HCC lesions 5 cm in diameter.[3] Based on the above data, we feel it is reasonable to utilize HCC lesions ≤4 cm as the key inclusion criteria for our study for both RFA and cryoablation (CRYO).

For patients with tumor diameters of 3.1 to 4.0 cm, the 1-, 3-, and 5-year tumor-free survival (TFS) rates were 86%, 51%, and 29% in the CRYO group and 84%, 49%, and 30% in the RFA group, respectively. There was no significant difference between these two groups ($P = 0.88$). Consistent with other studies,[2] we did not find that tumor size would significantly affect overall survival (OS) and TFS. But there was a significantly lower local tumor progression (LTP) rate in the CRYO group, compared to that in the RFA group (7 of 91 [7.7%] HCC lesions for cryoablation versus 14 of 77 [18.2%] for RFA, $P = 0.041$).

Regarding the effect of the alternative treatment for those with distant tumor recurrence (110 patients) on OS, Prof. Hyun is correct, in that combination therapy using transarterial chemoembolization (TACE), sorafenib, and local ablation could benefit the OS. Considering OS was influenced by many factors, we chose OS and TFS as the secondary endpoints. We did not collect more detailed information, such as the frequency of TACE or local ablation times, duration of sorafenib treatment, and time for conservative management. So, we did not analyze the effect of retreatments of HCC recurrence and metastasis on OS.

It should be noted that at the time this study was designed, the greatest concern was the procedure-related complications. Thus, it is important to systemically assess the safety of cryoablation through a randomized, controlled trial (RCT) with comparison to RFA. It is also important to evaluate the efficacy of cryoablation for HCC by a RCT, and the LTP has been a standard measure of the primary endpoints. Our study has demonstrated that percutaneous cryoablation is safe and effective and should be one of the standard ablation modalities for HCC.

Chunping Wang, M.Phil.[1]
Ke-Qin Hu, M.D.[2]
Yongping Yang, M.D., Ph.D.[1]
[1]*Center of Therapeutic Research for Hepatocellular Carcinoma the 302nd Hospital Beijing, China*
[2]*Division of GI/Hepatology University of California Irvine Irvine, CA*

References

1. Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma < or =4 cm. Gastroenterology 2004;127:1714-1723.
2. Lee DH, Lee JM, Lee JY, Kim SH, Yoon JH, Kim YJ, et al. Radiofrequency ablation of hepatocellular carcinoma as first-line treatment: long-term results and prognostic factors in 162 patients with cirrhosis. Radiology 2014;270:900-909.
3. Wang C, Lu Y, Chen Y, Feng Y, An L, Wang X, et al. Prognostic factors and recurrence of hepatitis B-related hepatocellular carcinoma after argon-helium cryoablation: a prospective study. Clin Exp Metastasis 2009;26:839-848.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.27713
Potential conflict of interest: Nothing to report.

Serum Alkaline Phosphatase Levels Accurately Reflect Cholestasis in Mice

To the Editor:

Well-characterized preclinical animal models are of utmost importance to explore the complex pathophysiology and novel treatment modalities in thus far difficult-to-treat cholangiopathies, such as primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC).[1,2] However, there is an ongoing discussion regarding the suitability of serum tests to monitor cholestasis in such models. Therefore, we compared serum samples of four different well established mouse models for sclerosing cholangitis, including lithocholic acid (LCA)-fed, 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-fed mice as a model for toxic/chemically induced cholangitis, multidrug resistance protein-2 (Mdr2; Abcb4) knockout mice ($Mdr2^{-/-}$), representing a genetic model with characteristic features of sclerosing cholangitis,[1,2] and common bile-duct-ligated mice (CBDL) in comparison to their respective controls. Aliquots of serum samples collected during harvesting for previous experiments have been kept frozen at −80°C. After 4-fold dilution of sera, alanine aminotransferase (ALT), alkaline phosphatise (ALP), serum bilirubin, and serum bile acids (SBAs) were measured enzymatically on a Cobas 501 analyzer (Roche Diagnostics, Mannheim, Germany). For ALP, the reagent, ALP2 (ALP IFCC Gen.2.), was used according to the recommendations of the International Federation of Clinical Chemistry (IFCC).[3,4] For statistical analyses, mouse models were summarized as toxic/chemically induced (4- and 7-days LCA-fed, and 1-, 4-, and 8-weeks DDC-fed mice), genetically induced (8-weeks- and 4-months-old $Mdr2^{-/-}$ mice) and surgically induced cholestasis (CBDL for 7 days and 6 and 8 weeks) with respective controls for every group. Statistical analysis included Student *t* test and Spearman's correlation coefficient, using IBM SPSS Statistics 21 (IBM Corp., Armonk, NY). A *P* value <0.05 was considered significant. Serum parameters for each experimental group are reported as arithmetic means ± standard deviation (Table 1). With the exception of the DDC- and LCA-fed group, where, in general, high standard deviations for serum liver tests were observed, ALP levels significantly correlated with SBA levels, which are highly specific and sensitive for cholestasis. We herein show that ALP is a sensitive parameter to accurately monitor cholestasis in mouse models of chemically, genetically, and surgically induced cholestatic liver injury. However, there is a wide variation in the degree of cholestasis, which is also reflected by variations in SBA levels, which follow closely those of ALP serum levels. Given that bile acid derivatives are increasingly tested as potential therapeutic agents in mouse models of cholestatic liver diseases, measurement of ALP might be seen as advantageous, because it is unlikely to be confounded by diet or treatment. In conclusion, ALP represents a specific marker to accurately assess cholestasis in mice.

