Angiogenin May be an Early Biomarker for Regeneration in Drug-Induced Liver Failure

Maureen R Newman[1]*, Heping Hu[2], Mark Yen[1], Jody A Rule[3], William M Lee[3], the Acute Liver Failure Study Group, and Geertrui F Vanhove[1]; *Presenting author

[1]Surrozen Inc., South San Francisco, CA 94080, USA; [2]XSH Solutions, Inc.; [3]University of Texas Southwestern Medical Center at Dallas

Background

- Acute liver failure (ALF) due to acetaminophen (APAP) overdose or other drug-induced liver injury (DILI) has limited treatment options.
  - Patients who are not expected to recover following first-line therapy such as intravenous N-acetylcysteine are listed for liver transplant (Tujios 2018)
  - Only 65% of patients with APAP hepatotoxicity recover without transplant, leading to approximately 500 deaths annually in the US
  - Estimates of 300–500 deaths due to DILI, with only 25% of patients surviving without transplant
- Wnt signaling plays a central role in hepatocyte expansion during development and tissue repair. Downstream canonical Wnt signaling mediated by β-catenin stabilization correlates with increased regeneration in ALF patients (Apte 2009, Bhushan 2014).
  - Angiogenin is a direct Wnt target secreted primarily by hepatocytes and stimulates angiogenesis, cell growth, and cell survival. No link between ALF and angiogenin has been reported.
  - Leukocyte cell derived chemotaxin 2 (LECT2) is a hepatokine secreted nearly exclusively by hepatocytes and is a direct Wnt target gene. LECT2 plays a key role in liver regeneration and is related to patient survival in ALF (Sato 2004).
- Here, we asked if there was a difference in serum levels of alpha-fetoprotein (AFP), cholinesterase (BChE), and markers of Wnt signaling (angiogenin and LECT2) between ALF patients who did not receive a liver transplant and those who went on to liver transplant or died.

Methods

Serum samples were selected from the US Adult Acute Liver Failure Study Group Registry (NCT 00518440). The etiology of liver failure was divided into two groups as adjudicated by the ALFSG: APAP or DILI/other/indeterminate. Spontaneous survivors (SS) were defined as patients who recovered without transplant and were compared to patients who went on to transplant or died (LT/D). The number of serum samples per category is shown in the table.

Days following enrollment	APAP	DILI/other	APAP	DILI/other
	Spontaneous Survival		Transplanted or Died	
Day 1	N=11	N=4	N=10	N=9
Day 3	N=10	N=5	N=10	N=9
Day 7	N=10	N=5		

AFP, angiogenin, and LECT2 were measured by enzyme-linked immunosorbent assay (ELISA), and BChE was measured by enzymatic activity assay.

To better account for differences in clinical course, hospital admission was used to interpret temporal changes in biomarker levels as the timing of study enrollment varies among patients. The log scale of biomarker levels were modeled by conducting a random-mixed effect model, with covariates including the terms for time since hospital admission and status group indicating SS or LT/D. Unstructured correlation is assumed to account for correlation due to repeated measurements over time within the same patient. The Kenward-Roger method is applied to adjust for degrees of freedom. Point estimates and 95% confidence intervals (CI) for least squared means for each status group and the difference between the two statuses were estimated.

Results

The profiles of AFP, angiogenin, LECT2, and BChE were plotted against time from hospital admission, separately for SS and LT/D. Point estimates and 95% CI of least squared means were compared between SS and LT/D. Differences for SS vs LT/D are summarized in the table.

Analyte	Fold SS vs LT/D	95% CI	P-value
AFP	1.72	(0.53, 5.57)	0.3583
Angiogenin	2.63	(1.76, 3.93)	<0.0001
LECT2	6.26	(1.68, 23.39)	0.0078
BChE	1.84	(0.96, 3.53)	0.0663

Blue highlighting indicates a significant difference between SS and LT/D.

The differences in point estimates for angiogenin (Figure 1) and LECT2 (Figure 2) between SS and LT/D were significant, while the differences for AFP (Figure 3) and BChE (Figure 4) were not significant.

Figure 1

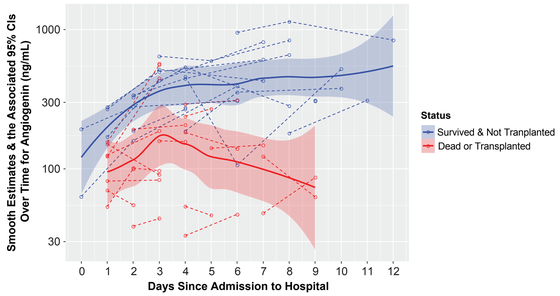


Figure 2

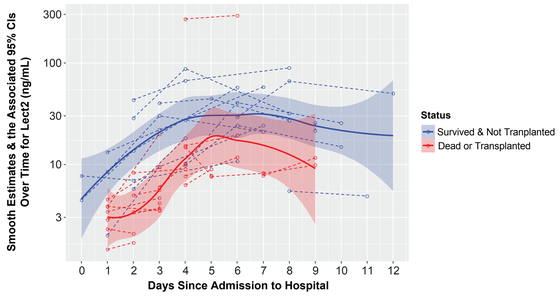


Figure 3

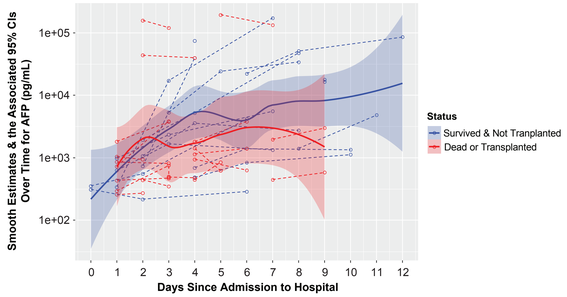
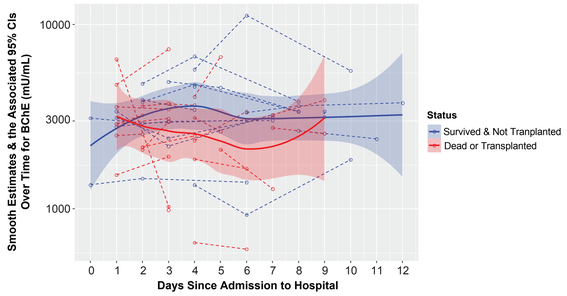


