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PSTC and SAFE-T Collaboration: Normal Ranges for 12 Novel Biomarkers for Liver Safety

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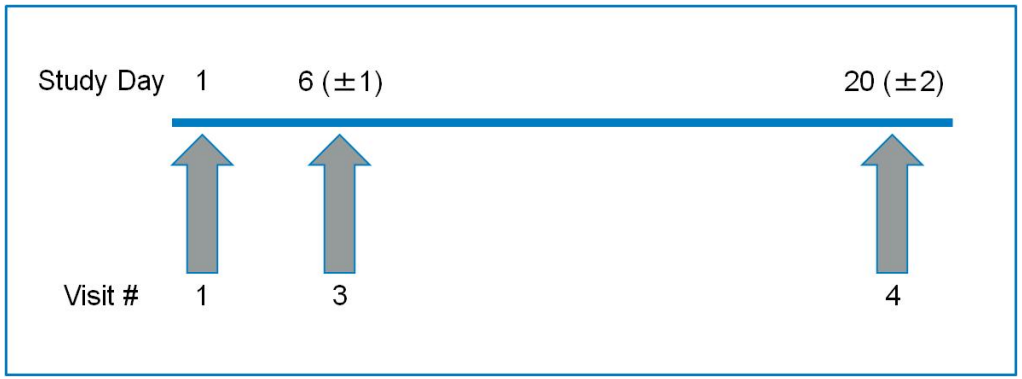
KEY MESSAGES

- Collaboration between Hepatotoxicity Working Group (HWG) of Predictive Safety Testing Consortium (PSTC) and Work Package 3 of Safer and Faster Evidence-Based Translation (SAFE-T) consortium
- 12 novel liver biomarkers were assessed by healthy volunteers (HV) with commercially available or newly developed assays
- Intra- and Inter-subject variability was low
- Only age related stratification was observed in AFP and PT
- Baseline estimation of HV liver biomarkers will be utilized by PSTC and SAFE-T consortia during biomarker qualification
- Ongoing SAFE-T clinical trials will assess performance of biomarkers in three context of use (COU) areas

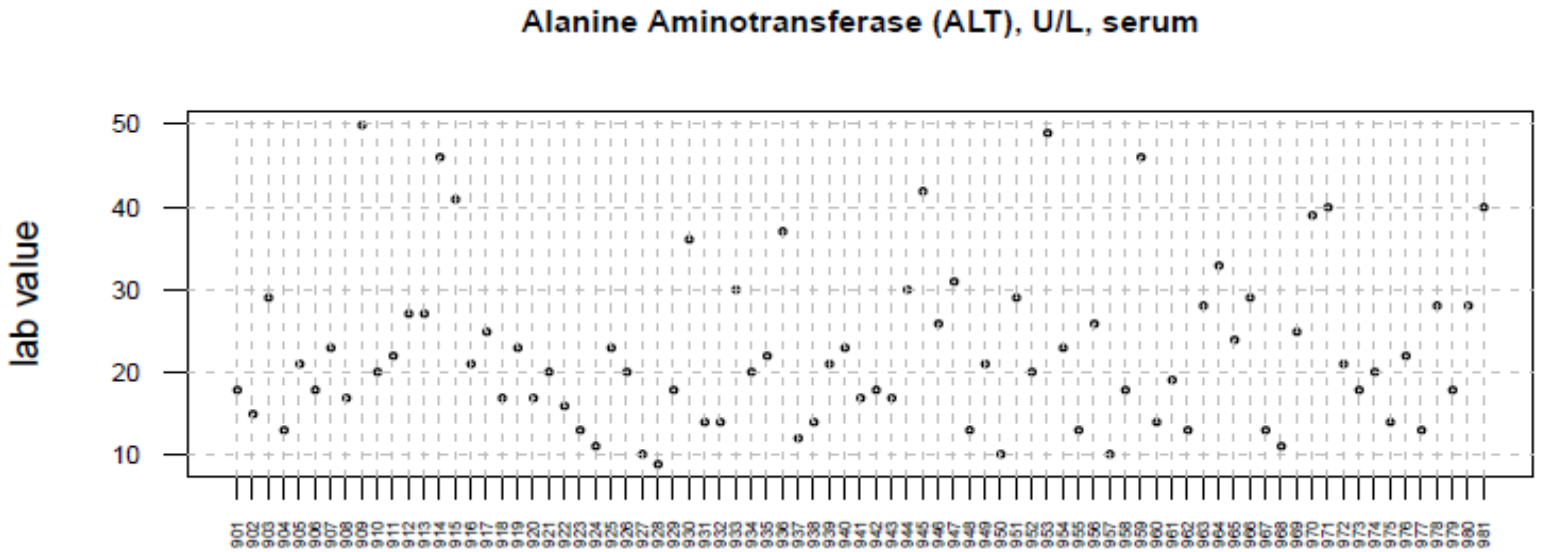
BACKGROUND OF PSTC HV STUDY:

