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Evidence for Serotonin as a Relevant Inducer of Liver Regeneration After Liver Resection in Humans

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Liver regeneration (LR) involves a complex interplay of growth factors and antagonists. In this context, platelet-derived serotonin (5-HT) has been identified as a critical inducer of LR in mice. Clinical evidence for a role of 5-HT in LR in humans is lacking. Accordingly, serum and plasma 5-HT was monitored perioperatively in 60 patients undergoing liver resection, of which 35 served as exploration and 25 as validation sets. Intraplatelet (IP) levels of 5-HT were calculated by subtraction of plasma 5-HT from serum values. Serum markers of liver function were used to evaluate LR and liver dysfunction (LD). In the exploration setting, IP 5-HT levels significantly decreased after liver resection ($P < 0.001$) and gradually recovered during the first week. IP 5-HT measured before surgery specifically predicted LD in the subsequent 7 days (area under the curve: 0.721; $P = 0.029$). Patients suffering from postoperative LD and morbidity were found to have reduced IP 5-HT levels during the entire perioperative period. Furthermore, we validated that reduced preoperative IP 5-HT (<73 ng/mL) was associated with an increased incidence of postoperative LD and morbidity ($P = 0.045$ and $P = 0.021$) and were able to demonstrate that IP 5-HT levels were an independent predictor of poor clinical outcome. *Conclusions:* These findings provide evidence that IP 5-HT correlates with LR in humans: Patients with low IP 5-HT before liver resection suffered from delayed hepatic regeneration. Therefore, IP 5-HT levels may prove a helpful clinical marker to predict postoperative LD and clinical outcome before hepatic resection and initiate suitable interventions. (HEPATOLOGY 2014;60:257-266)

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Liver resection is considered the only curative treatment option for several neoplastic entities of the liver.[1,2] Accordingly, hepatectomies are performed for eligible patients based on resection of all radiological and macroscopic detectable tumors while preserving at least 20%-25% of healthy total liver volume.[3,4] Despite substantial improvements in surgical techniques and perioperative care, postoperative morbidity and mortality remain an important concern after liver resection.[5,6] The most significant factor determining morbidity and mortality after hepatectomy is the ability of the remnant liver to regenerate. In this context, several cell types and growth factors have been shown to regulate the highly orchestrated process of liver regeneration (LR).[7,8]

Inhibition of platelet aggregation by clopidogrel has previously been shown to prevent hepatocyte proliferation after 70% partial hepatectomy (PH) in mice, emphasizing a critical role of platelets in LR.[9] Furthermore, several clinical studies have found that

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CCC, cholangiocellular carcinoma; CI, confidence interval; CTx, chemotherapy; GGT, gamma-glutamyl transferase; ELISA, enzyme-linked immunosorbent assay; HCC, hepatocellular carcinoma; ICG, indocyanine green; IP, intraplatelet; IV, intravenous; LD, liver dysfunction; LR, liver regeneration; mCRC, metastatic colorectal cancer; MVA, multivariate analysis; NPV, negative predictive value; PDR, plasma disappearance rate; PF-4, platelet factor 4; PH, partial hepatectomy; POD, postoperative day; PPV, positive predictive value; pre-OP, before surgery; PT, prothrombin time; ROC, receiver operating characteristic; R15, retention rate at 15 minutes; 5-HT, serotonin/5-hydroxytryptamine; SB, serum bilirubin; SSRIs, selective serotonin reuptake inhibitors; UVA, univariate analysis.

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circulating low platelet counts are associated with poor clinical outcome after liver resection.[10,11] Tryptophan hydroxylase knockout mice,[12] lacking the rate-limiting enzyme of serotonin (5-hydroxytryptamine; 5-HT) biosynthesis, have failed to show adequate LR after PH.[9] Accordingly, platelet-derived 5-HT has been postulated to be a relevant platelet-derived stimulus for hepatocyte growth. Many experimental models have now indisputably demonstrated that LR is affected by platelet-derived 5-HT and interaction with its receptors.[9,13-18]

Despite extensive experimental research, there is no clinical evidence to date on whether 5-HT might also be a relevant inducer of LR in humans. Therefore, we examined the relationship between postoperative LR and 5-HT levels in patients undergoing hepatectomy. Based on the preclinical data,[9,13-18] we hypothesized that a reduced preoperative 5-HT level would be associated with delayed LR as well as an increased incidence of postoperative liver dysfunction (LD), which, in turn, might result in increased postoperative morbidity.

Patients and Methods

Prospective Study Cohorts

From January 2012 until May 2013, a total of 60 patients undergoing liver resection were prospectively included in this study; 35 were included in the exploratory analysis and 25 were subsequently recruited as a validation set. To increase homogeneity of our collective, only patients with one of our three most-frequent neoplastic entities were included, namely, metastatic colorectal cancer (N = 44), hepatocellular carcinoma (HCC; N = 9), or cholangiocellular carcinoma (CCC; N = 7) patients. Liver resections were classified according to the International Hepato-Pancreato-Biliary Association Brisbane 2000 nomenclature in minor ($\leq$3 segments) and major (>3 segments) hepatectomy.[19] Baseline characteristics are listed in Table 1. Routine blood sampling as well as serum and plasma preparation were performed immediately before surgery (preOP) and on postoperative day (POD) 1 as well as on POD5 after liver resection. Analysis of blood samples and patient data were approved by the institutional ethics committee (#424/2010); all patients gave written informed consent. Furthermore, the study has been registered at the clinical trials registry (ClinicalTrials.gov Identifier: NCT01700231).

