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ORIGINAL RESEARCH—CLINICAL

The Ratio of Activin A and Follistatin-Like 3 Is Associated With Posthepatectomy Liver Failure and Morbidity in Patients Undergoing Liver Resection

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BACKGROUND AND AIMS: Activin A is a key regulator in liver regeneration, but data evaluating its role in humans after hepatic surgery are limited. In this study we explore the predictive role of circulating activin A, its antagonist follistatin-like 3 (FSTL-3), and their ratio for posthepatectomy liver failure (PHLF) and monitor their levels after surgery, to evaluate their role in human liver regeneration. **METHODS:** Activin A and FSTL-3 levels were assessed in 59 patients undergoing liver surgery. Using receiver operating characteristic analysis, we evaluated the predictive potential of activin A, FSTL-3, and their ratio. **RESULTS:** While perioperative dynamics of activin A and FSTL3 were significantly affected by hepatic resection (activin A $P = .045$, FSTL-3 $P = .005$), their functionally relevant ratio did not significantly change ($P = .528$). Neither activin A nor FSTL-3 alone but only their ratio exhibited a significant predictive potential for PHLF (area under the curve: 0.789, $P = .038$). Patients with low preoperative activin A/FSTL-3 ratio were found to more frequently suffer from PHLF (0.017) and morbidity (0.005). **CONCLUSION:** Activin A/FSTL-3 ratio predicts PHLF and morbidity. Its significance in preoperative patient assessment needs to be further validated in larger, independent cohorts.

Keywords: Activin A; Follistatin-like 3; Liver surgery; Posthepatectomy liver failure; Postoperative morbidity

Introduction

Curative treatment of liver tumors is highly dependent on successful resection, but immediate postoperative and long-term outcome hinges on the ability of the liver to retain its physiological functions.[1] Prior to surgery risk assessment via liver function assessment tests like indocyanine green (ICG) clearance and evaluation of blood-derived scores like Child-Turcotte-Pugh, Albumin-Bilirubin score, aspartate aminotransferase-to-platelet ratio index or Model for End-Stage Liver Disease (MELD) are frequently applied.[2] Particularly, the summative combination of aminotransferase-to-platelet ratio index and Albumin-Bilirubin score showed highest predictive potential for postoperative outcome.[3,4] But even with patients stratified for underlying liver diseases, the incidence of posthepatectomy liver failure (PHLF) vastly varies, with an incidence of 5%–15% being reported in the literature.[5]

Liver regeneration following liver surgery is a complex process that revolves around quiescent hepatocytes reentering the cell cycle and after proliferation returning to the G0 phase during the termination of liver regeneration.[6] Activin A is a member of the transforming growth factor β superfamily and is a dimeric polypeptide. It binds to the type II and I activin receptor and recruits mothers against decapentaplegic homolog (SMAD) 2 and 3, which, through multimerization with SMAD 4, translocate to the nucleus and modulate the expression of a plethora of genes.[7] Activin A can further activate other intracellular pathways, like p38 mitogen–activated protein kinases, extracellular signal-

Abbreviations used in this paper: **AUC, area under the curve; EKR, extracellular signal-regulated kinase; FDTL-3, follistatin-like 3; HCC, hepatocellular carcinoma; ICG, indocyanine green; MELD, Model for End-Stage Liver Disease; NPV, negative predictive value; PDR, plasma disappearance rate; PHLF, posthepatectomy liver failure; pHx, partial hepatectomy; POD, postoperative day; preOP, prior to the operation; PT, prothrombin time; ROC, receiver operating characteristic; SB, serum bilirubin.**

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regulated kinases 1 and 2 (ERK1/2), and c- Jun NH2-terminal kinase depending on cell type and physiological circumstances.[8,9] Activin A is linked to a plethora of different physiological functions. It influences reproductive physiology in a SMAD coindependent or independent manner, and has a regulatory role in inflammation, regeneration and apoptosis in various organ systems.[10] In the liver activin A is mainly expressed by hepatocytes.[11] It regulates, through SMAD activation, liver mass and initiates termination of cell division after the proliferative phase of liver regeneration.[11,12] Activin A has been extensively investigated in the context of chronic liver disease. Fibrotic and cirrhotic rat livers show enhanced activin A expression[13] and activin A concentrations are elevated in patients suffering from acute liver failure and nonalcoholic fatty liver disease.[14,15] However, only very limited evidence has been generated regarding the relevance of activin A in liver regeneration. Partial hepatectomy (pHx) in rats leads to downregulation of activin A expression. At 24 hours post pHx activin A expression is maximally decreased and only increases 168 hours post pHx.[16]

Follistatin-like 3 (FSTL-3) is an antagonistic protein to activin A. FSTL-3 is encoded by the follistatin related gene and binds activin A, making it impossible for activin A to interact with its receptors.[16,17] Upregulation of activin inhibition can be seen 24–48 hours after liver surgery.[16] Infusion of activin A antagonists into rats right after hepatectomy accelerates liver regeneration, while treatment with activin A produces the opposite effect.[18] Rats, who are treated with activin A inhibitors, show an immediate onset of DNA synthesis after pHx, in comparison to activin A–treated rats.[19] But when comparing liver architecture in both cohorts, activin A–treated rats returned to physiological liver architecture after 120 hours, while rats with inhibited activin A failed to do so at this time point. Also, mice where activin A was antagonized had elevated serum bilirubin (SB) concentrations and lower glucose levels. This indicates that increased regeneration through blockage of activin A may not be beneficial.[20]

The aim of this study was to explore perioperative dynamics of circulating activin A and its inhibitor FSTL-3 in humans undergoing liver resection and to examine their predictive potential for PHLF and morbidity.