- These well-annotated quality samples were used to evaluate renal safety biomarkers by PSTC Nephrotoxicity Working Group
- All subjects were recruited at the Jasper Clinic, Inc., Kalamazoo, MI
- A total of 81 volunteers completed the study
- Plasma, serum, urine and whole blood samples were collected over 21 day on days 1, 6±1, and 20±1



INCLUSION AND EXCLUSION

- Standard inclusion and exclusion criteria for HV study
- Original intent of the this HV study was to assess renal biomarkers thus there was emphasis on glomerular filtration rate (GFR) criteria
- Most ALP, ALT, AST and Bilirubin values were below ULN of Jasper clinic reference ranges, and no apparent liver injury was found among the 81 subjects



DEMOGRAPHICS

	Age Category		
	20 – 39 years N=41	40 – 70 years N=40	Total N=81
Age (years)			
Mean	29.4	50.8	40.0
Mode	27.0	40.0	27.0
Min, Max	20.0, 39.0	40.0, 69.0	20.0, 69.0
Sex, n (%)			
Male	20 (48.8%)	20 (50.0%)	40 (49.4%)
Female	21 (51.2%)	20 (50.0%)	41 (50.6%)
Race, n (%)			
White	32 (78.0%)	36 (90.0%)	68 (84.0%)
Non-white	9 (22.0%)	4 (10.0%)	13 (16.0%)
Ethnicity, n (%)			
Hispanic	1 (2.4%)	1 (2.5%)	2 (2.5%)
Non-Hispanic	40 (97.6%)	39 (97.5%)	79 (97.5%)
BMI (lb/in^2)*			
Mean	26.8	28.0	27.4
Mode	--	21.8	21.8
Median	27.76	27.89	27.76
Min, Max	19.1, 35.4	20.4, 37.6	19.1, 37.6
CDC BMI Category, n (%)			
Normal	18 (43.9%)	7 (17.5%)	25 (30.9%)
Overweight	10 (24.4%)	19 (47.5%)	29 (35.8%)
Obese	13 (31.7%)	14 (35.0%)	27 (33.3%)

Abbreviations: BMI = body mass index, CDC = Centers for Disease Control and Prevention, in = inches, lb = pounds, max = maximum, min = minimum

** - Subjects with BMI greater than 35 were included in the study*

12 LIVER BIOMARKERS ASSESSED

Biomarker	Type of Biofluid	Detection Method
Keratin 18, full length (K18)	Serum	ELISA (Peviva cat #10040)
Keratin 18, caspase cleaved fragment (ccK18)	Serum	ELISA (Peviva cat #10011)
Glutamate dehydrogenase (GLDH)	Serum	Enzyme activity assay (Roche cat #11929992)
α-Glutathion S-Transferase (GST-α)	Serum	Immunoassay (Myriad RBM; performed by NMI)
α-Fetoprotein (AFP)	Serum	Immunoassay (Myriad RBM; performed by NMI)
Arginase-1 (ARG1)	Serum	Immunoassay (developed by NMI)
Osteopontin (OPN)	Serum	Immunoassay (Myriad RBM; performed by NMI)
Macrophage colony-stimulating factor 1 receptor (M-CSF-R)	Plasma	Immunoassay (developed by NMI)
Paraoxonase 1 (PON1) and Prothrombin (PT)	Plasma	Immunoassays (developed by NMI)
Leucocyte cell- derived chemotaxin-2 (LECT2)	Plasma	Immunoassay (developed by NMI)
Sorbitol dehydrogenase (SDH)	Serum	Enzyme activity assay (Sekisui cat #740-10)