Assessment of Preoperative Liver Function

Preoperative liver function was assessed by the indocyanine green (ICG) clearance test. ICG measurement was performed as previously described.[20] Briefly, pulse spectrophotometry was used to measure blood ICG concentration. A dose of 25 mg of ICG was dissolved in 20 mL of distilled water and was injected intravenously (IV) based on body weight of patients. Plasma disappearance rate (PDR) and retention rate at 15 minutes (R15) were calculated automatically from blood ICG concentration-time course.

Definition and Classification of Postoperative LD and Morbidity

Postoperative LD was evaluated based on the previously proposed criteria by Balzan et al.[21] The so-called "50-50 criteria" identify patients with a prothrombin time (PT) <50% and a serum bilirubin (SB) level >50 μmol/L corresponding to an SB concentration >2.9 mg/dl. Balzan et al. were able to demonstrate that patients with an SB value >50 μmol/L and a PT <50% on POD5 had a significant increase of postoperative mortality. Furthermore, in patients with significant morbidity, this 50-50 criterium was met 3-8 days before clinical evidence of complications. We recorded respective liver function parameters during the first postoperative week. Because the focus of this study was to detect delayed hepatic regeneration and not only complete liver failure, an SB concentration >2.9 mg/dL or a PT value <50% on any day within the first postoperative week were defined as postoperative LD.

To evaluate postoperative morbidity, the classification described by Dindo et al.[22] was applied and the severity of postoperative complications was recorded in grade I-V. In case of multiple complications per patient, the most serious one was used for analysis.

Death within 90 days after surgery was classified as postoperative mortality.

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Additional Supporting Information may be found in the online version of this article.

Table 1. Patient Demographics

Parameter	Entire Collective (N = 60)	Evaluation Cohort (N = 35)	Validation Cohort (N = 25)	*P* Value
Sex				0.154
Male	40	21	19	
Female	20	14	6	
Neoplastic entity				<0.001
mCRC	44	19	25	
HCC	9	9		
CCC	7	7		
Hepatic resection				0.128
Major	28	19	9	
Minor	32	16	16	
Cofactors				
Cirrhosis	7	7	0	0.017
CASH	16	9	7	0.536
Smoker	13	8	5	0.525
PVE	7	5	2	0.375
CTx	37	17	20	0.013
SSRI	4	2	2	0.557
Preoperative parameters	Median (range)	Median (range)	Median (range)	
PDR, %	21 (7.6-38.3)	22 (7.6-38.3)	20 (14.2-36.4)	0.793
R15, %	4.3 (0.3-32.0)	3.6 (0.3-32.0)	5.4 (0.4-11.5)	0.753
Steatosis, %	10 (0-70)	20 (0-70)	5 (0-60)	0.660
Platelets ($\times 10^3/\mu L$)	227 (92-492)	220 (113-492)	238 (92-470)	0.198
Age, years	64 (22-84)	63 (22-84)	64 (42-78)	0.805

CASH, chemotherapy associated steatohepatitis; PVE, portal venous embolization.

Optimized Blood Sample Preparation to Measure the IP 5-HT Pool

Plasma and Serum Preparation. For all perioperative time points, serum and plasma samples were collected.

We have previously demonstrated that conventional plasma preparation frequently suffers from *in vitro* platelet activation.[23,24] Because 5-HT is stored in platelet alpha granules, its blood measurement is highly sensitive to artificial *in vitro* platelet activation, as has recently been addressed by Brand and Anderson.[25] Accordingly, an optimized plasma preparation technique was applied, as previously described by us.[23,24,26,27] Briefly, blood was drawn into prechilled tubes containing citrate, theophylline, adenosine, and dipyridamole and was immediately placed on ice and further processed within 30 minutes. After an initial centrifugation step at 1,000×*g* and 4°C for 10 minutes, the plasma supernatant was subjected to further centrifugation at 10,000×*g* and 4°C for 10 minutes (to remove remaining platelets). The supernatant was stored in aliquots at −80°C.

Serum samples were retrieved by blood collection without the addition of anti coagulants and by centrifugation (at 1,000×*g* and room temperature for 10 minutes) 30 minutes after collection. The supernatant was stored in aliquots at −80°C.

Platelet Extracts. In a subset of 20 patients, blood was drawn and anticoagulated with trisodium citrate. To obtain platelet-rich plasma, blood was centrifuged at 125×*g* for 20 minutes. To avoid contaminations with other cell types, only the upper two thirds of the platelet-rich plasma fraction were used. Platelets were isolated by repeated washing (three times) of platelets with HEPES-tyrode buffer in the presence of prostaglandin E1 (1 μM, 1,000×*g*, 90 seconds). Washed platelets were then resuspended in lysis buffer (phosphate-buffered saline plus 0.02% Triton plus protease inhibitor) to generate platelet extracts, which were then stored in aliquots at −80°C.

Quantification of the IP 5-HT Pool

Plasma and serum samples as well as platelet extracts were analyzed by commercially available enzyme-linked immunosorbent assay (ELISA) tests for human 5-HT (5-HT ELISA; IBL, Hamburg, Germany) and PF-4 (Quantikine; R&D Systems, Minneapolis, MN), according to the manufacturer's instructions. To calculate the actual IP 5-HT pool, plasma 5-HT levels, reflecting the actual circulating amount of 5-HT, were subtracted from serum 5-HT levels, which contain all 5-HT released by platelet activation during blood clotting.

