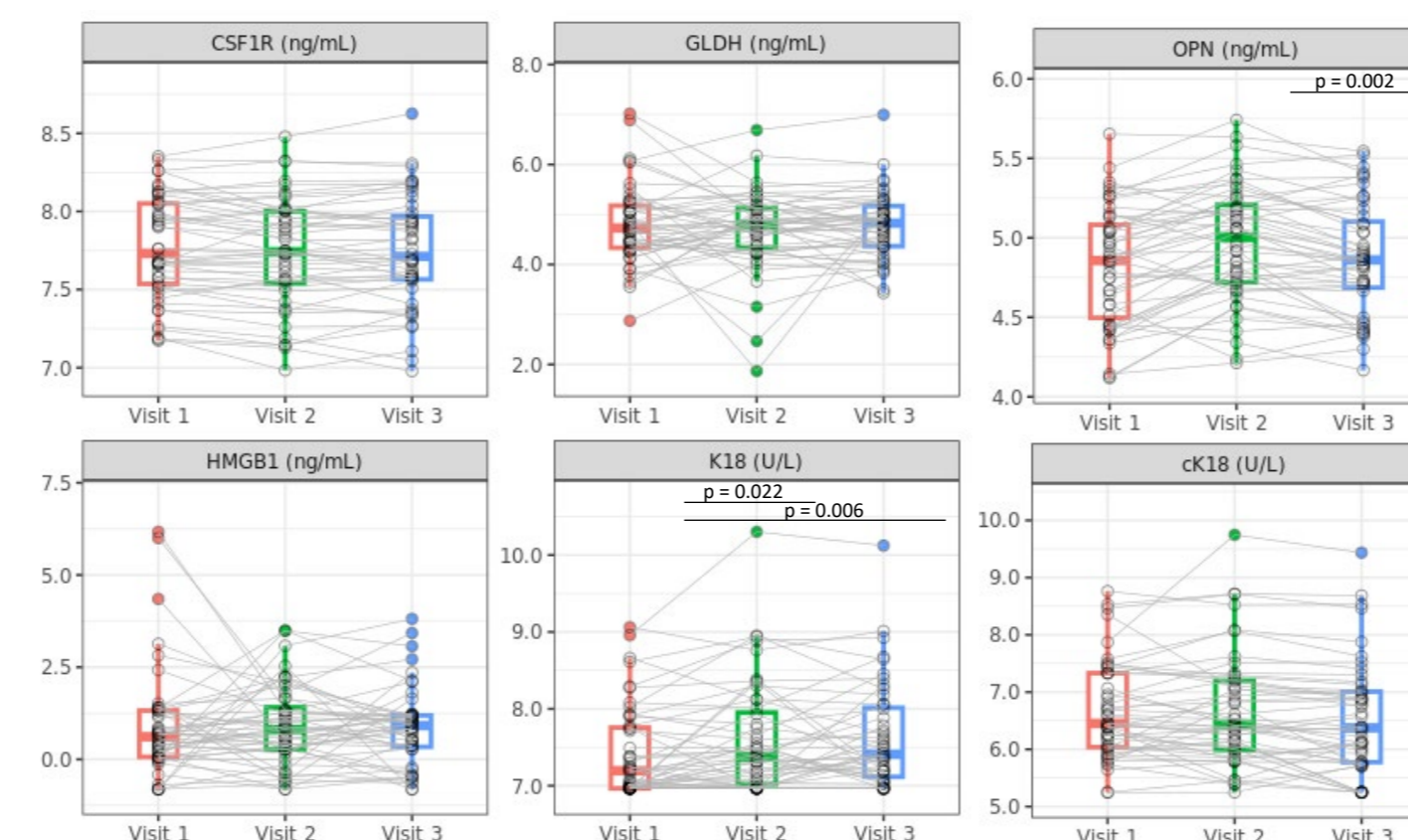


Figure 3: Biomarker variation during non-fasting (visit 1), fasting (visit 2), and post-prandial (visit 3) visits. The biomarker data were transformed by log₂. Biomarker variation per subject (gray line) and boxplot.

RESULTS

Using validated, mechanistic biomarker assays for drug-induced liver injury (DILI), learning-phase data were generated from single time point samples obtained from patients with suspected DILI (n = 100), patients with fatty liver disease (NAFLD/AFLD, n = 53), patients with psoriasis or rheumatoid arthritis (n = 29), and healthy volunteers (n = 50). Preliminary analyses indicate that median biomarker concentrations were increased in suspected DILI cases compared with healthy controls, including K18 (5.7-fold), ccK18 (10.5-fold), HMGB1 (6.9-fold), MCSF1R (12.5-fold), OPN (3.38-fold), and GLDH (11.4-fold). Comparable elevations were observed when suspected DILI cases were compared with non-DILI disease controls.