Acknowledgment: The authors thank Dr. Herbert Schwetz for helping with statistical analysis and Judith Gumhold for excellent technical assistance.

Table 1. Serum Parameters of Mouse Models for Cholestasis

	Laboratory Parameters			
	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dL)	SBA (μmol/L)
Toxic/chemically induced cholestasis				
Controls (n = 4)	20 ± 9	113 ± 49	0.07 ± 0.04	5 ± 2
DDC feeding 1w (n = 3)	176 ± 121 (*P* = 0.155)	628 ± 594 (*P* = 0.272)	0.17 ± 0.13 (*P* = 0.297)	43 ± 30 (*P* = 0.160)
DDC feeding 4w (n = 4)	344 ± 319 (*P* = 0.135)	807 ± 382 (*P* = 0.011)*	0.35 ± 0.17 (*P* = 0.020)*	76 ± 45 (*P* = 0.052)
DDC feeding 8w (n = 4)	360 ± 423 (*P* = 0.206)	660 ± 662 (*P* = 0.197)	0.12 ± 0.06 (*P* = 0.194)	140 ± 115 (*P* = 0.100)
LCA feeding 4d (n = 3)	1,001 ± 796 (*P* = 0.051)	613 ± 317 (*P* = 0.109)	0.80 ± 0.67 (*P* = 0.198)	200 ± 144 (*P* = 0.143)
LCA feeding 7d (n = 4)	683 ± 532 (*P* = 0.088)	481 ± 249 (*P* = 0.027)*	0.20 ± 0.22 (*P* = 0.287)	82 ± 42 (*P* = 0.010)*
Genetically induced cholestasis				
Mdr2 WT 8w (n = 4)	64 ± 9	114 ± 5	0.10 ± 0.05	9.5 ± 1.8
Mdr2 KO 8w (n = 4)	452 ± 163 (*P* = 0.017)*	235 ± 81 (*P* = 0.058)	0.14 ± 0.05 (*P* = 0.315)	64 ± 44 (*P* = 0.087)
Mdr2 WT 4m (n = 4)	76 ± 13	73 ± 7	0.04 ± 0.03	11 ± 5
Mdr2 KO 4m (n = 4)	357 ± 139 (*P* = 0.007)*	196 ± 76 (*P* = 0.047)*	0.16 ± 0.06 (*P* = 0.010)*	41 ± 36 (*P* = 0.152)
Surgically induced cholestasis				
SOP 7d (n = 4)	20 ± 9	20 ± 24	0.03 ± 0.04	7 ± 6
CBDL 7d (n = 4)	497 ± 256 (*P* = 0.010)*	337 ± 72 (*P* = 0.000)*	8.0 ± 1.4 (*P* = 0.002)*	583 ± 117 (*P* = 0.000)*
SOP 6w (n = 4)	43 ± 4	79 ± 11	0.08 ± 0.03	13 ± 6
CBDL 6w (n = 4)	306 ± 397 (*P* = 0.277)	1,083 ± 668 (*P* = 0.057)	9.0 ± 8.2 (*P* = 0.116)	462 ± 349 (*P* = 0.042)*
SOP 8w (n = 3)	48 ± 14	76 ± 11	0.04 ± 0.07	7 ± 3
CBDL 8w (n = 4)	119 ± 80 (*P* = 0.199)	1,374 ± 824 (*P* = 0.045)*	12 ± 8 (*P* = 0.054)	845 ± 692 (*P* = 0.096)