Perioperative parameters of liver function (SB, PT, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (AP), and albumin) were measured in serum samples by routine laboratory blood tests.

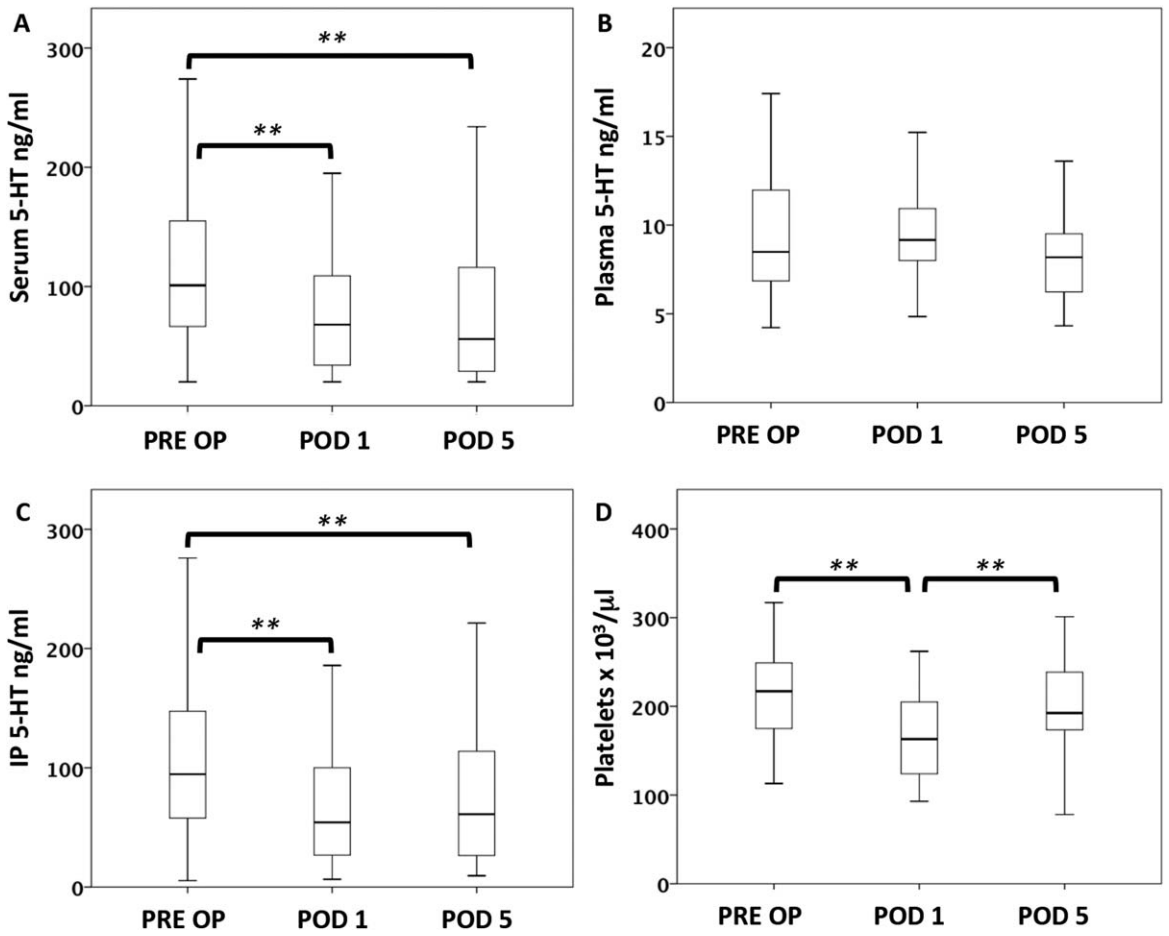

Fig. 1. Perioperative time course of serum (A), plasma (B), and IP (C) levels of 5-HT as well as platelet counts (D). Optimized plasma and serum preparation was performed 1 day before surgery (pre-OP), on the first POD (POD1), and on POD5. $^{**}P \leq 0.005$; $^{*}P \leq 0.05$.

Statistical Analysis

Statistical analyses were carried out with SPSS software (version 20; SPSS, Inc., Chicago, IL) and were based on nonparametric tests (Mann-Whitney's *U* test, Wilcoxon's test, and Spearman's correlation). The chi-squared test was used to evaluate frequencies between two groups. A receiver operating characteristic (ROC) analysis was performed to assess the specificity and sensitivity of IP 5-HT levels to predict postoperative LD. For multivariate analysis (MVA), the entire study collective (60 patients) and the first five most-significant parameters of univariate analysis (UVA) were included to achieve sufficient statistical power. To specifically test for independence of IP 5-HT to platelets, platelet count was included in MVA irrespective from its predictive potential in UVA. Box-plot illustrations are given without outliers and extreme values to improve the resolution of interquartile ranges. *P* values ≤ 0.05 were considered statistically significant.

Results

Patient Demographics. Basic characteristics of the entire collective, the exploration, as well as the validation cohorts are listed in Table 1 and were comparatively analyzed. Because the evaluation cohort also included HCC patients, a significantly higher incidence of cirrhosis was observed in the evaluation, as opposed to the validation cohort. Furthermore, because the validation cohort was exclusively composed of metastatic colorectal cancer (mCRC) patients, the incidence of preoperative chemotherapy (CTx) was significantly higher in the validation than the exploration cohort. No patient received platelet transfusion and no intraoperative cell-save devices were used. With reference to the subsequently defined cut-off value for the IP 5-HT level, we compared patients with preoperatively elevated IP 5-HT (IP 5-HT^{high}) to patients with lower IP 5-HT (IP 5-HT^{low}) levels (Supporting Table 1). No statistically significant difference in baseline

characteristics was observed between groups, apart from a significantly lower platelet count for IP 5-HT^{low} patients in the validation set. In particular, tumor type and liver function were comparable between IP 5-HT^{high} individuals and patients with lower preoperative IP 5-HT.