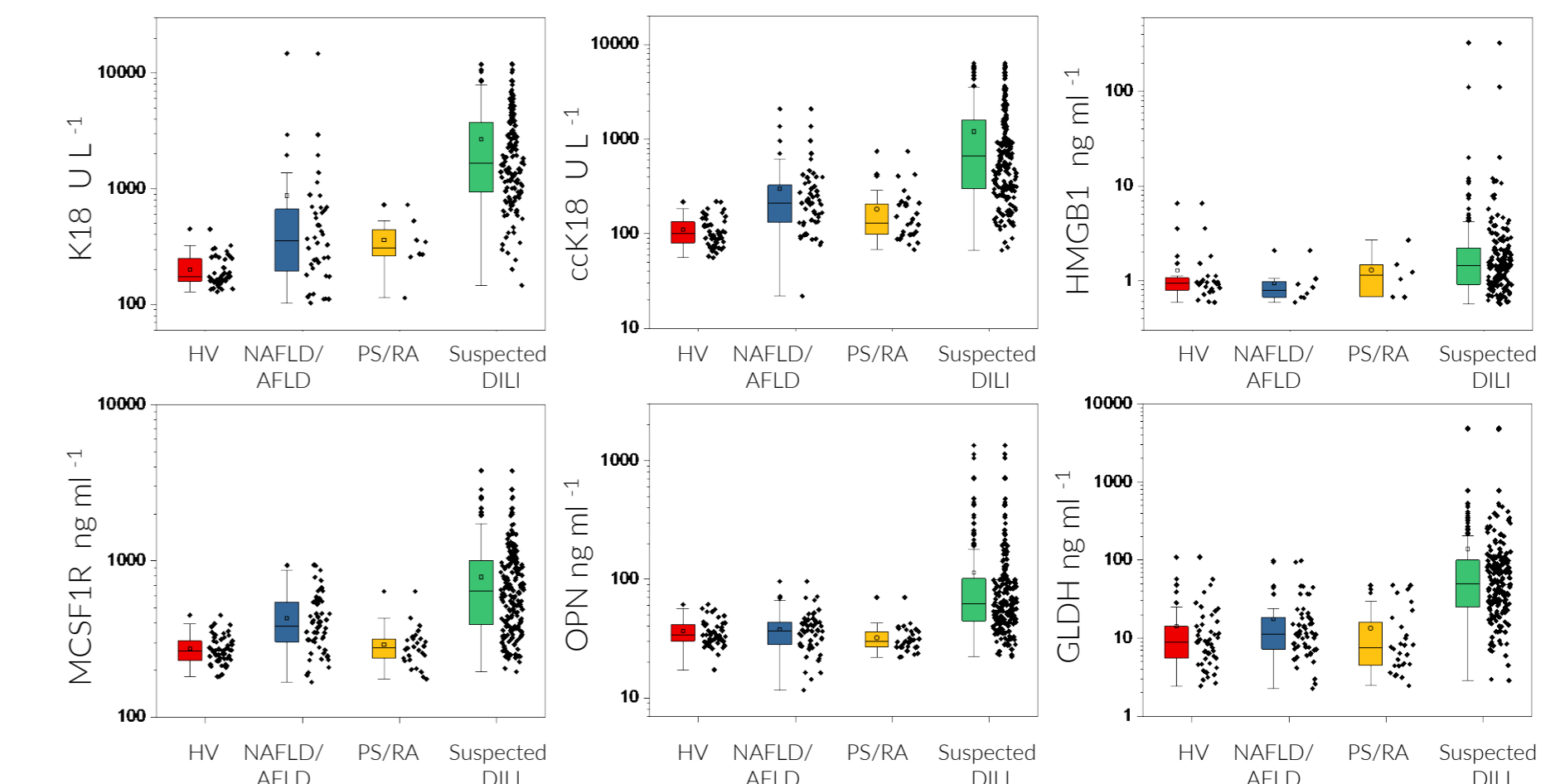


Figure 3: Analysis of the six novel mechanistic DILI biomarker proteins macrophage colony-stimulating factor 1 receptor (MCSF1R), osteopontin (OPN), high mobility group protein B1 (HMGB1), glutamate dehydrogenase (GLDH), caspase-cleaved keratin 18 (ccK18) and keratin 18 (K18) in EDTA plasma samples of patients with suspected DILI (n=50) with fatty liver disease (NAFLD/AFLD, n=53), with psoriasis or rheumatoid arthritis (n=29) and healthy volunteers (n=50). Biomarkers MCSF1R, OPN, GLDH and HMGB1 were assessed using a multiplexed immunoassay with mass spectrometric readout, biomarkers ccK18 and K18 were assessed using a commercially available immunoassay.

OUTLOOK

Ongoing work will assess these biomarkers in a prospectively collected, deeply phenotyped case-control repository, including longitudinal samples captured during liver injury progression, to support biomarker validation and qualification within the TransBioLine project.

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