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ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

The Impact of Modern Chemotherapy and Chemotherapy-Associated Liver Injuries (CALI) on Liver Function: Value of 99mTc-Labelled-Mebrofenin SPECT-Hepatobiliary Scintigraphy

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ABSTRACT

Background. Chemotherapy is increasingly used before hepatic resection, with controversial impact regarding liver function. This study aimed to assess the capacity of 99mTc-labelled-mebrofenin SPECT-hepatobiliary scintigraphy (HBS) to predict liver dysfunction due to chemotherapy and/or chemotherapeutic-associated liver injuries (CALI), such as sinusoidal obstruction syndrome (SOS) and nonalcoholic steatohepatitis (NASH) activity score (NAS).

Methods. From 2011 to 2015, all consecutive noncirrhotic patients scheduled for a major hepatectomy ($\geq$ 3 segments) gave informed consent for preoperative SPECT-HBS allowing measurements of segmental liver function. As primary endpoint, HBS results were compared between patients with versus without (1) preoperative chemotherapy ($\leq$ 3 months); and (2) CALI, mainly steatosis, NAS (Kleiner), or SOS (Rubbia-Brandt). Secondary endpoints were (1) other factors impairing function; and (2) impact of chemotherapy, and/or CALI on hepatocyte isolation outcome via liver tissues.

Results. Among 115 patients, 55 (47.8%) received chemotherapy. Sixteen developed SOS and 35 NAS, with worse postoperative outcome. Overall, chemotherapy had no impact on liver function, except above 12 cycles. In patients with CALI, a steatosis $\geq$ 30% significantly compromised function, as well as NAS, especially grades 2–5. Conversely, SOS had no impact, although subjected to very low patients number with severe SOS. Other factors impairing function were diabetes, overweight/obesity, or fibrosis. Similarly, chemotherapy in 73 of 164 patients had no effect on hepatocytes isolation outcome; regarding CALI, steatosis $\geq$ 30% and NAS impaired the yield and/or viability of hepatocytes, but not SOS.

Conclusions. In this first large, prospective study, HBS appeared to be a valuable tool to select heavily treated patients at risk of liver dysfunction through steatosis or NAS.

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Postoperative liver failure (PHLF) is a prominent cause of death following hepatic resection, mostly due to an insufficient functional volume of remnant liver and inadequate liver regeneration capacities. Besides the deleterious effect of diabetes mellitus or chronic liver disease, the impact of preoperative chemotherapy on the liver

regeneration capacities remains unclear. Many drugs used to treat hepatic malignancies, such as 5-fluorouracil, oxaliplatin, or irinotecan, have been reported to cause some degree of liver damage, mainly steatosis, sinusoidal obstruction syndrome (SOS, especially for oxaliplatin) or nonalcoholic steatohepatitis (NASH, in particular for irinotecan). However, only some of the patients who received preoperative chemotherapy developed CALI. Moreover, the basic pathogenesis of neoadjuvant chemotherapy and chemotherapy-associated liver injury (CALI) is still poorly understood, with controversial data on its effect on liver function and regeneration capacities: deleterious effect versus no deleterious effect.[1–4] Recently, nuclear imaging techniques have been developed for quantitative assessment of liver function in liver surgery, using mainly (99 m)Tc-labeled agents, such as (99 m)Tc-Iminodiacetic acid (IDA) compounds (mebrofenin).[5] In particular, technical improvements in gamma-camera have enabled faster single photon emission CT (SPECT), providing valuable informations about the distribution of function within the liver.

In this prospective study, we assessed whether (99 m)Tc-mebrofenin hepatobiliary scintigraphy (HBS) with SPECT was a valuable tool to evaluate the impact of modern chemotherapy and/or CALI on liver function in patients scheduled for anatomic liver resection. In parallel, as we provided since 2006 samples for hepatocytes isolation from specimens obtained during partial hepatectomy, we could analyze the effect of neoadjuvant chemotherapy and/or CALI on hepatocellular viability and yield.

PATIENTS AND METHODS

From November 2011 to December 2015, 115 patients with a median age of 62 years [19–88] scheduled for an anatomical major hepatectomy ($\geq$ 3 segments) in a non-cirrhotic liver were enrolled in a prospective pilot HBS study, after obtaining patient's consent and institutional review board approval of the "Comité de Protection des Personnes." Enrolled patients underwent SPECT-HBS in the 3 weeks before surgery, allowing measurements of total and segmental liver function. All examinations were performed after PVE when performed (i.e., in case of future remnant liver (FRL) volume $\leq$ 25% of total liver volume and/or 0.5% of body weight[6, 7] in average 3 to 4 weeks before the hepatectomy) and after biliary drainage in jaundiced patients considering the competition between mebrofenin and bilirubin on hepatic receptors (hyperbilirubinemia $>$ 75 mg/dl was an exclusion criterion).[8] Volumetric assessments were performed as previously described.[6] Neoadjuvant chemotherapy was defined as chemotherapy $\pm$ biotherapy (anti-VEGF or anti-EGFR) administered within 3 months before hepatic resection;[9] cessation period of chemotherapy before surgery was on average 1 month according to the current recommendations.[10,11] The 3-month morbi-mortality was graded according to Clavien classification and clinically significant PHLF as 50–50 criteria and/or a peak serum bilirubin $>$ 7 mg/dL (PeakBili $>$ 7).[12–15]