Perioperative Time Course of 5-HT Levels Shows a Rapid Reduction in IP 5-HT After Liver Resection. Initially, we characterized the perioperative time course of 5-HT in patients undergoing liver resection. Accordingly, blood was drawn 1 day before surgery as well as on POD1 and POD5 in an exploration set of 35 patients. A significant decrease of serum 5-HT was observed immediately after surgery on POD1 ($P < 0.001$), and serum levels remained decreased until POD5 ($P < 0.001$; Fig. 1A). Free circulating 5-HT levels were determined by optimized plasma preparation, preventing artificial platelet activation during processing. Plasma 5-HT concentrations were not significantly altered during the observation period (Fig. 1 B). To reflect the IP 5-HT pool, circulating plasma 5-HT levels were subtracted from serum 5-HT levels, which contains all platelet-released 5-HT. IP 5-HT levels decreased significantly after liver resection ($P < 0.001$) and, while tending to recover, remained decreased until POD5 ($P < 0.001$; Fig. 1C). Total platelet counts, as the major source of serum 5-HT, significantly decreased after surgery ($P < 0.001$), but were able to recover until POD5 ($P = 0.002$; Fig. 1 D). To further test for increased platelet activation after liver resection, plasma platelet factor 4 (PF-4) levels were analyzed. PF-4 levels were found to increase significantly after surgery on POD1 ($P = 0.050$) and tended to remain elevated until POD5 (Supporting Fig. 1), confirming an increased activation of platelets after LR.

To demonstrate that IP 5-HT levels are independent of the extension of liver resection, IP 5-HT levels were comparatively analyzed in patients undergoing minor versus major resections (Supporting Fig. 2). Importantly, no significant differences were found in the time course of IP 5-HT in patients undergoing major or minor resection.

IP 5-HT Reflects Total Available 5-HT Amount in Platelet Extracts. To evaluate whether calculated IP 5-HT values reflect actual 5-HT platelet content, platelet extracts were additionally generated from 20 patients. The calculated IP 5-HT concentration was adjusted for total circulating platelet counts to deduce 5-HT content per platelet. Per platelet IP 5-HT values significantly decreased immediately after surgery ($P < 0.001$) and further decreased until POD5 ($P < 0.001$; Fig. 2A). 5-HT levels determined in platelet extracts were found to be higher, but followed the same time course ($P = 0.003$ and $P = 0.001$; Fig. 2B) and significantly correlated with the calculated per platelet IP 5-HT levels ($P < 0.001$; $k = 0.649$; Fig. 2C).

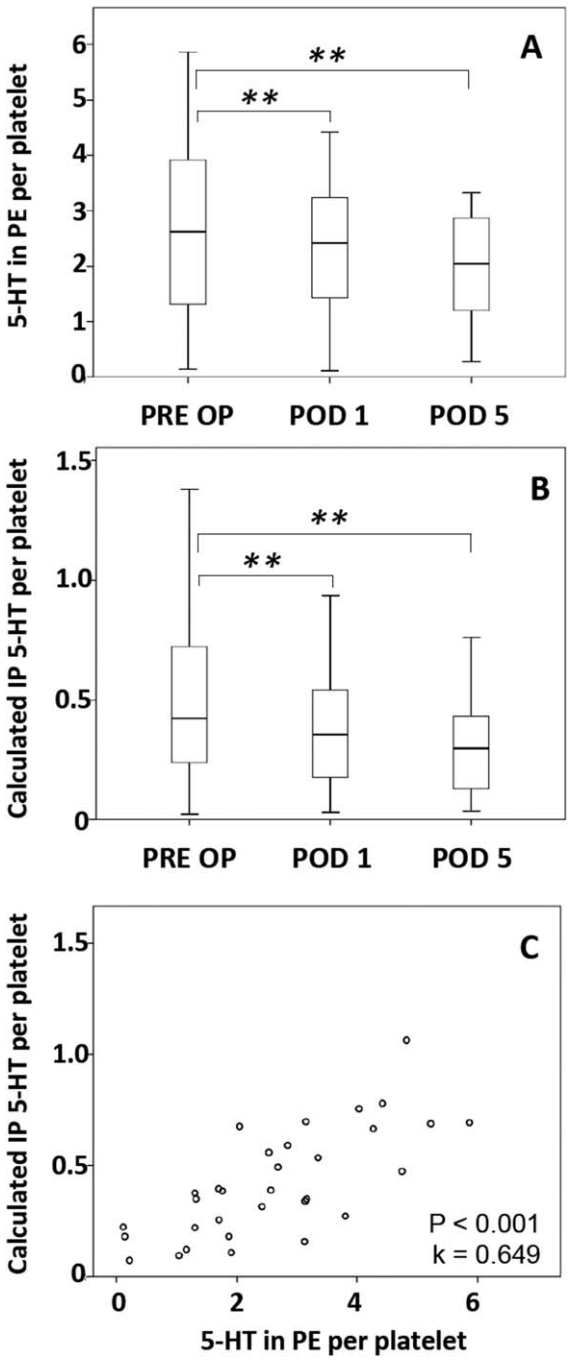

Fig. 2. Perioperative time course of calculated IP 5-HT values per platelet (A) and of 5-HT concentrations determined in platelet extracts (PE) per platelet (B). Optimized plasma, serum, and PE preparation was performed 1 day before surgery (pre-OP), on the first POD (POD1), and on POD5. Correlation of perioperative IP 5-HT values per platelet and of 5-HT concentrations determined in PE is illustrated (C). $^{**}P \leq 0.005$; $^{*}P \leq 0.05$.