Patients and methods

Study cohort

Between March 2013 and March 2018, 59 patients underwent liver surgery at the clinic Landstrasse in Vienna. Patient data were prospectively maintained in a biobank and used for retrospective analyses. The study was approved by the institutional ethics committee (# EK 16-253-0117) and all patients gave a written informed consent. Exclusion criteria included pregnancy, age less than 18 years, and decompensated liver cirrhosis.

Plasma preparation

Blood samples were collected prior to the operation (preOP) and on the first and the fifth postoperative day (POD). Blood was then used for optimized plasma preparation, as described earlier,[21,22] and stored at −80 °C.

Determination of activin A and FSTL-3 concentrations

Activin A and FSTL-3 were analyzed with enzyme-linked immunosorbent assay using commercially available enzyme-linked immunosorbent assay kits (Quantikine; R&D Systems, Minneapolis, Minnesota) as per the manufacturer's instructions.

Quantification of routine blood parameters

Concentrations for SB, prothrombin time (PT), platelet counts, and alanine aminotransferase were measured in appropriate samples by routine laboratory blood tests.

Assessment of liver function

Perioperative liver function was evaluated by ICG-clearance testing as previously described.[23] Briefly, pulse spectrometry was used to quantify the patient's blood ICG concentration. Particularly, a dose of 25 mg dye was dissolved in 20 mL of distilled water, immediately before injection. The injected amount was adjusted to the body weight ratio of the patient (0.25 mg/kg). The 2 parameters, plasma disappearance rate (PDR) and retention 15 min after administration (R15), were recorded with a Limon device (Pulsion Medical System SE, Germany) and automatically calculated in accordance with the time course of blood ICG concentration.

Definition and classification of PHLF and morbidity

PHLF was defined by the criteria put forth by the international study group on liver surgery.[24] Patients were classified as suffering from PHLF, when elevated levels of SB and prolonged PT occurred on or after POD5. If these parameters differed from the normal range preoperatively, the parameters had to be deranged on 2 consecutive days after POD5. Of note, all patients had to have normalized SB and PT prior to discharge and showing good clinical performance, to be classified as no PHLF.

Morbidity after surgery was defined with the classification established by Dindo et al.[25] The severity of morbidity was then further categorized into grade I–V. If more than one complication was recorded, the more severe complication was documented. Postoperative mortality was defined as death within 90 days after surgery.

Statistical analysis

All analyses are performed using data from subjects with valid marker values only. All statistical tests were carried out on SPSS software (SPSS, Inc., Chicago, Illinois, Version 27). Analysis was based on nonparametric tests to compare related or independent samples (Mann–Whitney U test, Wilcoxon signed rank test, and Kruskal-Wallis test). To compare the predictive potential for PHLF of the activin A/FSTL-3 ratio to routine blood parameters and clinically established liver function assessment tests, receiver operating characteristic (ROC) curves for activin A/FSTL-3 ratio, PDR, R15, SB, PT, and platelets were plotted. To identify a cut-off for the ratio of

Table. Patient Demographics

Cohort (N = 59)

Parameter	Median (IQR)/N (%)
Age (y)	64.69 (53.13–71.71)
Sex	
Male	34 (57.6)
Female	25 (42.4)
Tumor entity	
HCC	24 (40.7)
CCC	22 (37.3)
Benign tumor	13 (22)
Hemangioma	5 (8.3)
Hepatocellular adenoma	3 (5)
Focal nodular hyperplasia	3 (5)
Liver cyst	2 (3.3)
Hepatic resection	
Minor	15 (25.4)
Major	44 (74.6)
Histology	
Fibrosis	32 (54.2)
Fibrosis grade	1 (0–4)
Steatosis (%)	5.00 (0.00–25.00)
Steatohepatitis	25 (41.7)
Morbidity	
No morbidity	29 (49.2)
I	7 (11.9)
II	15 (25.4)
IIIa	4 (6.8)
IIIb	2 (3.4)
IVa	0
IVb	0
V	2 (3.4)
PHLF	
No PHLF	52 (88.1)
PHLF total	7 (11.7)
ISGLS A	2 (3.4)
ISGLS B	4 (6.8)
ISGLS C	1 (1.7)
Preoperative parameters	
MELD-Na preop	6.7 (6.3–9.0)
PDR in %/min	21 (17–24.8)
R15 in %	4.7 (2–8.7)
SB in mg/dL	0.56 (0.12–0.90)
PT in %	96.50 (86.25–109.75)
AP in U/L	93.50 (66.50–132.50)
AST in U/L	37.45 (26.25–69.25)
ALT in U/L	32.30 (17.00–56.00)
GGT in U/L	117.00 (58.00–258.00)
Albumin in g/L	43.95 (40.50–46.18)
Platelets in G/L	237.00 (188.50–280.00)

IQR, interquartile range; HCC, hepatocellular carcinoma; CCC, cholangiocellular carcinoma; PHLF, posthepatectomy liver failure; ISGLS, international study group on liver surgery; MELD, model for end-stage liver disease; Na, sodium; PDR, plasma diseappearance rate; R15, retention 15 min after administration; SB, serum bilirubin; PT, prothrombin time; AP, alcalic phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase.

activin A and FSTL-3, distinguishing our cohort into high-risk and low-risk groups for PHLF and postoperative morbidity, we used Youden J statistic. As our analysis was exploratory, reported P values are unadjusted and P values $< .05$ were considered statistically significant.