Hepatobiliary Scintigraphy (HBS)

After injection of 130 MBq of (99 m)Tc-mebrofenin, a dynamic acquisition was performed (45 frames of 10 s) with a gamma-camera SYMBIA S (Siemens®) in anterior and posterior view. The liver uptake phase was followed by a tomographic study (32 projections of 10 s each). Activity curves were obtained from the dynamic acquisition after drawing regions of interest (ROI) on the parenchymal liver, heart, and total field-of-view. The total liver uptake (TLU) rate was calculated between 150 and 350 s after injection as described by Ekman et al.[16] The FRL was outlined on the tomoscintigraphic study after registration with the contrast-enhanced computed tomography. The FRL uptake was calculated by dividing counts within the delineated FRL by the total liver counts and multiplying this factor by the TLU. The intrinsic liver function (function per unit of volume of the liver, expressed as uptake per 100 g of liver; %/min) was calculated as the ratio of the uptake values to the respective liver volume; this enabled the normalization of the HBS computations to the volume of the corresponding liver segments. HBS results were compared between patients preoperatively treated by chemotherapy $\pm$ biotherapy versus untreated ones and between CALI versus no CALI cases.

Liver Resection

The surgical techniques and the various vascular control methods used to reduce the intra operative bleeding have been described elsewhere.[6,17] For the purposes of the study, all specimens of partial hepatectomy in the context of chemotherapy were reanalyzed retrospectively by two expert pathologists (E.L., V.G.) blinded to the clinical data to document CALI, mainly steatosis (considered pathologic if $\geq$ 30%), SOS, and NAS. SOS was graded according to the sinusoidal pathological score reported by Rubbia-Brandt et al.[18] as follows: 0, absent; 1, mild (centrilobular involvement limited to one-third of the lobule); 2, moderate (centrilobular involvement extending to two-thirds of the lobule); and 3, severe (complete lobular involvement). NASH was graded according to the nonalcoholic fatty liver disease (NAFLD) activity score (NAS), as defined by Kleiner et al.[19] which is based on steatosis, lobular inflammation, and ballooning. Other factors likely to

impact liver function and regeneration capacities were systematically analyzed, mainly age, initial jaundice, body mass index (BMI), diabetes mellitus, alcohol or tobacco consumption, or fibrosis quantified according to the METAVIR score.[19,20]

Tissue Processing and Human Hepatocyte Isolation

Since 2006, liver tissues were obtained from an historical cohort of fully consenting patients ($n = 164$) undergoing hepatic resection, allowing hepatocytes isolation and measurements of cell viability and yield (with exclusion of cirrhosis, hepatitis B and C, or cholestatic livers as associated with a poor isolation outcome[21]). Upon removal of the liver specimen from the abdominal cavity, the liver tissue was immediately taken from the macroscopically tumor-free margin of the sample and kept into an ice-cold medium under sterile conditions during transport to the processing laboratory (BIOPREDIC, Rennes, France). Warm ischemic time outside the body did not exceed 20 min. After a 6- to 8-h cold ischemic transport period, primary human hepatocytes were isolated from the resected liver tissues by using a modified two-step collagenase perfusion procedure, as previously described.[22,23] After washing, the isolated hepatocytes were suspended in a cell culture medium and the number of viable cells (number of cells in millions), cell viability (% of viable cells), and yield (million(s) of viable cells per gram of tissue; Mc/g) were determined by trypan blue dye exclusion test.

Endpoints

As primary end-point, HBS results were compared between patients with versus without preoperative chemotherapy ± biotherapy (within 3 months) and with versus without CALI. Secondary endpoints were 1) other factors impairing liver function; 2) impact of chemotherapy and/or CALI on hepatocyte isolation outcome via liver tissues.

Statistical Analysis

Continuous variables were expressed as the mean (± standard deviation) and compared using the independent-samples Mann–Whitney U test or Kruskal–Wallis test. Categorical variables were expressed as percentages and compared using χ^2 tests or Fisher exact tests, as appropriate. All analyses were performed using SPSS, version 22.0 (SPSS, Chicago, IL). The significance threshold was set to $p < 0.05$.