Hepatic Biomarker	Origin of Biomarker	Clinical Significance
Keratin 18, full length (K18)	K18 is abundant within hepatocytes and constitutes around 5% of total hepatic protein.	The full-length protein is released from necrotic cells. It is significantly elevated in acetaminophen overdose patients that die/require a liver transplant compared to spontaneous survivors. (Antoine et al. 2012)
Keratin 18, caspase cleaved fragment (ccK18)	ccK18 is abundant within hepatocytes and constitutes around 5% of total hepatic protein.	The caspase-cleaved fragment is released from apoptotic cells and helps define the type of cytotoxicity. CK-18 fragments in blood predict severity of disease in NASH and in hepatitis C. (Joka et al. 2012)
Glutamate dehydrogenase (GLDH)	Mitochondrial enzyme located primarily in the centrilobular region of the liver	a sensitive marker of liver toxicity with hepatocellular damage in preclinical species; shown to be elevated in humans with hepatic ischemia or hepatitis; shown to correlate with ALT in patients with a broad range of clinically demonstrated liver injuries including acetaminophen-induced liver injury and to detect mild hepatocyte necrosis in patients treated with heparin. (Schomaker et al. 2013)
α-Glutathion S-Transferase (GST-α)	Centrilobular region of the liver; accounts for up to 90% of all GST in the liver	Hepatotoxicity biomarker shown in rats to have enhanced specificity and sensitivity compared to ALT; humans with acetaminophen overdose show elevated GSTα levels earlier than ALT ; GSTα may offer a better assessment of rapid changes in liver damage due to the shorter half-life of plasma GSTα compared to ALT or AST (Bailey et al. 2012)
α-Fetoprotein (AFP)	Liver (hepatocytes)	Increase of AFP has been detected in many types of liver disease incl. APAP overdose. Data about sensitivity to detect DILI do not exist. Data from literature suggest that AFP is expressed after the onset of liver injury and during regeneration with increased serum/plasma levels. AFP may have value as a prognostic marker in liver injury. (Schmidt et al. 2005)
Arginase-1 (ARG1)	Two isoforms of arginase are known: ARG1 and ARG2. ARG1 is highly abundant in liver cytosol and is expressed at low levels in erythrocytes	ARG1 has been shown to be highly sensitive for acute liver damage (leakage marker). Circulating concentrations increase in patients with various hepatic disorders, such as hepatoma and viral or alcoholic hepatitis. A significant correlation with AST and ALT activities was described following partial resection and orthotopic liver transplantation. (Bailey et al. 2012)

Hepatic Biomarker	Origin of Biomarker	Clinical Significance
Macrophage colony-stimulating factor 1 receptor (M-CSF-R)	Monocytes / macrophages	Data from the ximelagatran biomarker discovery study suggest that CSF1R is shedded from macrophages during DILI. CSF1R serum/plasma levels may have value as a prognostic marker for liver disease associated with inflammation. (Andersson et al. 2009)
Paraoxonase 1 (PON1) / Prothrombin (PT)	Strongest expression in hepatocytes, but not liver-specific	PON1 is not a leakage enzyme, but is constitutively released into the circulation. Decreases in serum PON1 reflect liver injury or dysfunction and have been linked to chronic hepatic damage. The biomarker serves two purposes: 1) as a diagnostic marker for repressed liver function; 2) together with prothrombin as a marker to differentiate between healthy controls and subjects with all types of NAFLD and NASH. (Keskin et al. 2009)
Leucocyte cell- derived chemotaxin-2 (LECT2)	hepatocytes	Prognostic indicator of liver regeneration and injury. Serum Lect2 levels are inversely proportional to ALT and decrease at the peak of liver regeneration after hepatectomy. (Sato et al. 2004)
Sorbitol dehydrogenase (SDH)	Liver; multiple tissues	Sensitive enzymatic serum marker of liver toxicity increasing with hepatocelluar damage in preclinical species. Shown to be elevated in humans with various liver diseases and to detect mild hepatocyte necrosis in patients treated with heparin. The biomarker serves two purposes: 1) as an early marker of hepatocellular injury, possibly preceding ALT on a temporal scale 2) as a specific marker of hepatocellular injury. (Harrill et al. 2012)