Figure 4



- Differences in point estimates were compared between SS and LT/D separately for etiology of liver failure (APAP and DILI/other).
  - There were significant differences for angiogenin in both APAP patients and in DILI/other patients, but for LECT2 a significant difference was only observed in APAP patients (Figures 5 and 6)
  - No other markers showed significant differences in any population

Analyte	Etiology	Fold SS vs LT/D	95% CI	P-value
AFP	APAP	2.24	(0.79, 6.33)	0.1246
AFP	DILI/other	0.98	(0.07, 13.54)	0.9874
Angiogenin	APAP	2.09	(1.31, 3.34)	0.0032
Angiogenin	DILI/other	2.55	(1.23, 5.30)	0.0154
LECT2	APAP	3.64	(2.10, 6.33)	<0.0001
LECT2	DILI/other	0.98	(0.28, 3.42)	0.9766
BChE	APAP	1.16	(0.79, 1.73)	0.4349
BChE	DILI/other	1.35	(0.70, 2.63)	0.3445

Blue highlighting indicates a significant difference between SS and LT/D.

Figure 5. APAP: Smooth Estimates & the Associated 95% CIs

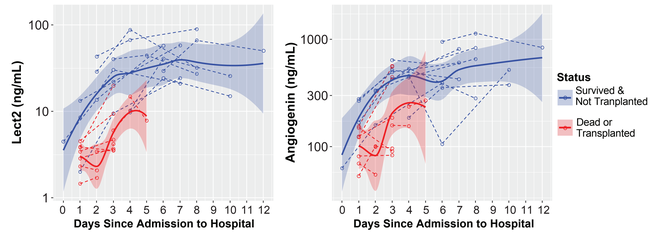
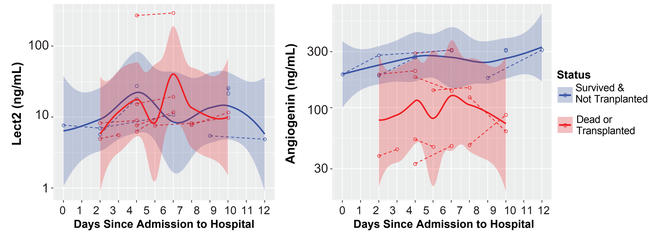


Figure 6. DILI/other: Smooth Estimates & the Associated 95% CIs



Conclusions

- **In ALF, a >2.5-fold increase in circulating angiogenin and a >6-fold increase in circulating LECT2 was seen early after hospital admission in patients who recovered without a liver transplant.**
  - **When analyzed by population, a 2-2.5-fold increase in circulating angiogenin was seen in both APAP patients and DILI/other patients while an increase in LECT-2 was only observed in APAP patients (3.6-fold).**
- **These results suggest angiogenin and LECT2 may function as prognostic indicators of regeneration and could function as biomarkers for the activation of Wnt signaling.**
- **Additional sample analysis and statistics focusing on the first 7 days of hospitalization will be conducted to confirm these findings.**

Presenting author: Maureen Newman, email: maureen@surrozen.com
Surrozen Inc., South San Francisco, CA 94080
www.surrozen.com

Acknowledgement: The authors acknowledge the ALFSG NIDDK grant U-01 DK 058369 to UT Southwestern Medical Center.

References

Tujios, S., Lee, W. Acute liver failure induced by idiosyncratic reaction to drugs: challenges in diagnosis and therapy. Liver International. 2018. 38(1):6-14. DOI: 10.1111/liv.13535. PMID: 28771932.

Apte, U; Singh, S; Zeng, G; Cieply, B; Virji, M; Wu, T; Monga, S. Beta-catenin activation promotes liver regeneration after acetaminophen-induced injury. The American Journal of Pathology. 2009. 175(3):1056-1065. DOI: 10.2353/ajpath.2009.080976. PMID: 19679878.

Bhushan, B; Walesky, C; Manley, M; Gallagher, T; Borude, P; Edwards, G; Monga, S; Apte, U. Pro-regenerative signaling after acetaminophen-induced acute liver injury in mice identified using a novel incremental dose model. The American Journal of Pathology. 2014. 184(11):3013-3025. DOI: 10.1016/j.ajpath.2014.07.019. PMID: 25193591.

Schiet, F; Ostapowicz, G; Murray, N; Satyanarana, R; Zaman, A; Munoz, S; Lee, W. Alpha-fetoproitein and prognosis in acute liver failure. Liver Transplantation. 2006. 12(12):1776-1781. DOI: 10.1002/lt.20886. PMID: 17133565.

Sato, Y.; Watanabe, H.; Kameyama, H.; Kobayashi, T.; Yamamoto, S.; Takeishi, T.; Hirano, K.; Oya, H.; Nakatsuka, H.; Watanabe, T.; Kokai, H.; Yamagoe, S.; Suzuki, K.; Oya, K.; Kojima, K.; Hatakeyama, K. Serum LECT2 level as a prognostic indicator in acute liver failure. Transplantation Proceedings. 2004. 36(8):2359-2361. DOI: 10.1016/j.transproceed.2004.07.007. PMID: 15561249.

SURROZEN