Results

Patients and cohorts

In total, this study included 59 patients who were retrospectively included out of a prospectively maintained biobank. Primary liver cancer patients were included (N = 46, 24 hepatocellular carcinoma [HCC], 22 cholangiocellular carcinoma) and benign liver tumors as noncancer controls (N = 13). Baseline characteristics of patients are given in Table.

Activin A and FSTL-3 levels decreased after surgery

Circulating activin A and FSTL-3 both showed a significant decrease from preOP to POD1 (Figure 1A and B) ($P = .045$, $P = .005$). FSTL-3 levels also show a significant increase from POD1 to POD5 (Figure 1B) ($P = .004$) and returned to concentrations comparable to preOP ($P = .167$). There was no significant change in activin A concentrations from POD1 to POD5 ($P = .174$) and activin A preOP and POD5 levels were also comparable ($P = .668$). FSTL-3 is known to be an important binding protein of circulating activin A[10] and the ratio of activin A and FSTL-3 has been evaluated as a predictive parameter in studies of acute liver failure due to paracetamol overdose or hepatitis-related liver injury.[14] In this context, no significant changes in the ratio of activin A and FSTL-3 from preOP to POD1 ($P = .528$), from POD1 to POD5 ($P = .954$), or from preOP to POD5 ($P = .797$) could be recorded (Figure 1C).

The ratio of activin A to FSTL-3 was significantly lower in patients suffering from PHLF

Given the pathophysiological relevance of activin A and FSTL-3 during liver regeneration, we further aimed to explore if patients with PHLF would differ in their perioperative time course (Figure 2A and B). While activin A levels tended to be lower in patients without PHLF, no statistically significant difference could be observed preOP, on POD1, or on POD5 between patient groups (preOP $P = .067$, POD1 $P = .131$, POD5 $P = .600$). FSTL-3 also did not show any significant difference on any of the 3 time points (preOP $P = 1.000$, POD1 $P = .702$, POD5 $P = .608$). However, activin A concentrations decreased from preOP to POD1 in patients who did not develop PHLF ($P = .005$). FSTL-3 also decreased from preOP to POD1 exclusively in no-PHLF patients ($P = .002$). FSTL-3 then rose from POD1 to POD5 ($P = .005$) (Figure 2A and B). As the ratio of activin A/FSTL-3 reflects the active fraction of activin A, we examined differences in our patients in the ratio of activin A and FSTL-3 (Figure 2C). Preoperatively, the ratio of activin A/FSTL-3 was significantly lower in patients who developed postoperative PHLF (Figure 2A) ($P = .037$). At the other time

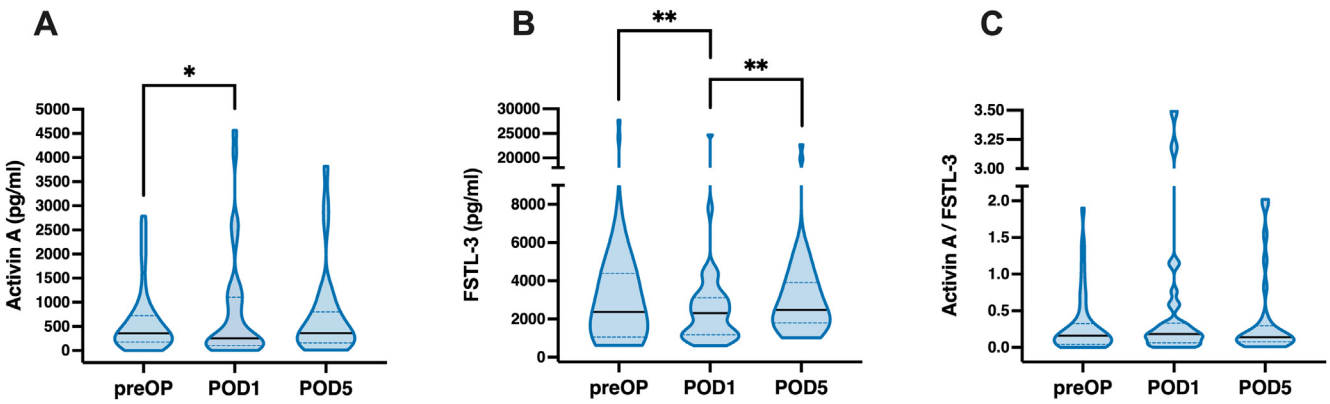

Figure 1. Perioperative activin A and FSTL-3 concentrations, Violin plots of the perioperative levels of activin A (A) and FSTL-3 (B) and activin A/FSTL-3 (C). *$P < .05$, **$P < .00$.

points, no statistically significant differences were observed ($P = .316$, $P = .483$).