	Correlation Coefficients		
	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dL)
All groups			
SBA, μmol/L	0.77†	0.85†	0.78†
Toxic/chemically induced cholestasis			
SBA, μmol/L	0.59†	0.43	0.42
Genetically induced cholestasis			
SBA, μmol/L	0.89†	0.95†	0.26
Surgically induced cholestasis			
SBA, μmol/L	0.12	0.73†	0.41

Laboratory parameters are expressed means ± standard deviation; correlation coefficient according to Spearman for all groups (including control groups) and each experimental group (without respective controls).

*$P < 0.05$, statistically significant difference between intervention and corresponding control group.

†$P < 0.01$, statistically significant correlation between respective serum parameters.

Abbreviations: WT, wild type; KO, knockout; SOP, sham operating procedure; d, days; w, weeks; m, months.

Elisabeth Krones, M.D.[1]
Wolfgang Erwa, M.D.[3]
Michael Trauner, M.D.[4]
Peter Fickert, M.D.[1,2]
[1]*Research Unit for Experimental and Molecular Hepatology*
Division of Gastroenterology and Hepatology
Department of Internal Medicine
Medical University of Graz
Graz, Austria
[2]*Department of Pathology*
Medical University of Graz
Graz, Austria
[3]*Clinical Institute of Medical and Chemical Laboratory Diagnostics*
Medical University of Graz
Graz, Austria
[4]*Hans Popper Laboratory of Molecular Hepatology*
Division of Gastroenterology and Hepatology
Department of Internal Medicine III
Medical University of Vienna
Vienna, Austria

References

1. Pollheimer MJ, Trauner M, Fickert P. Will we ever model PSC?-"it's hard to be a PSC model!". Clin Res Hepatol Gastroenterol 2011;35: 792-804.
2. Fickert P, Pollheimer MJ, Beuers U, Lackner C, Hirschfield G, Housset C, et al. Characterization of animal models for primary sclerosing cholangitis (PSC). J Hepatol 2014;60:1290-1303.
3. Tietz NW, Rinker AD, Burtis C, Duncan P, Ervin K, Ewen L, et al. Transferability studies for the AACC reference method and the IFCC method for measurement of alkaline phosphatase activity. Clin Chem 1984;30:704-706.
4. Duncan PH, McKneally SS, MacNeil ML, Fast DM, Bayse DD. Development of a reference material for alkaline phosphatase. Clin Chem 1984;30:93-97.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.27622
This work was supported by a grant (P24809-B19) from the Austrian Science Foundation (to P.F.).

All experiments have been approved by the local animal care and use committees according to criteria outlined by the National Academy of Sciences (BMWF-66.010/0045-II/10b/2010 and GZ-BMWF-66. 010/0012-II/3b/2014).

Potential conflict of interest: Prof. Trauner consults for, advises, is on the speakers' bureau of, and received grants from Falk. He consults for and advises Albireo, Genfit, Intercept, and Phenex. He is on the speakers' bureaus of Gilead, MSD, and Roche. He received grants from Intercept and Albireo.

Evidence Against a Role of Serotonin in Liver Regeneration in Humans

To the Editor:

We read with great interest the article by Starlinger et al., in which evidence for a role of serotonin in liver regeneration in humans was provided.[1] We would like to report on our findings in a prospective study on serotonin levels in platelet-rich plasma in adult patients undergoing a (extended) right hemihepatectomy (n = 16) in comparison to levels in patients undergoing a pylorus-preserving pancreaticoduodenectomy (PPPD; n = 10) and healthy controls (n = 22). Patient characteristics were published elsewhere.[2] We drew blood samples after induction of anesthesia, at the end of the surgery, and at postoperative days 1, 3, 5, 7, and 30. In addition, we took blood samples from the portal and from the hepatic vein just before the start and just after completion of parenchymal transection in the patients undergoing a hemihepatectomy. Serotonin levels in platelet-rich plasma were determined by liquid chromatography/tandem mass spectrometry, and levels were corrected for platelet count. The study protocol was approved by the local medical ethical committee, and informed consent was obtained from each participant before inclusion in the study.