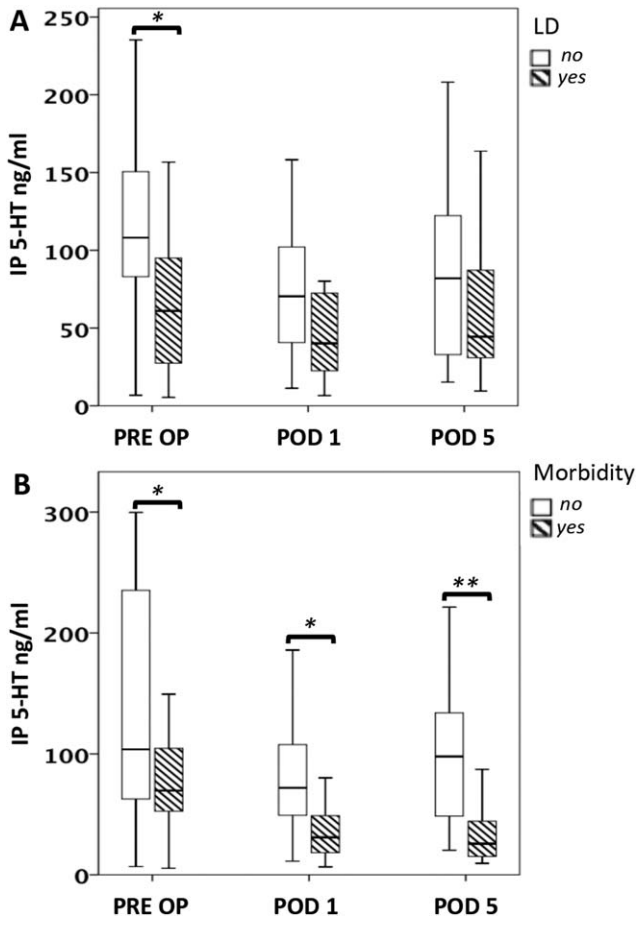

Fig. 3. Perioperative IP 5-HT levels according to postoperative LD and morbidity. Patients were divided into groups with or without postoperative LD (A) or morbidity (B). Samples were collected 1 day before surgery (pre-OP), on the first POD (POD1), and on POD5. $*P \leq 0.05$; $**P \leq 0.005$.

Low Preoperative IP 5-HT Levels and Failure of Postoperative 5-HT Recovery Is Associated With Poor Clinical Outcome. Given the preclinical evidence that platelet-derived 5-HT is a major inducer of LR, we addressed the question of whether patients with a reduced IP 5-HT pool would suffer from postoperative LD more frequently than patients with high IP 5-HT levels. When comparing patients with and without postoperative LD, individuals suffering from LD were found to have significantly reduced preoperative IP 5-HT levels (median IP 5-HT LD: 61 ng/mL; no LD: 108 ng/mL; $P = 0.028$), which remained reduced after liver resection (median IP 5-HT LD: POD1, 40 ng/mL, POD5, 44 ng/mL; no LD: POD1, 70 ng/mL; POD5, 82 ng/mL). The perioperative time course according to postoperative LD is illustrated in Fig. 3A.

When we further compared patients with postoperative morbidity of grade I-V according to Dindo et al.,[22] we found that preoperative IP 5-HT levels were substantially reduced in patients with postoperative complications (median IP 5-HT morbidity: 58 ng/mL; no morbidity: 108 ng/mL; $P = 0.041$). Intriguingly, in patients suffering from postoperative morbidity, IP 5-HT failed to recover after liver resection, reflected by a significant reduction of IP 5-HT at POD1 and POD5 (median IP 5-HT morbidity: POD1, 27 ng/mL; POD5, 26 ng/mL; no morbidity: POD1, 73 ng/mL; POD5, 94 ng/mL; POD1, $P = 0.008$; POD5, $P = 0.001$). The perioperative time course according to postoperative morbidity is illustrated in Fig. 3B.

A Preoperative IP 5-HT Value Below 73 ng/mL Specifically Identifies Patients With Postoperative LD and Morbidity. Because we had observed significantly lower preoperative IP 5-HT levels in patients with LD (Fig. 3A), we aimed to further characterize the potential of preoperative IP 5-HT values to predict postoperative LD. Therefore, an ROC analysis was performed, revealing a significant predictive value of IP 5-HT pool levels (area under the curve: 0.721; $P = 0.029$, Fig. 4A). Using ROC analysis, a cut-off level of 73 ng/mL of IP 5-HT was chosen to identify patients with postoperative LD with a specificity of 76% and a sensitivity of 64%. Based on this cut-off value, a high-risk LD group of 14 patients was identified (IP 5-HT^{low}); the remaining patients were classified as a low-risk group (IP 5-HT^{high}). Accordingly, we found IP 5-HT^{low} patients to suffer more frequently from postoperative LD ($P = 0.017$; 5 of 21 [24%] in IP 5-HT^{high} vs. 9 of 14 [64%] in IP 5-HT^{low} patients; Fig. 4B). This translated to a positive predictive value (PPV) of 0.643 (confidence interval [CI]: 0.356-0.860) and a negative predictive value (NPV) of 0.762 (CI, 0.525-0.909).