RESULTS

The main clinicopathological data of the 115 enrolled patients are shown in Table 1. All patients underwent a major hepatectomy. Most patients were operated on for a malignant tumor, and approximately half had received neoadjuvant chemotherapy within 3 months. Sixteen developed SOS and 35 NAS, with grades > 1 in 5 and 18 patients, respectively (Table 1). The baseline characteristics of patients who received chemotherapy and those who did not were globally similar, except for a higher rate of primary malignant tumor requiring more frequently preoperative biliary drainage in preoperatively-untreated patients (Table 1). Postoperatively, CALI had a significant impact on patients' outcome, with increased morbidity in particular PHLF according to 50–50 criteria in patients with SOS (16.7% vs. 1.5% in patients without SOS, $p = 0.004$) and to PeakBili > 7 in patients with NAS grades 2–5 (8.7% vs. 1.9% in patients without NAS, $p > 0.05$).

HBS

In 115 HBS patients, results were analyzed in terms of intrinsic liver function, enabling the normalization of the HBS computations to the volume of the corresponding liver segments for the total liver, the FRL and the segments to be resected (Table 2). Overall, the intrinsic liver function was better preserved in the FRL than in the liver segments to be resected with borderline significance, possibly due to the tumor burden in the diseased part of the liver. Regarding the main endpoint, HBS results were compared between patients with and without neoadjuvant chemotherapy (within 3 months) and according to the presence or not of CALI. Overall, there was no impact of neoadjuvant chemotherapy (with or without biotherapy) on the HBS results, except for intensive chemotherapy ≥ 12 cycles ($n = 22$ patients) that significantly impaired liver function. When focusing on patients with CALI, a steatosis $\geq 30\%$ significantly compromised the intrinsic liver function in the FRL, as well as NAS, and this was even more pronounced for NAS grades 2–5 (Fig. 1). By contrast, liver function was not altered by SOS, although subjected to the low number of patients with significant SOS grades 2–3 ($n = 5$).

As secondary endpoints, the other factors impairing the FRL function were diabetes, overweight or obesity, or the presence of fibrosis (Table 2). There was no impact of gender or age (less vs. more than 60, 70, or 80), dyslipidemia, or intermittent portal trial clamping (data not shown). Due to the competition between mebrofenin and bilirubin on hepatic receptors, initial jaundice significantly impacted liver function in the segments to be resected that

TABLE 1 Clinicopathological features and postoperative outcome of the 115 patients who had preoperative HBS and of subgroups with versus without chemotherapy

Clinical parameters	Overall population (n = 115)	Preoperative chemotherapy (n = 55)	No preoperative chemotherapy (n = 60)
Age	62 ± 13	62 ± 10.6	62 ± 15
Gender (female/male)	45/70	22/33	23/37
ASA score > 2 (%)	24 (20.9)	10 (18.2)	14 (23.3)
Diabetes (%)	21 (18.3)	10 (18.2)	11 (18.6)
Hypertension (%)	48 (41.7)	19 (34.5)	29 (48.3)
Hyperlipidemia (%)	23 (20)	9 (16.4)	14 (23.3)
Chronic obstructive pulmonary disease (%)	7 (6.1)	3 (5.5)	4 (6.7)
Jaundice* (%)	8 (7)	0	8 (13.3)
BMI (%)			
> 25	65 (56.5)	33 (60)	32 (53.3)
> 30	22 (19.1)	13 (23.6)	9 (15)
Smoking habits (%)	26 (22.6)	13 46.4)	13 (40.6)
Alcohol (%)	19 (16.5)	6 (10.9)	13 (21.7)
Preoperative biliary drainage* (%)	6 (5.2)	0	6 (10)
Preoperative PVE (%)	25 (21.7)	15 (27.3)	10 (16.7)
Preoperative chemotherapy (%)			
No. of patients	55 (47.8)		–
No. of cycles	9.6 ± 7.4 [3–30]		–
≥ 12 cycles	22 (19.1)		
FOLFOX or FOLFIRI	41 (35.6)		
FOLFOX + FOLFIRI	2 (1.7)		
With biotherapy	25 (21.7)		
Hepatic resection			
Right/extended right (%)	47 (40.9)/33 (28.7)	25 (45.5)/17 (30.9)	22(36.7)/16(26.7)
Left/extended left (%)	22 (19.1)/5 (4.3)	7 (12.7)/1 (1.8)	15 (25)/4 (6.7)
Other (%)	8 (7)	5 (9.1)	3 (5)
CALI/underlying liver disease (%)			
Steatosis ≥ 30%	18 (15.7)	12 (21.8)	6 (10)
Fibrosis (F1-F3)	30 (26.1)	13 (24.5)	17 (30.9)
Fibrosis ≥ F2	10 (8.7)	3 (5.7)	7 (11.7)
SOS[μ]	16 (29.1)		–
SOS grades 2–3	5 (9.1)		
NAS[μ]	35 (63.6)		–
NAS grades 2–5	18 (15.6)		
Tumor type* (%)			
Benign tumor	8 (7)	0	8 (13.3)
Malignant tumor			
Primary	45 (39.1)	0	45 (75)
Secondary	62 (53.9)	55 (100)	7 (11.7)
Postoperative outcome (%)			
Overall complication	64 (55.6)	27 (49.1)	37 (61.7)
Clavien ≥ IIIB	19 (16.5)	7 (12.7)	12 (20)
Death	6 (5.2)	1 (1.8)	5 (8.3)