Activin A and FSTL-3 levels showed no difference between patients with and without postoperative morbidity

Subsequently, we evaluated levels of activin A and FSTL-3 in the context of postoperative morbidity (Figure 2D and E). There were no differences in activin A concentrations at any time point (preOP $P = .149$, POD1 $P = .746$, POD5 $P = 1.000$). Similarly, FSTL-3 showed no differences between cohorts on any time point (preOP $P = .949$, POD1 $P = .705$, POD5 $P = .699$). When examining perioperative dynamics, activin A decreased from preOP to POD1 (Figure 2D) but only in patients who did not develop postoperative morbidity ($P = .047$). FSTL-3 also decreased from preOP to POD1 (Figure 2E), but in contrast to activin A, this dynamic could only be seen in patients who suffered from postoperative morbidity ($P = .042$). FSTL-3 then rose in patients with postoperative morbidity (Figure 2E) ($P = .019$). When exploring differences of activin A/FSTL-3 ratio in postoperative morbidity, values did not show any differences between time points (Figure 2F), only preOP ratio values appeared to be lower in patients who suffered from postoperative morbidity (preOP $P = .093$, POD1 $P = .232$, POD5 $P = .821$).

Activin A, FSTL-3, and activin A/FSTL-3 did not differ between malign or benign liver tumors and groups of high-grade and low-grade fibrosis

To further explore our findings in the context of the underlying liver pathology, we compared preoperative activin A and FSTL-3 and their ratio with regards to indication for surgery (HCC vs cholangiocellular carcinoma vs benign liver tumors) (Figure 3A–C). Activin A did not differ between tumors ($P = .537$). FSTL-3 also did not differ between different indications ($P = .149$). When looking at the ratio of activin A and FSTL-3, no significant differences could be observed ($P = .430$). We further aimed to characterize activin A, FSTL-3, and their ratio in patients with respect to underlying liver fibrosis grade. We did not see any difference in measured activin A and FSTL-3 or their ratio, when comparing different fibrosis grades separately (data not shown). Next, a low-grade group of patients with no or grade 1 fibrosis and a high-grade group with fibrosis grades 2–4 were compared during the perioperative time course (Figure 3D–F). There was no significant difference between these 2 groups preoperatively (activin A preOP $P = .806$, FSTL-3 preOP $P = .732$, activin A/FSTL-3 preOP $P = .891$) (Figure 4D–F). Values also did not differ on POD1 or POD5 (activin A POD1 $P = .142$, FSTL-3 POD1 $P = .198$, activin A/FSTL-3 POD1 $P = .113$) (activin A POD5 $P = .755$, FSTL-3 POD5 $P = .204$, activin A/FSTL-3 POD5 $P = .932$) (Figure 4D–F). We then evaluated changes over time in concentrations of activin A and FSTL-3 and their ratio (Figure 4 D–F). Activin A levels in patients with low-grade fibrosis rose from preOP to POD1 ($P = .047$) but did not show any other significant concentration changes. Patients with high-grade fibrosis did not differ in activin A concentrations over time. When looking at FSTL-3 concentrations, patients with low-grade fibrosis showed no difference in measured FSTL-3 from preOP to POD1 ($P = .099$), while patients with high-grade fibrosis showed a significant decline in FSTL-3 levels from preOP to POD1 ($P = .047$). Patients with high-grade fibrosis also showed a significant increase in FSTL-3 from POD1 to POD5 ($P = .027$). There was no difference in perioperative activin A and activin A/FSTL-3 ratio levels.

Preoperative activin/FSTL-3 was predictive for postoperative PHLF and morbidity

Using ROC analysis, we aimed to further investigate the predictive potential for PHLF of the preoperative activin A/FSTL-3 ratio (Figure 4A). The activin A/FSTL-3 ratio was able to significantly predict postoperative PHLF (area under the curve [AUC] = 0.789, $P = .038$, 95% CI: 0.609–0.969). Furthermore, the activin A/FSTL-3 ratio