To evaluate whether a low preoperative IP 5-HT pool would further translate into poor clinical performance, we compared incidence of postoperative morbidity of grade I–V, according to Dindo et al.,[22] in high- versus low-risk patients, based on our cut-off level. IP 5-HT^{low} patients were found to develop postoperative morbidity more often than IP 5-HT^{high} patients ($P = 0.046$; 5 of 21 [24%] in IP 5-HT^{high} vs. 8 of 14 [57%] IP 5-HT^{low} patients; Fig. 4C), which translated to a PPV of 0.571 (CI, 0.296- 0.811) and a NPV of 0.762 (CI, 0.525-0.909).

Validation Collective Confirms Predictive Potential of Preoperative IP 5-HT Levels for Postoperative LD and Morbidity. Because we had observed a predictive potential of preoperative IP 5-HT values for postoperative LD in our exploration set of 35 patients based on the detailed analysis of three time points, we selectively investigated the preoperative IP 5-HT

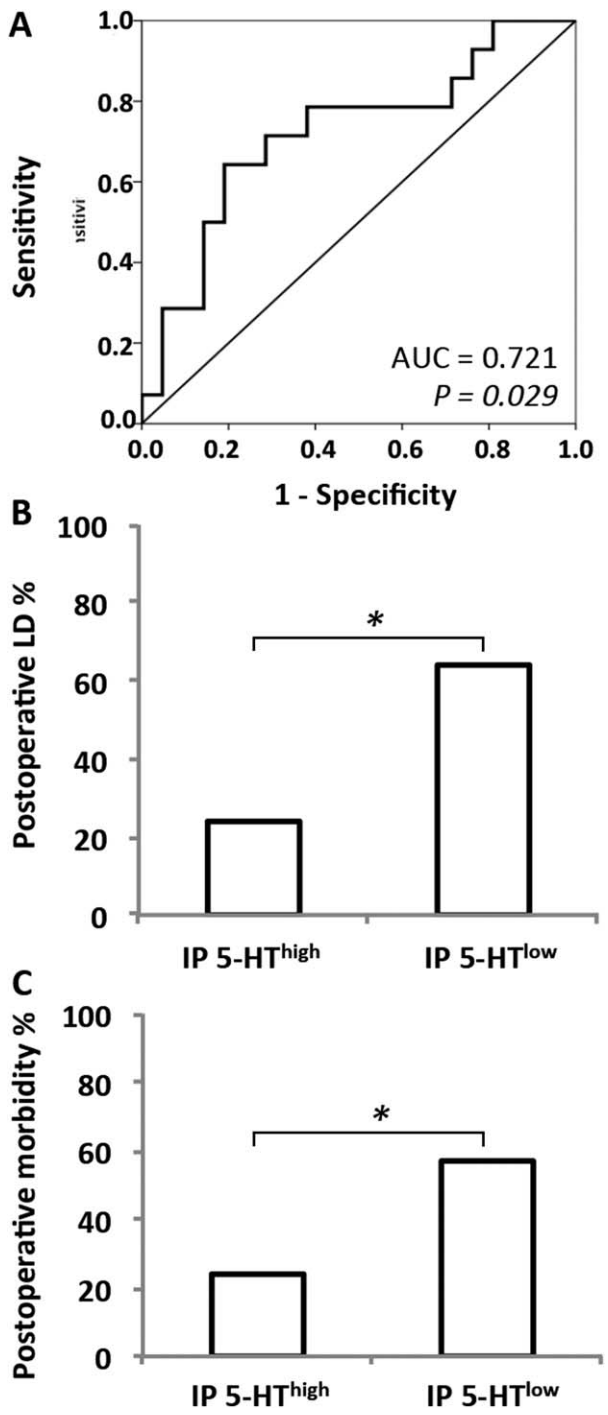

Fig. 4. Preoperative IP 5-HT levels and postoperative LD and morbidity. ROC curve analysis for IP 5-HT levels to predict postoperative LD (A) and preoperative IP 5-HT levels in patients with or without postoperative LD (B) or morbidity (C) are illustrated. $^{**}P \leq 0.005$; $^{*}P \leq 0.05$.

concentrations in a further 25 patients to validate our results. In the validation collective, IP 5-HT^{low} patients were found to have significantly higher postoperative SB and lower PT levels within the first postoperative week than the low-risk, IP 5-HT^{high} group (SB, $P < 0.001$; PT, $P = 0.049$), reflecting a delayed postoperative recovery of liver function. Thus, IP 5-HT^{low} patients suffered from a significantly increased risk of postoperative LD ($P = 0.045$; 5 of 18 patients [27%] in IP 5-HT^{high} vs. 5 of 7 patients [71%] in IP 5-HT^{low} patients; PPV, 0.714; CI, 0.303-0.949; NPV, 0.722; CI, 0.464-0.893; Supporting Fig. 3A). Furthermore, we were also able to confirm that IP 5-HT^{low} patients suffered from an increased risk of postoperative morbidity ($P = 0.021$; 4 of 18 patients [22%] in IP 5-HT^{high} vs. 5 of 7 patients [71%] IP 5-HT^{low} patients; PPV, 0.714; CI, 0.303-0.949; NPV, 0.778; CI, 0.519-0.926; Supporting Fig. 3B).