Serotonin levels at baseline were comparable between patients undergoing hemihepatectomy, patients undergoing PPPD, and healthy subjects (Fig. 1). In contrast to the Starlinger et al. study, no changes in serotonin were observed in the early postoperative period. Only at postoperative days 5 and 7 had serotonin levels clearly decreased; but, importantly, the decrease was similar between the hemihepatectomy and PPPD patients. Serotonin content was identical between samples taken in the afferent and efferent liver veins before and after hemihepatectomy, indicating that there was no detectable serotonin consumption by the liver directly after hemihepatectomy. Although the number of patients we studied was smaller compared to the Starlinger study, we studied more time points in a more homogeneous cohort consisting of patients without cirrhosis undergoing a major hepatectomy, included an appropriate control group, and studied the serotonin gradient over the liver before and just after hemihepatectomy. Technical differences between the studies included measurement of serotonin in platelet-rich plasma versus a calculated serum-platelet–poor plasma difference, as studied by Starlinger et al. Importantly, we calculated serotonin content per platelet, thereby correcting for consumption of platelets as a result of dilution or consumption.

Although we do not dispute that platelets are likely important for liver regeneration in humans,[3] our data do not support the notion that platelet serotonin is key in this process.

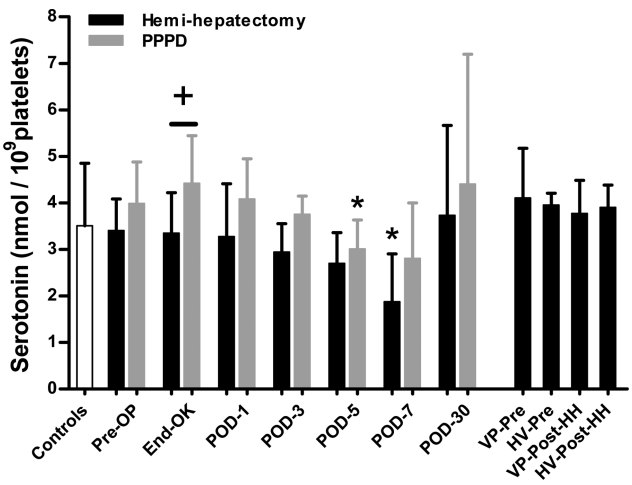

Fig. 1. Median serotonin levels in controls, patients undergoing hemihepatectomy, and patients undergoing PPPD. Shown are serotonin levels corrected for platelet count. $*P < 0.05$ versus pre-op (Friedman's test). $+P < 0.05$, hemihepatectomy versus PPPD (Mann-Whitney's U test). Abbreviations: End-OK, end of surgery; HH, hemihepatectomy; HV, hepatic vein; POD, postoperative day; Pre-OP, preoperative; VP, vena porta.

Edris M. Alkozai, M.D.[1]
Martijn van Faassen, B.A.Sc.[2]
Ido P. Kema, Ph.D.[2]
Robert J. Porte, M.D., Ph.D.[3]
Ton Lisman, Ph.D.[1,3]
[1]*Surgical Research Laboratory*
Department of Surgery
University of Groningen
University Medical Center Groningen
Groningen, The Netherlands
[2]*Department of Laboratory Medicine*
University of Groningen
University Medical Center Groningen
Groningen, The Netherlands
[3]*Section of Hepatobiliary Surgery and Liver Transplantation*
Department of Surgery
University of Groningen
University Medical Center Groningen
Groningen, The Netherlands

References

1. Starlinger P, Assinger A, Haegele S, Wanek D, Zikeli S, Schauer D, et al. Evidence for serotonin as a relevant inducer of liver regeneration after liver resection in humans. Hepatology 2014;60:257-266.
2. **Potze W, Alkozai EM,** Adelmeijer J, Porte RJ, Lisman T. Hypercoagulability following major partial liver resection—detected by thrombomodulin-modified thrombin generation testing. Aliment Pharmacol Ther 2015;41:189-198.
3. Alkozai EM, Nijsten MW, de Jong KP, de Boer MT, Peeters PM, Slooff MJ, et al. Immediate postoperative low platelet count is associated with delayed liver function recovery after partial liver resection. Ann Surg 2010;251:300-306.

Author names in bold designate shared co-first authorship.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.27673
This study was sponsored, in part, by a Mozaiek grant from the Dutch Organization of Scientific Research awarded to Edris Alkozai.
Potential conflict of interest: Nothing to report.