PVE portal vein embolization. [μ] Percentages of patients with SOS or NAS calculated within the subgroup of patients who had preoperative chemotherapy only. *$p < 0.05$ when comparing patients with versus without preoperative chemotherapy

TABLE 2 HBS results in terms of intrinsic liver function in the total liver, the FRL and in the segments to be resected according to chemotherapy and/or CALI or other perioperative factors

		Liver function of the total liver		Liver function of the future remnant liver		Liver function of the segments to be resected	*p*
Overall population		1.17 ± 0.6		1.21 ± 0.6		1.13 ± 0.7	0.098*
According to the following factors:			*p*		*p*		*p*
Initial jaundice	No	1.2 ± 0.6	0.08	1.2 ± 0.6	0.6	1.2 ± 0.7	**0.02**
	Yes	0.63 ± 0.4		1 ± 0.6		0.56 ± 0.4	
Alcohol abuse	No	1.18 ± 0.65	0.89	1.2 ± 0.6	0.97	1.15 ± 0.76	0.58
	Yes	1.09 ± 0.5		1.3 ± 0.53		1.1 ± 0.62	
Smoking	No	1.34 ± 0.9	0.6	1.2 ± 0.7	0.18	1.3 ± 1	0.7
	Yes	1.2 ± 0.4		1.3 ± 0.5		1.2 ± 0.6	
Diabetes	No	1.23 ± 0.66	**0.008**	1.25 ± 0.6	**0.015**	1.2 ± 0.8	**0.05**
	Yes	0.94 ± 0.38		1 ± 0.43		0.88 ± 0.4	
Overweight	No	1.37 ± 0.84	**0.001**	1.35 ± 0.7	**0.008**	1.3 ± 0.9	**0.005**
(BMI > 25)	Yes	1 ± 0.33		1.1 ± 0.4		0.97 ± 0.5	
Obesity	No	1.24 ± 0.7	**0.001**	1.27 ± 0.6	**0.008**	1.2 ± 0.8	0.12
(BMI > 30)	Yes	0.9 ± 0.19		0.95 ± 0.21		0.9 ± 0.3	
Neoadjuvant chemotherapy	No	1.2 ± 0.7	1	1.2 ± 0.6	0.9	1.1 ± 0.9	0.4
	Yes	1.1 ± 0.4		1.1 ± 0.4		1.2 ± 0.5	
	≥ 6 cycles	1.1 ± 0.5	0.09	1.1 ± 0.4	0.09	0.2 ± 0.6	0.18
	≥ 12 cycles	1 ± 0.5	**0.036**	0.99 ± 0.4	**0.021**	1.1 ± 0.6	0.24
Targeted therapy	No	1.1 ± 0.7	0.5	1.2 ± 0.6	0.5	1 ± 0.8	0.064
	Yes	1.2 ± 0.4		1.1 ± 0.4		1.3 ± 0.54	
Fibrosis	No	1.27 ± 0.7	0.69	1.29 ± 0.6	**0.005**	1.24 ± 0.8	0.19
	Yes	0.97 ± 0.39		1 ± 0.4		0.97 ± 0.5	
Fibrosis ≥ F2	No	1.2 ± 0.65	0.95	1.23 ± 0.58	0.162	1.2 ± 0.8	0.73
	Yes	0.98 ± 0.41		1 ± 0.39		1 ± 0.6	
Steatosis ≥ 30%	No	1.2 ± 0.43	0.26	1.2 ± 0.4	**0.048**	1.2 ± 0.5	0.55
	Yes	0.98 ± 0.28		0.89 ± 0.2		1.1 ± 0.5	
Malignant tumor	No	1.6 ± 1.7	0.65	1.5 ± 1.5	0.7	1.8 ± 2.2	0.7
	Yes						
	Primary	1 ± 0.48		1.2 ± 0.4		0.94 ± 0.45	
	Secondary	1.2 ± 0.4		1.2 ± 0.4		1.2 ± 0.6	
SOS	No	1.2 ± 0.4	0.75	1.2 ± 0.4	1	1.2 ± 0.5	0.66
	Yes	1.2 ± 0.48		1.1 ± 0.48		1.3 ± 0.7	
	SOS 2–3	1.15 ± 0.34	1	1.1 ± 0.4	1	1.3 ± 0.55	0.7
NAS	No	1.31 ± 0.49	1	1.35 ± 0.47	**0.017**	1.3 ± 0.6	0.5
	Yes	1.12 ± 0.36		1.06 ± 0.94		1.2 ± 0.6	
	NAS 2–5	1.04 ± 0.32	0.23	0.99 ± 0.29	**0.034**	1.1 ± 0.4	0.5