STATISTICAL ANALYSIS

- A mixed effect model for log transformed data was used to obtain the variance components for between subject variation and within subject variation assuming a log-normal distribution

- The 95th percentile was obtained using the estimated mean and standard deviation for the log-normal distribution

- The 95th percentile was taken as the estimate of the ULN

- For two of the markers (CCK18 and GSTA) a substantial number of values were below LLoQ, so a maximum likelihood estimate for a truncated log-normal distribution was used to estimate the ULN

- For one of the markers (K18) a nonparametric approach was used to obtain the 95th percentile because approximately 93% of the data were below LLoQ

GEOMETRIC MEAN, CV AND ULN

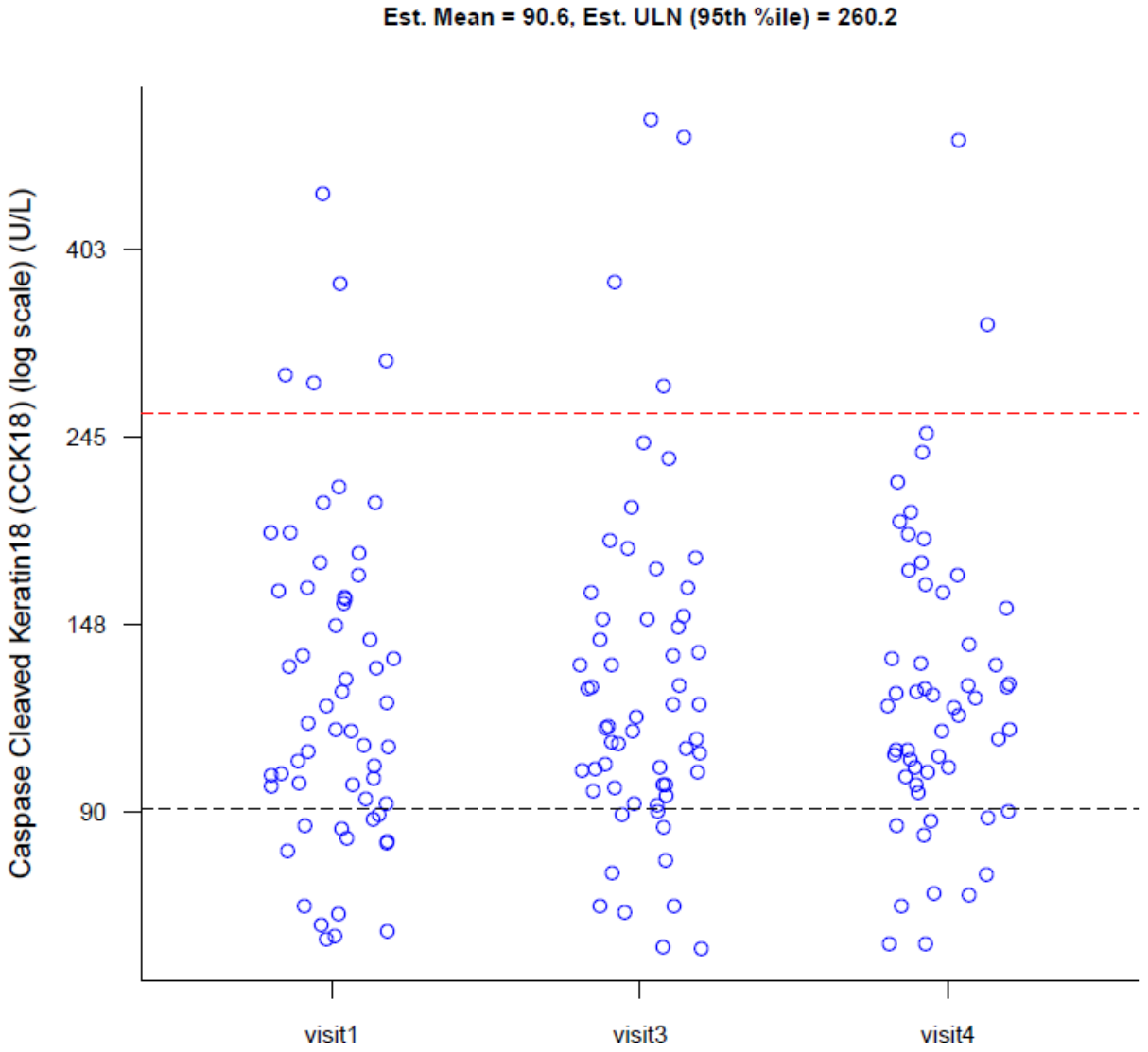
Biomarker	Unit	est. geometric mean	intra-subj CV	inter-subj CV	est. ULN (95th percentile)
AFP	ng/mL	0.68	31.93%	61.53%	1.98
ARG1	ng/mL	7.63	37.46%	46.03%	19.46
GLDH	U/L	2.71	34.53%	52.74%	7.24
GSTA	ng/mL	6.31	71.86%	119.54%	60.00
CCK18	U/L	90.65	23.71%	37.12%	260.16
K18	U/L				122.80
LECT2	ng/mL	252.27	28.64%	20.97%	447.96
MCSFR1	ng/mL	334.81	13.89%	30.08%	571.64
OSTEOPON	ng/mL	4.13	26.61%	52.15%	10.31
PON1	ng/mL	294.97	31.67%	43.33%	690.82
PT	µg/L	60.07	13.48%	17.58%	86.29
SDH	U/L	3.02	41.01%	43.43%	7.75