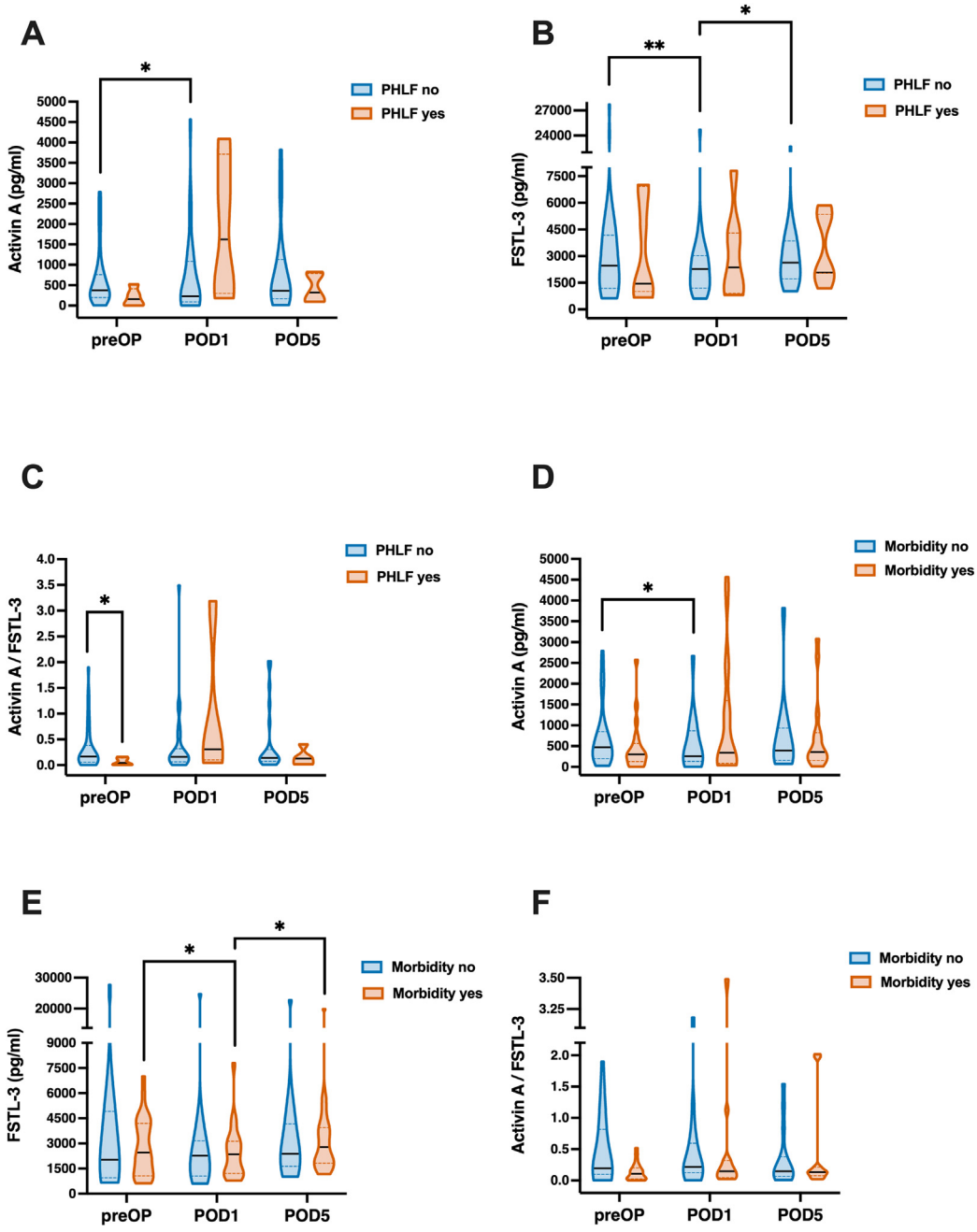

Figure 2. Perioperative activin A and FSTL-3 concentrations, outcome subgroups, Violin plots of activin A (A and D) and FSTL-3 (B and E) concentrations and Activin/FSTL-3 values (C and F) on all perioperative time points (preop, POD1, POD5) in accordance to status of PHLF and morbidity. *$P < .05$, **$P < .005$.

was compared to already established parameters for liver function assessment such as routine blood parameters and ICG clearance (Figure 4B and C) (PDR AUC = 0.531, P = .823, 95% CI: 0.273–0.788, R15 AUC = 0.614, P = .408, 95% CI: 0.336–0.893), preOP SB (Figure 4D) (AUC = 0.552, P = .669, 95% CI: 0.345–0.759), preOP PT (Figure 4E) (AUC = 0.556, P = .663, 95% CI: 0.258–0.855), and preOP platelet count (Figure 4F) (AUC = 506, P = .959, 95% CI: 0.290–0.722) in our cohort. We further divided the cohort in a high and low activin A/FSTL-3 ratio group using a cut-off of 0.16 pg/mL (Figure 5). When comparing the 2 groups, we could identify all patients with postoperative PHLF in the low activin A/FSTL-3 ratio group, with none of the patients in the high activin A/FSTL-3 ratio group developing PHLF (5 of 21 [23.8%] vs 0 of 21 [0.0%], P = .017, Sensitivity = 100%, Specificity = 57%, positive predictive value = 24%, negative predictive value [NPV] = 100%). Furthermore, patients in the low activin A/FSTL-3 ratio group were found to suffer from a significantly higher risk for postoperative morbidity (14 of