*In the first raw, the *p* value refers to the comparison of liver function between the FRL and the segments to be resected in the overall population; in the rest of the table, the comparisons are made for each region of interest in presence or not of a factor likely to impact liver function. Significant *p* values (< 0.05) are indicated in bold. For all results, liver function was expressed as intrinsic liver function (i.e., function per unit of volume of the liver, expressed as uptake per 100 g of liver; %/min)

were not drained preoperatively (in contrast to the FRL). Overall, most of these factors more frequently influenced liver function within the FRL than in the segments to be resected (Table 2), likely due to the preeminence of tumor burden in the latter.

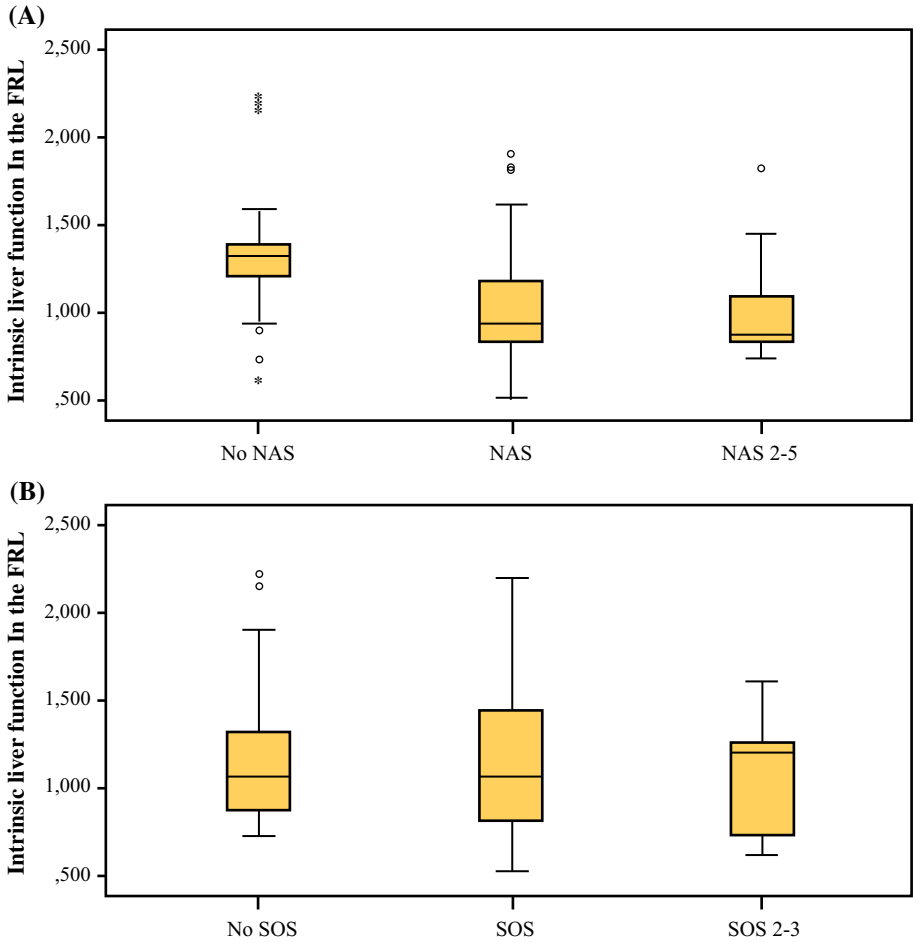

FIG. 1 Box plot showing the intrinsic FRL function (function per unit of volume of the liver, expressed as uptake per 100 g of liver; %/min) according to **a** the presence or absence of NAS or NAS grade 2–5 ($p < 0.05$ between patients without vs. with NAS—whatever the grade); **b** the presence or absence of SOS or severe SOS grade 2–3 (nonsignificant differences) in patients who received chemotherapy in the 3 months before surgery

Hepatocyte Isolation Data

In 164 patients, the mean cell yield of hepatocyte isolation was 7.2 ± 6.3 Mc/g and viability 66.4% ± 20.1%. Overall, neoadjuvant chemotherapy in 73 patients had no effect on any parameters of hepatocytes isolation, and this was true whatever the regimen used and cycles number (Table 3); targeted therapies (anti-VEGF or anti-EGFR) had no impact either. Regarding specifically the impact of CALI, a steatosis ≥ 30% in preoperatively treated patients significantly impaired the number and yield of hepatocytes, but not their viability. In addition, NAS in 37 patients significantly impaired both cell viability ($p = 0.046$), and yield for NAS grades 2–5 ($n = 8$; $p = 0.009$; Fig. 2). By contrast, hepatocytes isolation outcome was not affected by SOS in 44 patients compared to patients without SOS, even for SOS grades 2–3 ($n = 12$).