PERCENTAGE OF STUDY SAMPLES BELOW LLOQ BY VISIT

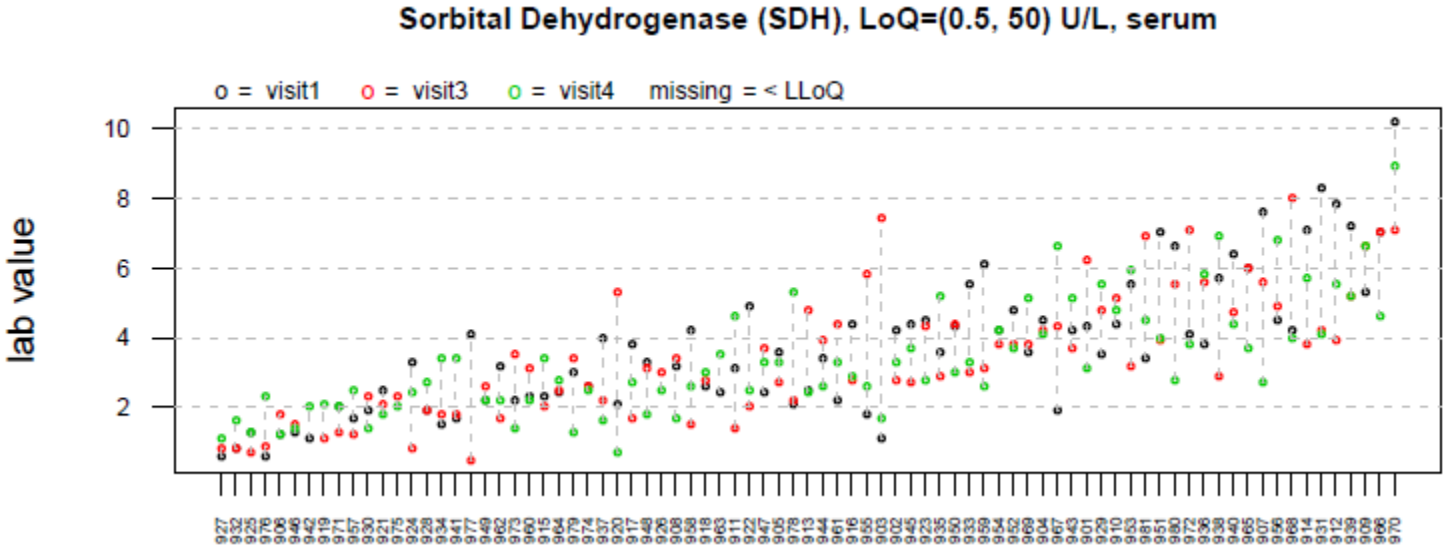
	AFP	CCK18	GSTα	K18	SDH
# of Samples	243	243	243	243	243
# below LLOQ	3 (1.2%)	72(29.6%)	28(11.5%)	226(93%)	6(2.5%)