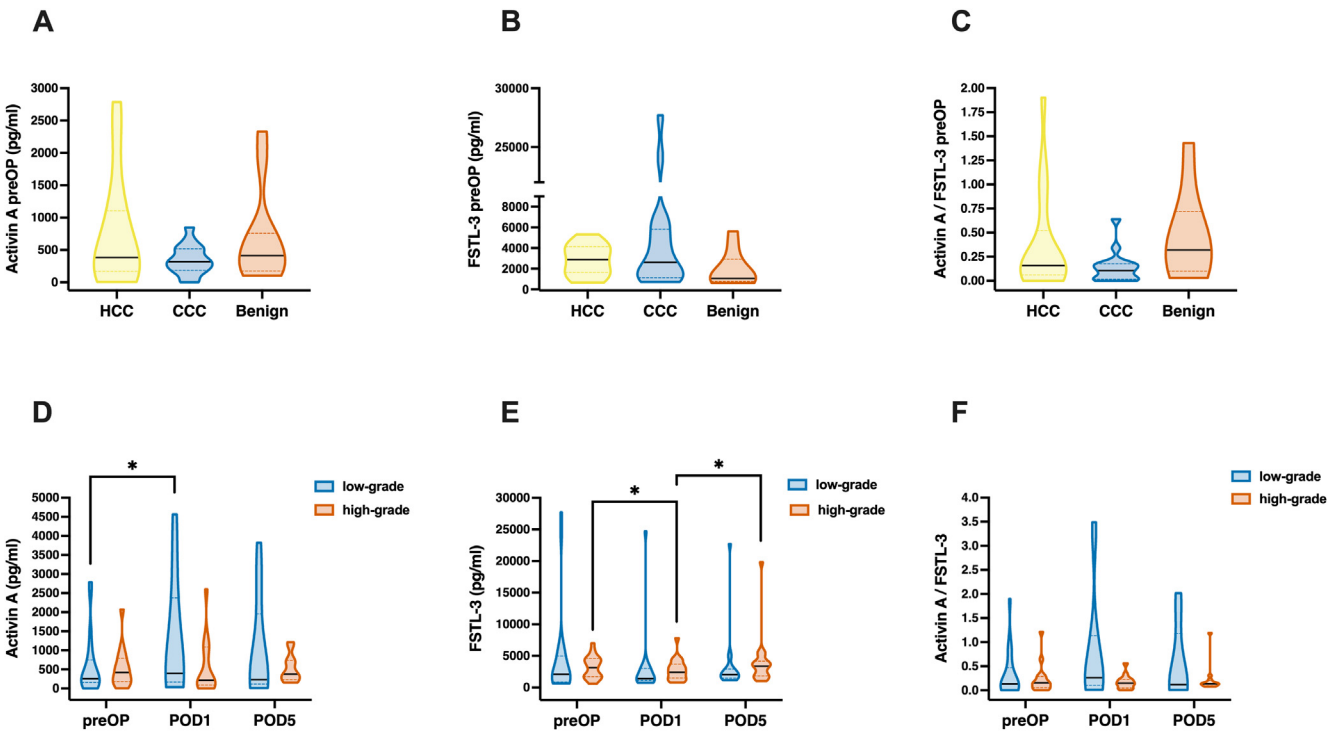

Figure 3. Perioperative activin A and FSTL-3 concentrations, grouped by tumor type and fibrosis grade, Violin plots of the concentrations of activin A (A), FSTL-3 (B), and activin A/FSTL-3 (C) grouped around the underlying malign or benign liver entity. Violin plots of activin A (D) and FSTL-3 (E) levels and activin A/FSTL-3 ratio (F) values of patients grouped into low-grade (Fibrosis grade 0–1) and high-grade (Fibrosis grade 2–4). Both cohorts are compared over the perioperative time-course. *$P <$ .05, **$P <$ .005.

21 [66.7%] vs 5 of 21 [23.8%], $P = .005$, Specificity = 40%, Sensitivity = 74%, positive predictive value = 67%, NPV = 76%).

Discussion

While the role of activin A and its inhibition has been extensively assessed in chronic liver disease and regeneration in experimental models, the relevance of this tightly regulated system in human liver regeneration still needs to be elucidated. Here, we provide evidence that the ratio of activin A and its inhibitor FSTL-3 are associated with postoperative PHLF and postoperative morbidity outperforming established liver function assessment tests, with no observable difference regarding patients with or without chronic liver disease and among different tumor entities.

In the liver, activin A is produced mainly in hepatocytes, with an additional minor source of activin A coming from mast cells.[11] When activin A is blocked by its antagonists such as FSTL-3, either by infusion through the portal vein or adenovirus-mediated overexpression, DNA synthesis is stimulated and the liver increases in size.[12,26] This means that activin A functions as a regulator of proliferation and organ size in the liver. In rodents after pHx, activin A mRNA expression in the liver decreases.[16] In line with these results, we found a decrease of circulating activin A levels on POD1. In intact livers antagonizing activin A initiates DNA replication and increases liver weight after 48 hours.[26] FSTL-3 is one of many Follistatin-related proteins but FSTL-3 being the only Follistatin-related protein which exhibits activin binding properties.[27] There are limited data available regarding the perioperative time course of activin A, FSTL-3, and their ratio in human liver regeneration. We report a postoperative decrease and subsequent rise from POD1 to POD5 in FSTL-3 concentrations in human liver regeneration. This is of particular interest as the ratio of these 2 proteins, reflecting the functionally available activin A, did not differ significantly during the observed time frame. While it is likely that other factors influence the complex functional homeostasis of activin A, this nicely illustrates that singular assessment of activin A is probably insufficient to reflect its functional state. Our exploratory data would suggest that inhibitors of activin A also dynamically change perioperatively and might thereby significantly affect biological activity.