When looking at other factors likely to impact the liver regeneration capacities, parameters of hepatocytes isolation were mainly impaired by a history of alcohol abuse (data not shown), or the presence of malignant tumor in the resected liver. Moreover, there was a trend toward lower hepatocytes yield and viability in case of fibrosis (Table 3).

DISCUSSION

The current report is the first HBS prospective study to elucidate the pathogenesis and impact of chemotherapy with or without CALI on hepatocytes function. We first found that neoadjuvant chemotherapy had a deleterious effect on liver function only if intensive (12 cycles or more). Regarding CALI, the presence of steatosis ≥ 30% or NAS significantly impaired liver function in the FRL, and this was even more pronounced for NAS grades 2–5. By contrast, there was no deleterious effect of SOS on liver function, although subjected to the low number of patients with significant SOS. In parallel, in one of the largest collections of hepatocytes originating from a comparable cohort of patients, we observed a decrease in hepatocytes yield and/or viability in patients with steatosis or NAS, while SOS had no impact. Overall, these results are in lines with the differential impact of CALI on liver cells affecting more or less hepatocytes (steatosis, NAS) and/or endothelial cells (SOS).

Chemotherapy for hepatic malignancies is associated with hepatic injuries in approximately 30 to 50% of cases, in agreement with the current results, with CALI types

TABLE 3 Impact of chemotherapy and histopathological parameters on hepatocytes isolation in terms of number of cells (millions), yield (million(s) of viable cells per gram of tissue), and cell viability (%)

		No. of cells	*p*	Isolation yield	*p*	Viability	*p*
Neoadjuvant chemotherapy	No	438 ± 451.5	0.24	7 ± 6.3	0.84	67.4 ± 18.8	0.55
	Yes	336 ± 322.1		7.3 ± 6.4		65.1 ± 21.6	
	≥ 6 cycles	346 ± 306.8	0.4	7.4 ± 5.4	0.37	69.7 ± 17	0.9
	≥ 12 cycles	385 ± 354	0.8	6.5 ± 4.8	0.8	70 ± 14	0.8
Targeted therapy	No	418.6 ± 431.3	0.29	7.2 ± 6.3	0.75	67.3 ± 18.7	0.73
	Yes	337.7 ± 328.5		7 ± 6.4		64.4 ± 22.8	
Fibrosis	No	407.5 ± 400.5	0.4	7.7 ± 6.5	0.064	67.3 ± 20	0.051
	Yes	385.1 ± 436.5		5.6 ± 5.8		60.9 ± 20.9	
Fibrosis ≥ F2	No	413 ± 411.4	0.2	7.5 ± 6.3	0.057	67 ± 19.6	0.096
	Yes	305.4 ± 350.5		5.2 ± 6.9		56.4 ± 25.4	
Steatosis ≥ 30%	No	367.6 ± 336	**0.021**	8 ± 6.6	**0.004**	65.4 ± 22.2	0.4
	Yes	121.3 ± 112.6		2.1 ± 2.3		60.8 ± 21.7	
Malignant tumor	No	794.1 ± 715	**0.022**	8.3 ± 6.3	**0.041**	69.9 ± 18.7	0.216
	Yes						
	Primary	312.5 ± 401		4.9 ± 5.2		61.6 ± 22.6	
	Secondary	370.3 ± 327.2		7.7 ± 6.5		67.3 ± 19.5	
SOS	No	331 ± 352.3	0.59	7.3 ± 7.4	0.53	63.9 ± 24.3	0.72
	Yes	334.6 ± 297		7.1 ± 5		68.2 ± 18	
	SOS 2–3	233.9 ± 155.2	0.57	6.7 ± 4.6	0.89	70.3 ± 15.4	0.7
NAS	No	350 ± 320.8	0.39	7.2 ± 5.2	0.41	69.7 ± 18.7	**0.046**
	Yes	311.6 ± 327		7.1 ± 7.4		61.7 ± 23.6	
	NAS 2–5	146 ± 273.5	**0.011**	2.9 ± 5.4	**0.009**	48 ± 27.9	**0.042**

For each raw, the *p* value is given for the comparison between the absence (No) versus the presence (Yes) of the factor to a varying degree. Significant *p* values (< 0.05) are indicated in bold

differing according to the regimen used.[18,24] Previous studies reported globally similar rates of post-hepatectomy morbi-mortality between patients with versus without preoperative chemotherapy.[25,26] Nevertheless, prolonged chemotherapy for more than 6 or 12 cycles reportedly elevated the risk of morbidity, in particular PHLF.[27,28] Accordingly, we showed here via HBS that the impairment in liver function involved only the heavily treated patients who had received 12 or more cycles of chemotherapy; this functional impairment was observed despite a mean cessation period of chemotherapy before surgery of 1 month and the previously reported reversibility of CALI.[29,30] Besides, there was no impact of preoperative biotherapy on HBS results, in agreement with the absence of deleterious effect of bevacizumab or cetuximab on the liver regeneration capacities.[31,32] Considering the current results, HBS may be worth in the selection of heavily treated patients before surgery and in the decision of postponing surgery 6–8 weeks after the cessation of chemotherapy to permit recovery of liver function.[11]