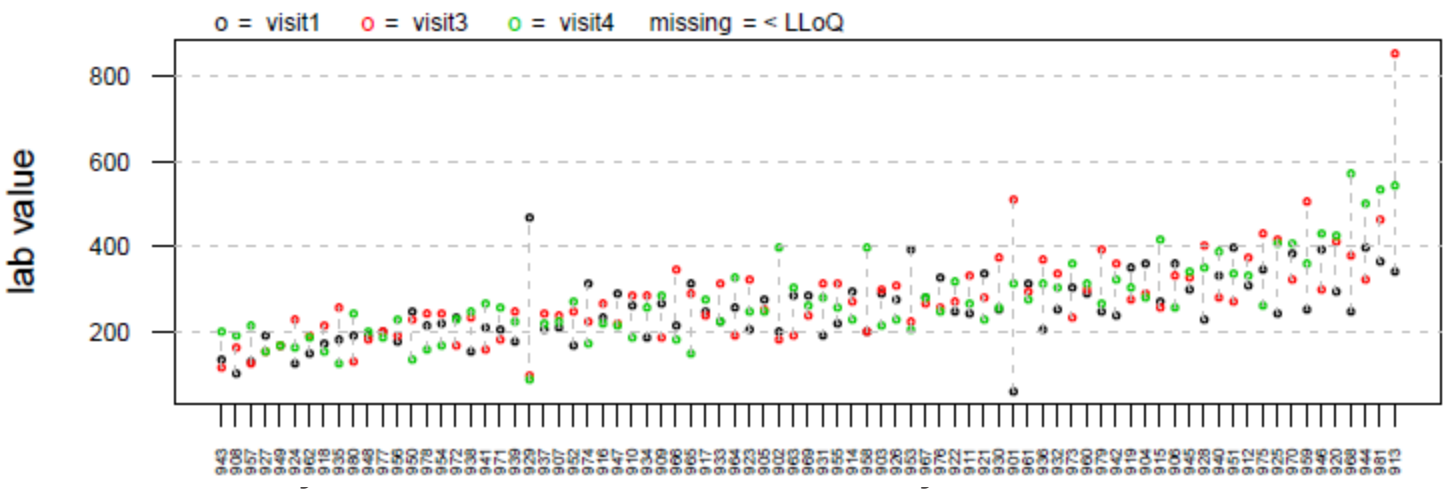
•All samples assessed for for ARG1, GLDG, LECT2, MCSFR1, OPON, PON1 and PT were above the LLOQ