Activin A is overexpressed in cirrhotic and fibrotic rat livers. In humans, increased activin A concentrations are observed in patients with alcoholic cirrhosis, nonalcoholic steatohepatitis, viral hepatitis, or patients suffering from acute liver failure.[15,28–30] Regarding the underlying mechanisms by which activin A influences fibrosis and cirrhosis, Sugiyama et al could show that activin A expression is high in fibrotic areas of the liver and that activin A promotes type

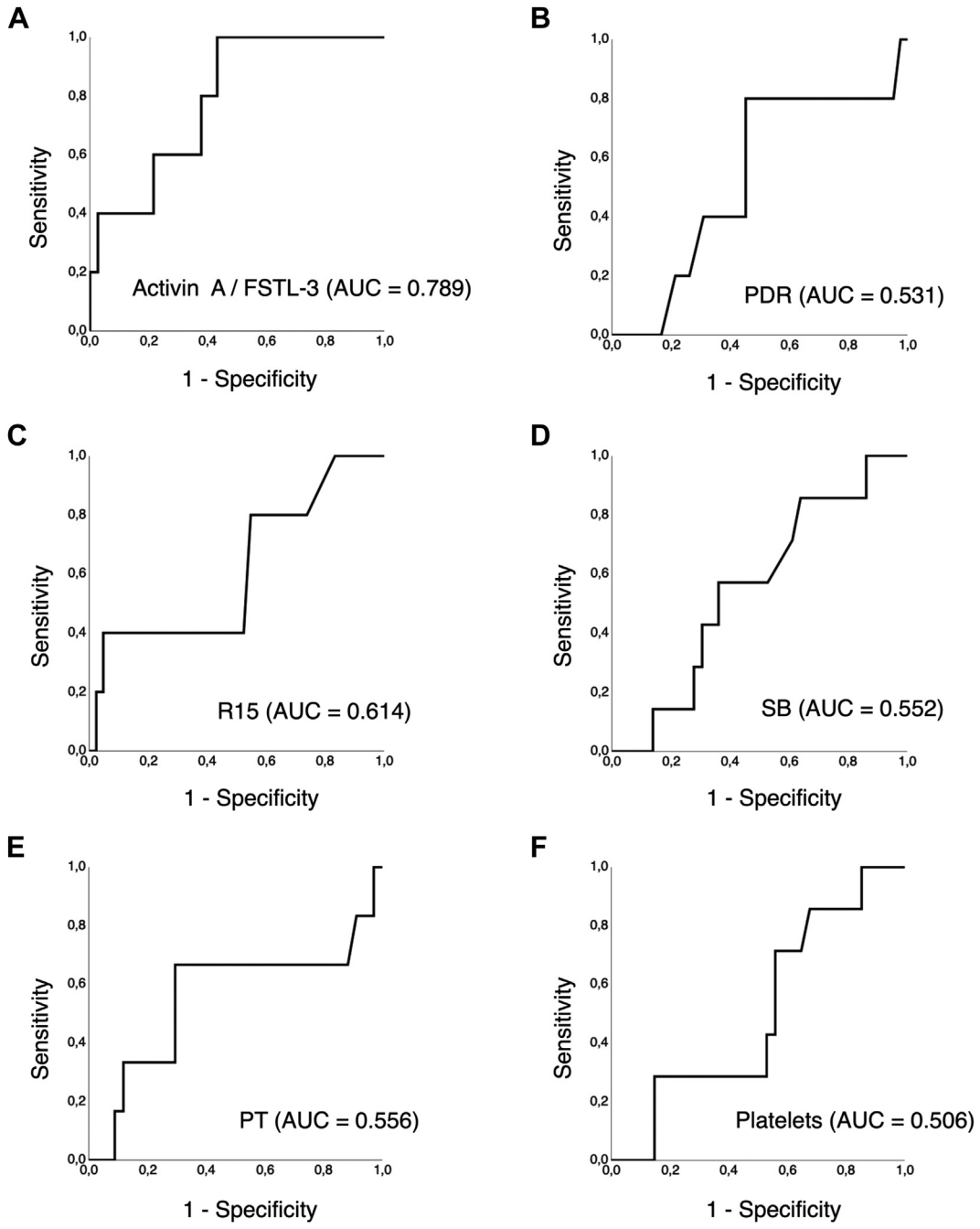

Figure 4. Comparison of ROC curves for the discriminatory potential for posthepatectomy liver failure, ROC statistics for the predictive values of preop activin A/FSTL-3 ratio (A), preOP PDR (B), preOP R15 (C), preOP SB (D), preOP PT (E), preOP Platelets (F). *$P < .05$, **$P < .005$.

1 collagen mRNA expression in Ito-cells.[13] Although changes in activin A and its inhibition are described in the context of underlying liver disease, we did not observe any difference in activin A, FSTL-3, or in the ratio of activin A and FSTL-3 in our cohort in patients grouped depending on high-grade or low-grade fibrosis. Similarly, we did not see an elevation of preoperative activin A levels in patients with steatohepatitis in our cohort ($P = .685$). This inconsistency with the available literature could be caused by multiple factors. On the one hand, patients amendable to undergoing liver resection only suffer from early-stage liver disease which might not have developed a striking increase in activin A as observed in patients with advanced cirrhosis and steatohepatitis. Indeed, nearly 40% of our patients did not suffer from fibrosis or only from grade 1 fibrosis and only about 23% presented with cirrhosis; the median degree of underlying steatosis was 5%. In line with this hypothesis, activin A levels were rather low in comparison to other studies primarily reporting on patients with chronic liver diseases. In particular, Voumvoraki et al present a median of