Regarding more specifically CALI, we confirmed that CALI were associated with increased morbidity, in particular PHLF. Accordingly, we showed here that liver function assessed via HBS was impaired in case of NAS, and this was even more pronounced for NAS grades 2–5. By contrast, other authors reported that there was no correlation between the liver function assessed by ICG-R15 value and steatohepatitis.[33,34] On the other hand, deterioration of the ICG-R15 has been reported in some patients with post-chemotherapy SOS,[35,36] although not confirmed or with borderline significance in other studies [33,34] or in the current one (subjected to the low number of patients with significant SOS). As a potential explanation for these discrepancies, SOS primarily affects non-hepatocellular (endothelial) cells, impairing less the hepatocellular mebrofenin uptake than steatosis and/or NAS.[37] Moreover, ICG-R15 is known to depend on the portal flow, suggesting that abnormal ICG-R15 value may rather reflect the consequences of SOS, namely portal hypertension, than SOS itself.[38–41] Narita et al. [35] thus demonstrated a strong correlation between ICG-R15 and the expression of CD34, an antibody used to detect sinusoidal capillarization of the

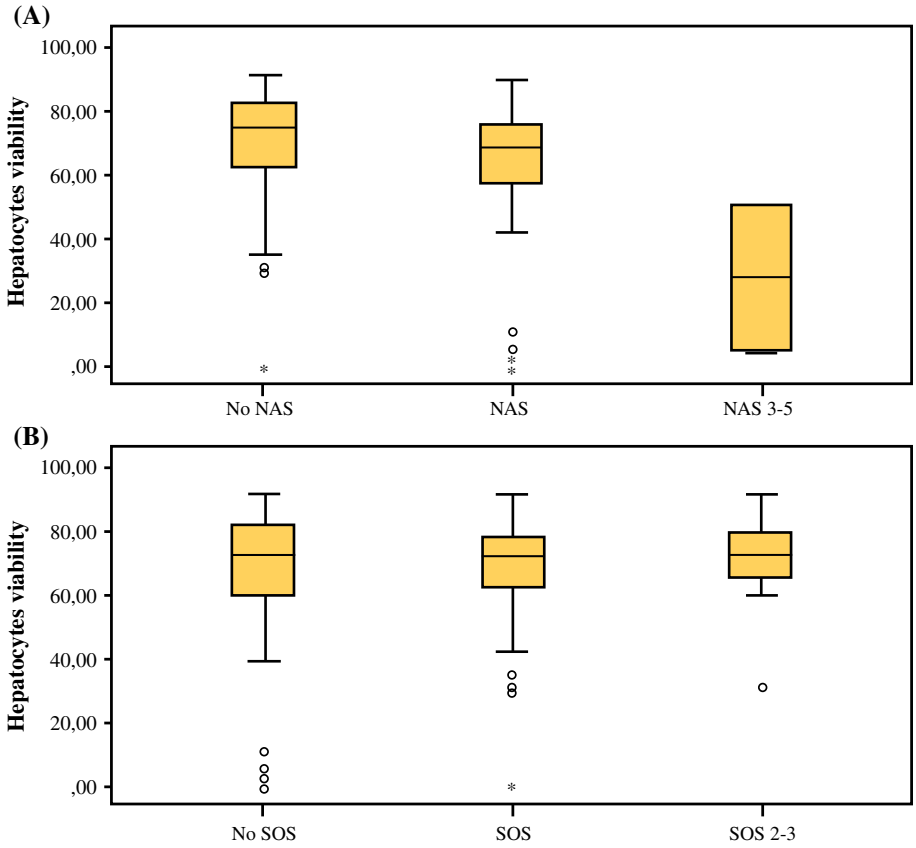

FIG. 2 Box plot showing the hepatocytes viability according to **a** the presence or absence of NAS or NAS grade 2–5 ($p < 0.05$ between patients without versus with NAS—whatever the grade); **b** the presence or absence of SOS or severe SOS grade 2–3 (nonsignificant differences) in patients who received chemotherapy in the 3 months before surgery

sinusoidal epithelium within nontumoral liver parenchyma adjacent to SOS. More importantly, ICG-R15 reflects the whole liver function, with no consideration of the heterogeneous distribution of hepatocellular function within the liver, which was clearly shown here. HBS—by allowing segmental analysis of liver function, in particular that of the FRL—is likely to overcome the limitations of ICG-R15 and to identify precisely patients with CALI.