Given the differences in the exploration and validation set, we separately evaluated, if HCC patients with postoperative LD or morbidity showed a similar reduction in IP 5-HT levels as the remaining patients. Accordingly, we found that HCC patients exhibited a comparable reduction of IP 5-HT levels in patients with postoperative LD/morbidity as the remaining patients (Supporting Fig. 4).

Preoperative IP 5-HT Is an Independent Predictor of Postoperative LD and Morbidity. Because we had observed a striking association of IP 5-HT levels with postoperative LD and morbidity, it was of interest to investigate, if preoperative IP 5-HT levels could independently predict postoperative outcome. Therefore, we performed an MVA based on all recruited patients. Because postoperative SB and PT were used to define LD, those parameters were not included in UVA and MVA for postoperative LD. Besides IP 5-HT, the four most-significant parameters upon UVA were included in MVA (Table 2A). To specifically test for independence of IP 5-HT from platelets, platelet count was also included in MVA, irrespective of its predictive potential in UVA. Strikingly, preoperative IP 5-HT was able to predict postoperative LD independently from platelets and other parameters ($P = 0.031$; CI = 0.972-0.999; Table 2A). Similarly, we performed MVA for postoperative morbidity (Table 2B). In accord, IP 5-HT was the only parameter to independently predict postoperative morbidity ($P = 0.047$; CI = 0.972-1.000; Table 2B).

Discussion

In this article, we present the first clinical evidence for a central relevance of 5-HT in LR using a collective of 60 patients undergoing hepatectomy. Several animal studies and *in vitro* experiments demonstrate that platelet-derived 5-HT is a critical inducer of LR,[9,13-18] but, to date, these data were never confirmed in humans. In this pilot trial, we were able to

Table 2A. Preoperative Predictors of LD After Hepatectomy

	Univariate				Multivariate			
Variables	**B**	**Exp(B)**	**95% CI**	***P* Value**	**B**	**Exp(B)**	**95% CI**	***P* Value**
IP 5-HT, ng/mL	−0.011	0.989	0.980-0.997	0.010	−0.015	0.985	0.972-0.999	0.031
Platelets $10^3/\mu L$	−0.005	0.995	0.988-1.003	0.210	−0.002	0.998	0.988-1.008	0.699
Age	0.023	1.023	0.977-1.071	0.338				
Steatosis, %	0.014	1.014	0.987-1.041	0.305				
Cirrosis	0.788	2.200	0.446-10.860	0.333				
CASH	−0.143	0.867	0.267-2.812	0.812				
PDR, %	0.013	1.013	0.936-1.097	0.745				
R15, %	0.027	1.028	0.935-1.130	0.571				
GGT, U/L	0.004	1.004	0.998-1.010	0.188				
AST, U/L	0.032	1.032	0.999-1.067	0.055	0.006	1.006	0.969-1.044	0.748
ALT, U/L	0.011	1.011	0.991-1.031	0.296				
ALP, U/L	0.003	1.003	0.995-1.011	0.411				
Albumin, g/L	−0.050	0.951	0.808-1.119	0.544				
Type of resection	1.708	5.519	1.782-17.092	0.003	1.667	5.294	1.095-25.60	0.038
Smoker	−0.511	0.600	0.162-2.228	0.445				
PVE	1.498	4.474	0.791-25.317	0.090	3.274	26.412	0.936-745.6	0.055
CTx	−0.821	04406	0.151-1.282	0.132	−0.856	0.425	0.092-1.966	0.273

CASH, chemotherapy-associated steatohepatitis; PVE, portal venous embolization.

show that low IP 5-HT levels preceding liver resection significantly predicted postoperative LD and were associated with delayed postoperative hepatic recovery as well as poor clinical outcome.

Platelets, which are incapable of synthesizing 5-HT themselves, absorb 5-HT efficiently from the plasma pool and store it in their dense-body granules. Little is currently known on the biochemical and -physical behavior of the granule contents and the dynamics of granule content uptake and secretion.[28] Interestingly, comparable to neurons, platelets are able to reversibly release and take up 5-HT, independent of the release of other dense granule constituents.[29] Accordingly, IP 5-HT levels may remain decreased postoperatively, because of intrahepatic release, whereas circulating platelet counts recover until POD5. Intriguingly, although we found a weak, but significant, correlation of preoperative platelet counts and IP 5-HT ($k = 0.406$; $P < 0.001$), only IP 5-HT levels were able to independently predict postoperative LD and morbidity.

Table 2B. Preoperative Predictors of Morbidity After Hepatectomy

	Univariate				Multivariate			
Variables	**B**	**Exp(B)**	**95% CI**	***P* Value**	**B**	**Exp(B)**	**95% CI**	***P* Value**
IP 5-HT, ng/mL	−0.012	0.988	0.980-0.997	0.010	−0.015	0.986	0.972-1.000	0.047
Platelets, $10^3/\mu L$	−0.007	0.993	0.985-1.001	0.079	−0.011	0.989	0.976-1.002	0.110
Age	0.042	1.043	0.992-1.097	0.099				
Steatosis, %	−0.004	0.996	0.969-1.023	0.757				
Cirrosis	0.870	2.386	0.482-11.803	0.286				
CASH	−0.824	0.439	0.122-1.576	0.207				
PDR, %	−0.023	0.978	0.901-1.060	0.584				
R15, %	0.060	1.061	0.959-1.174	0.248				
SB, mg/dL	0.955	2.599	0.963-7.016	0.059	−0.239	0.788	0.233-2.657	0.700
PT, %	−0.015	0.985	0.959-1.011	0.250				
GGT, U/L	0.014	1.014	1.002-1.027	0.025	0.016	1.016	0.997-1.035	0.093
AST, U/L	0.022	1.023	0.994-1.052	0.123				
ALT, U/L	0.011	1.011	0.991-1.032	0.272				
ALP, U/L	0.010	1.010	0.999-1.022	0.074	0.004	1.004	0.980-1.029	0.749
Albumin, g/L	−0.034	0.966	0.821-1.137	0.679				
Type of resection	1.561	4.762	1.548-14.648	0.006	0.526	1.692	0.368-7.787	0.500
Smoker	0.007	1.007	0.285-3.559	0.991				
PVE	0.870	2.386	0.482-11.803	0.286				
CTx	−0.947	0.388	0.132-1.142	0.086				