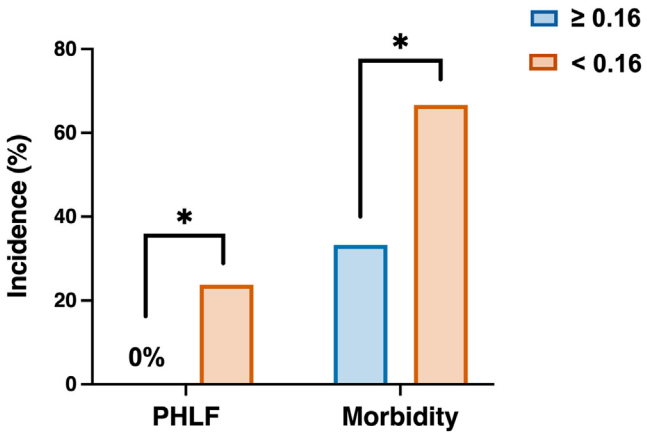

Figure 5. Patients grouped by Activin A/FSTL-3 cutoff into high-risk and low-risk groups, incidence of PHLF, and morbidity below and above the Median in the patient cohort. *$P < .05$, **$P < .005$.

637.50 pg/mL in patients with alcoholic cirrhosis,[31] almost double the median of 337.93 pg/mL in our cohort, with other studies reporting even higher activin A levels in patients suffering from acute liver failure more than 1500 pg/mL.[30] In this study, only patients with compensated cirrhosis were included, as decompensated cirrhosis would have made them ineligible for liver resection. This is reflected in the low median MELD-sodium (Na) in our patients, with MELD-Na being a surrogate parameter for the degree of existing liver disease.[32] This underlines a clear difference of our patient cohort from studies evaluating activin A in the context of chronic on acute liver disease. Ultimately, our study is certainly limited in statistical power given the low sample size, particularly as our patients mainly presented with low-grade fibrosis and steatosis.

Grusch et al report of downregulation of activin A mRNA expression in human HCC samples. Interestingly, they also report downregulation of FSTL-3 mRNA in these samples,[27] with FSTL-3 being abundantly expressed in normal liver tissue in comparison. There are limited data available, regarding circulating activin A in HCC. A study by Yuen et al, while showing elevated activin A levels in HCC patients in comparison to healthy controls, could not show significant differences between activin A concentrations in HCC patients and patients with cirrhosis due to other causes, also suggesting that chronic liver disease might be the predominant driver of Activin A elevation.[28] We believe the reason we did not see any difference in activin A levels with respect to underlying tumor type is due to more advanced stages of liver disease in their cohort.

A ratio reflecting activin A and its inhibition has been evaluated for its possible prognostic potential for acute liver failure.[30] With reference to the physiological properties of activin A, the ratio of activin A to FSTL-3 might be most relevant to accurately describe the biological activity of circulating activin A. Importantly, the ratio of activin A to FSTL-3 appeared far more sensitive to detect clinical differences, potentially due to better characterization of intrahepatic dysregulation of the activin A system compared to either parameter alone. Of note, the negative effect of dysregulation of the activin A system has been demonstrated by Endo et al.[20] Elevated inhibition of activin A delays the return to physiologic liver architecture in rats, while abundant activin A inhibits early regeneration. As patients with a lower ratio of activin A to FSTL-3 preOP exhibited an increased inhibition of circulating activin A, our results appear to highlight the critical relevance of this tightly regulated hepatic balance to respond to a regeneratory stimulus. Exhaustion of FSTL-3, due to constant overexpression, could influence postoperative liver regeneration, with possibly the antimitotic effect of activin A being unchecked by a lack of FSTL-3.

Blood-derived parameters, which are routinely evaluated in the clinical setting, for example, SB, platelets, or PT and liver function assessment tests like ICG clearance, have all been tested for their predictive potential in the context of PHLF.[1,33] When using ROC analysis, we could show that preoperative activin A/FSTL-3 was highly predictive for PHLF. We also analyzed the predictive potential of ICG clearance, namely PDR and R15, SB, PT, and platelets in our cohort. When comparing the AUC, activin A/FSTL-3 outperformed all analyzed parameters. We then separated our cohort in an activin A/FSTL-3 high and low group. This allocated all PHLF patients in the high-risk group below the cut-off, together with 74% of patients who developed postoperative morbidity. Activin A/FSTL-3 performed particularly strong as a negative predictor of postoperative outcome with an NPV of 100%. The possibility for a marker to preoperatively assess the risk for PHLF or postoperative morbidity after liver surgery could benefit from clinical decision-making. Of course, our ratio needs further independent validation, before any kind of clinical implementation or addition to existing scores.

Conclusion

In summary, we present perioperative data on activin A and FSTL-3 concentrations in humans undergoing liver resection. Although our data remain hypothesis-generating, it urges further research on activin A and FSTL-3 in postoperative liver regeneration. Examining not only supportive intervention to aid postoperative liver regeneration after human liver resection but also the tightly regulated return to physiologic liver mass seems equally important and beckons for further research. We present data documenting a promising potential of activin A/FSTL-3 ratio as a marker for preoperative patient risk stratification prior to hepatic resection. Activin A/FSTL-3 represents the state of quiescence vs proliferation in the liver and as such offers an interesting angle into the current knowledge of hepatic physiology.

Supplementary materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2023.02.011.

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The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
The data, analytic methods, and study materials will not be made available to other researchers.

Reporting Guidelines:
Helsinki Declaration.