Clinically significant steatosis ($\geq$ 30%) was among the factors affecting the hepatic function. The link between chemotherapy and steatosis is not clear, with some studies suggesting that patients who receive chemotherapy develop steatosis, in contrast to others [24, 42–45]; indeed, other variables, such as steroids administered to manage nausea, weight gain during chemotherapy, high BMI or diabetes mellitus may interfere.[46–48] In a series of 146 patients undergoing liver resection within 3 months of preoperative chemotherapy, being overweight (BMI > 27) was the only risk factor associated with steatosis ($\pm$ steatohepatitis).[29] Whatever the mechanisms, a major resection in patients with severe steatosis is reportedly more difficult, with an increased risk of bleeding and complications [46,47] and a slower regeneration rate.[20] This is in agreement with the negative impact of steatosis on liver function observed in the current study, as well as the deleterious effect of high BMI or diabetes mellitus that are often intricate. Likewise, some recent studies showed an impaired liver function in obese patients scheduled for bariatric surgery, which was corrected by weight loss following Roux-en-Y gastric bypass.[49] Regarding the impact of diabetes, liver perfusion was shown to be reduced in diabetic patients compared with nondiabetic ones,[50, 51] potentially explaining the impaired hepatic function observed herein.

Besides, parameters of hepatocytes isolation may give a good idea of hepatocytes viability and function: the hepatocyte isolation outcome directly impacted the results of hepatocytes engraftment in mice[52] and seemed to be predictive of the postoperative outcome in 51 patients,[53] although these preliminary results need to be confirmed. Regarding the impact of chemotherapy, Hewes et al.[54] previously reported no influence of pre-resection chemotherapy on the culture integrity of isolated hepatocytes in agreement with the current results. The impact of CALI was so far unknown. We showed that only NAS significantly impaired the hepatocytes viability, and this was paralleled by impaired function. In literature, such CALI indeed appeared to be a much more dangerous entity associated with a higher mortality.[24, 48, 55] In patients with steatohepatitis, it has been reported that the ability of the liver to recover from adenosine 5'-triphosphate depletion is severely impaired, and liver regeneration is diminished.[56, 57] Presumably, the yield and viability of hepatocytes may

depend on their ability to overcome challenging conditions, such as alteration of the vessels network (e.g., severe steatosis narrowing vessels, histopathological alterations associated with malignant tumors) in association with lobular inflammation in case of NAS.[55, 58]

Overall, the results of the current study prompt gastroenterologists, oncologists, and surgeons to a heightened awareness of CALI, especially in patients who have received long courses of chemotherapy and those at risk of NAFLD as a result of obesity, diabetes, or hyperlipidemia. Radiographic methods cannot accurately identify NAS or SOS; liver function tests are generally not helpful in diagnosis, because many patients have normal laboratory values despite substantial hepatic injury. Biopsy is the definitive method for the diagnosis of CALI, but it has been associated with a risk of sampling error due to the heterogeneous distribution of liver injury. Nevertheless, Bedossa et al. recently showed that, while only 65% of biopsies relying on 15-mm samples led to correct diagnosis, 75% of biopsies relying on 25-mm samples provided accurate information.[59] In patients with suspicion of or at high risk for hepatic injury, laparoscopy combining direct inspection and a liver biopsy measuring at least 25 mm may be a useful method for evaluation of CALI, as proposed by previous authors.[24]

There are several limitations to our study. Jaundiced patients may have impaired HBS results in the group without neoadjuvant chemotherapy, although all HBS were performed after biliary drainage. Furthermore, we used a consistent chemotherapy regimen to allow accurate correlation with pathologic injuries, but in practice many patients now are exposed to multiple chemotherapeutic agents before surgery. Nevertheless, results were analyzed according to the CALI observed, irrespective of the chemotherapy used. Moreover, we did not perform a systematic reanalysis of SOS and NAS in untreated patients, although Rubbia-Brandt et al. reported no CALI in patients treated by surgery alone.[45] Only few patients had both HBS and hepatocytes isolation as treated during different time periods—precluding any comparative analysis—although with the same chemotherapy regimen. Last, we did not analyze the all types of CALI; in particular, we had too few patients with severe NAS (grades 4–5), severe oxaliplatin-related lesions (which are much more frequent after hepatic arterial infusion of oxaliplatine[60]), peliosis, or nodular regenerative hyperplasia—but only the most frequent ones and those that have been associated with a worse postoperative course. Overall, the study population was likely representative regarding the significant number of chemotherapy cycles (median of 9.6 ± 7.4) in preoperatively treated patients.

CONCLUSIONS

HBS may provide an important functional test of liver reserve in patients treated by chemotherapy, especially those with prolonged chemotherapy or at risk of steatosis or NAS. Laparoscopy before laparotomy in patients with preoperative HBS that suggests CALI should be considered to directly evaluate the liver and carry out a large liver biopsy. Considering the reversibility of CALI, postponing surgery in patients with impaired HBS function is recommended.

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