CASH, chemotherapy-associated steatohepatitis; PVE, portal venous embolization.

This might suggest that not only platelet count itself, but primarily the composition of platelet granula might determine proregenerative activity of platelets. Indeed, Alkozai et al. also failed to document an association of preoperative platelets and postoperative LD in a large clinical trial.[30] Besides many inherited and acquired dense granule storage pool diseases, with variable reduction in number and contents of dense granules,[31] pharmacological interventions, such as selective serotonin reuptake inhibitors (SSRIs),[29] have been shown to significantly decrease the platelet storage pool of 5-HT. Of note, 4 of our patients received SSRI treatment perioperatively. Concurrent with the literature,[29] we found that those patients had highly reduced IP 5-HT levels ($P < 0.001$; Supporting Fig. 4). Furthermore, 3 of 4 SSRI-treated patients developed postoperative LD. This suggests that SSRI therapy might affect postoperative LR by reducing the IP 5-HT pool. However, because only 4 patients received SSRI treatment, this is an exploratory finding and has to be validated within a prospective trial.

5-HT has been shown to be of critical relevance in cell-cycle regulation of hepatocytes. In particular, 5-HT antagonists inhibit hepatocyte proliferation when administered close to the G_1/S phase and the intrahepatic 5-HT concentration peaks around the G_1/S transition point.[14,16] In line with this, hepatic 5-HT content has been found to correlate directly with hepatocyte proliferation in preclinical studies.[14,16] Accordingly, 5-HT should act to promote hepatocyte growth during the entire period of LR. In accord, reduced IP 5-HT levels, preoperatively as well as on POD1 and POD5, were found to be associated with poor postoperative outcome. Indeed, looking at the time course of IP 5-HT levels, patients developing postoperative LD or morbidity started with a reduced IP 5-HT content, but also seemed to recover their IP 5-HT levels far less rapidly than patients without postoperative complications. This might either be the result of a pronounced 5-HT release in the liver, reflecting a prolonged and probably pathologic LR, or might reflect a reduced capacity of restoring the platelet 5-HT pool, which, in turn, would result in a delayed LR.

Furthermore, we assessed whether preoperative IP 5-HT values were able to identify high-risk patients with postoperative LD. Balzan et al. have previously developed a standardized definition of postoperative LD using SB and PT.[21] Therefore, we have comparably based our definition of LD on the so-called 50-50 criteria proposed by Balzan et al. In particular, postoperative LD was defined as SB >50 μmol/L or PT $<50\%$ within the first postoperative week. It should be stressed that this classification was intended to identify patients with delayed postoperative hepatic recovery, rather than patients with complete LD and mortality, as originally specified by the 50-50 criteria on POD5 proposed by Balzan et al.[21] Interestingly, preoperative IP 5-HT levels were significantly reduced in patients with postoperative LD and were suited to predict postoperative LD with 76% specificity and 64% sensitivity, based on the selected cut-off value (73 ng/mL). Furthermore, we found that IP 5-HT^{low} patients suffered from an increased incidence of postoperative morbidity.

Furthermore, 1 patient died within 90 days after liver resection, suffering from fulminant postoperative liver failure. Importantly, this patient was found to have a reduced preoperative IP 5-HT level of 69 ng/mL and therefore classified as a high-risk IP 5-HT^{low} patient. This further supports the clinical relevance of IP 5-HT.

Of note, serum 5-HT levels, without subtraction of circulating plasma levels, also tended to predict postoperative LD and morbidity (data not shown). This might be of relevance for future clinical evaluations, because the preparation of serum is less sensitive to artifacts during sample preparation than plasma.

Because 5-HT activity has been shown to induce LR in mice,[9] IV injection of 5-HT could serve as an attractive approach to increase LR after liver resection. However, IV injection of 5-HT can cause significant neurological and hemodynamic side effects.[33] Although preoperative platelets failed to directly correlate with postoperative LD, a systemic increase of platelet counts might be a useful way to increase total serotonin pool. Because platelet transfusion can cause relevant morbidity,[34] indirect ways to increase circulating platelet counts could be an attractive tool to increase systemic 5-HT levels. Indeed, thrombopoietin receptor agonists have been shown to promote LR in mice.[35] However, this should be addressed by a prospective clinical trial.

Taken together, preoperative IP 5-HT was found to be a valuable and independent marker for prediction of LD and poor clinical outcome after liver resection. Because 5-HT is easily assessable in plasma and serum, it may represent a useful parameter to identify high-risk patients before surgery that require consideration and close monitoring for potential complications. Importantly, the predictive potential of IP 5-HT will have to be further validated in large-scale studies. Moreover, involvement of 5-HT might represent a novel therapeutic target to accelerate LR after hepatectomy, because it has already been suggested by preclinical models.[18,36]

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