ESTIMATED ULN AND MEAN FOR CCK18

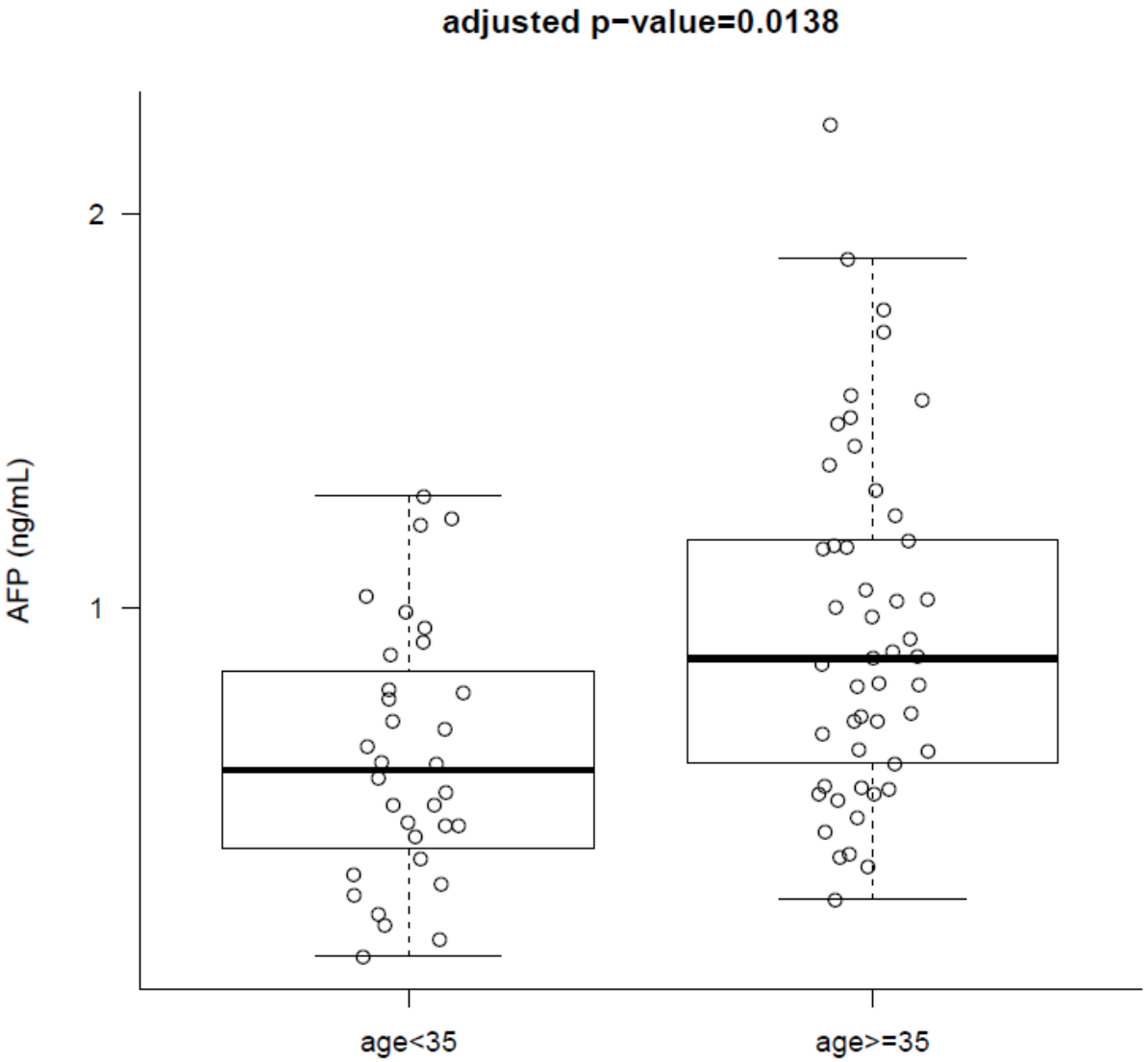


RAW DATA: LECT2 AND SDH

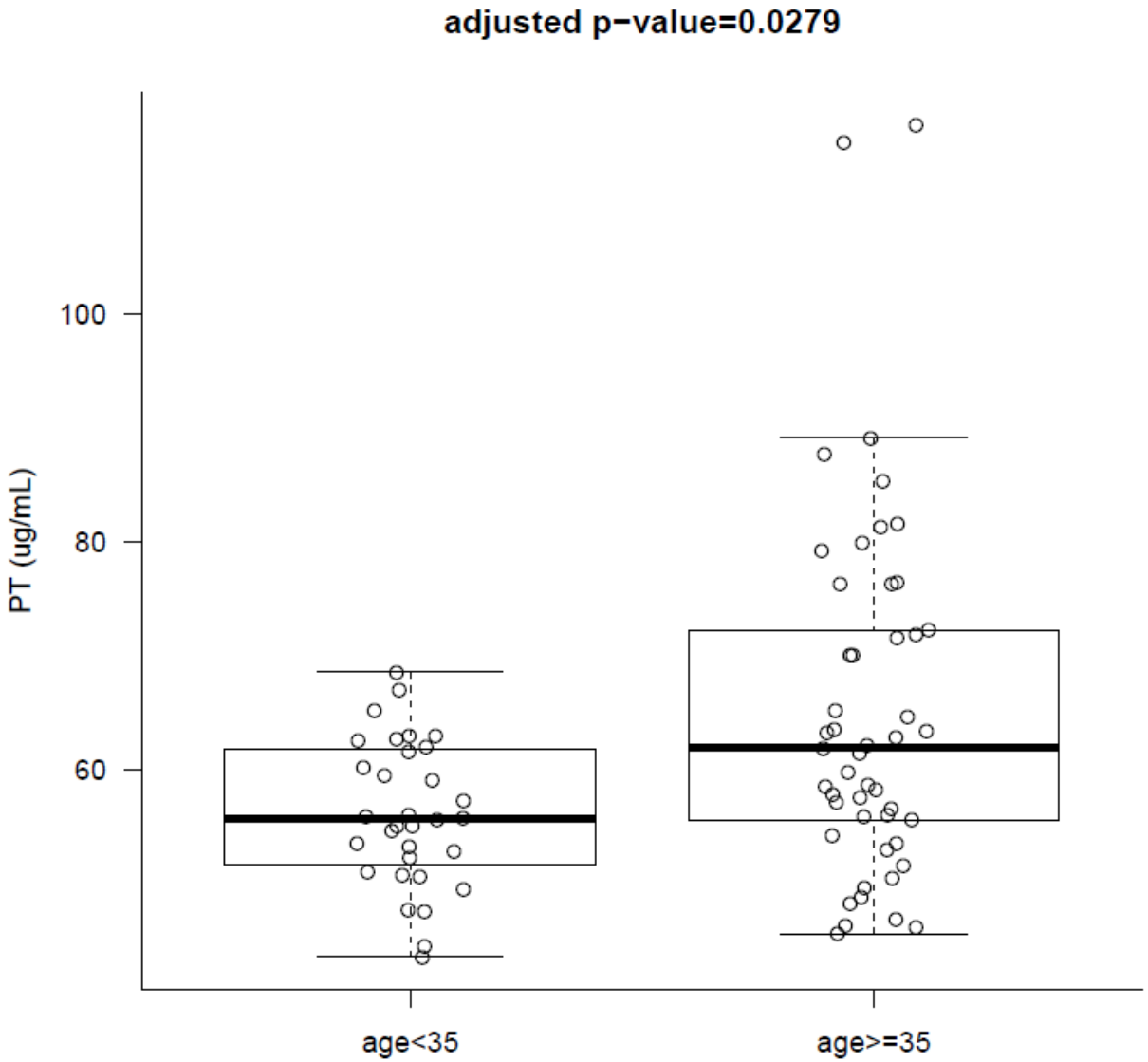




AFP VS. AGE



PT VS. AGE



NEXT STEPS

- Mir-122 and individual bile acid quantification from PSTC HV study sample
- SAFE-T assessment of clinical performance performance of 12 liver biomarkers

SAFE-T CONTEXT OF USE (COU) ASSESSMENT

Context-of-use statement "A":

Biomarker X (or panel of biomarkers) ……. is qualified as a clinical safety biomarker that sponsors may choose to incorporate into their clinical trials ***to confirm the diagnosis of drug induced liver injury as indicated by elevations of the standard markers ALT and/or Total bilirubin (TBIL).***

Context-of-use statement "B":

Biomarker X (or panel of biomarkers) … is qualified as a clinical safety biomarker that sponsors may choose to incorporate into their clinical trials ***to indicate a risk for the progression of hepatocellular injury to severe DILI in patients in whom an initial DILI diagnosis has been established based on elevations of standard markers ALT and/or TBIL.***

Context-of-use statement "C":

Biomarker X (or panel of biomarkers) … is qualified as a clinical safety biomarker that sponsors may choose to incorporate into their clinical trials ***to indicate subclinical DILI with mild ALT elevations < 3 x ULN in patients receiving hepatotoxic drugs OR to detect (subclinical) DILI before ALT increases.***

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