Assessment of Hepatic Steatosis by Expert Pathologists

The End of a Gold Standard

Ashraf Mohammad El-Badry, MB BCh, MCh, MD, Stefan Breitenstein, MD,* Wolfram Jochum, MD,† Kay Washington, MD,‡ Valérie Paradis, MD,§ Laura Rubbia-Brandt, MD,¶ Milo A. Puhan, MD, PhD,‖** Ksenija Slankamenac, MD,* Rolf Graf, PhD,* and Pierre-Alain Clavien, MD, PhD**

Background: The presence of fat in the liver is considered a major risk for postoperative complication after liver surgery and transplantation. The current standard of quantification of hepatic steatosis is microscopic evaluation by pathologists, although consistency in such assessment remains unclear. Computerized image analysis is an alternative method for objective assessment of the degree of hepatic steatosis.

Methods: High resolution images of hematoxylin and eosin stained liver sections from 46 consecutive patients, initially diagnosed with liver steatosis, were blindly assessed by 4 established expert pathologists from different institutions. Computerized analysis was carried out simultaneously on the same sections. Interobserver agreement and correlation between the pathologists' and computerized assessment were evaluated using intraclass correlation coefficients (ICC), Spearman rank correlation coefficients, or descriptive statistics.

Results: Poor agreement among pathologists (ICC: 0.57) was found regarding the assessment of total steatosis, (ICC >0.7 indicates acceptable agreement). Pathologists' estimation of micro- and macrosteatosis disclosed also poor correlation (ICC: 0.22, 0.55, respectively). Inconsistent assessment of histological features of steatohepatitis (lobular inflammation, portal inflammation, hepatocyte ballooning, and Mallory hyaline) was documented. Poor conformity was also shown between the computerized quantification and ratings of 3 pathologists (Spearman rank correlation coefficients: 0.22, 0.82, 0.28, and 0.38).

Conclusion: Quantification of hepatic steatosis in histological sections is strongly observer-dependent, not reproducible, and does not correlate with the computerized estimation. Current standards of assessment, previously published data and the clinical relevance of hepatic steatosis for liver surgery and transplantation must be challenged.

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Most surgeons rely on histologic diagnosis of hepatic steatosis to make a decision on surgical intervention on the liver or to accept a potential liver graft. The gold standard of assessment of hepatic steatosis is histologic evaluation by pathologists.[1] Hepatic steatosis is characterized quantitatively (percentage of hepatocytes containing lipid droplets) and qualitatively according to the size of the droplets (micro- and macrosteatosis).[2]

Although hepatic steatosis is a widely accepted risk factor for postoperative complications after major hepatectomy and orthotopic liver transplantation (OLT)[3–7]; studies have been inconsistent regarding the relevant amount of fat or type of fat necessary to cause injury; even a few studies have failed to document such a negative effect, all those observations leading to controversies in the field.[8–10] Moreover, the influence of micro- versus macrosteatosis remains debatable.[1] For example, Yoong et al[11] showed that liver grafts containing moderate degrees of microsteatosis significantly increase the rate of organ failure after OLT, while other groups demonstrated comparable 1-year graft survival with those receiving nonfatty grafts, and thus recommended the use of microsteatotic grafts, regardless of the total amount, to safely expand the donor pool.[12,13]

Pitfalls in the assessment of steatosis include liver tissue sampling and histologic work-up. For instance, a single biopsy may not mirror the fatty change in the whole organ.[14] Varieties of hepatic steatosis such as focal steatosis,[15] hypersteatosis,[16] or hepatic fatty sparing[17] could lead to misleading interpretation. Tissue fixatives can induce factitious fusions or the collapse of lipid droplets.[18] Visualization of lipid droplets is obviously influenced by the staining method.[19] Moreover, the histologic evaluation of hepatic steatosis completely ignores the lipid composition. Depending on the presence or absence of certain unsaturated fatty acids, hepatocytes may be susceptible, or protected, from ischemia/reperfusion injury.[20–22]

All those potential pitfalls might be corrected by multiple sampling and standardization in processing biopsies. However, another possible bias, which might definitively question the value of histology in assessing fat in the liver, is the degree of consistency among pathologists in interpreting the biopsies. This aspect has been poorly evaluated, particularly regarding interpretation of pathologists among different centers. Therefore, we have focused the current study on this specific aspect. To exclude interobserver variability, computerized programs were developed to objectively quantify hepatic steatosis by determining the area occupied by lipid droplets in a given field of a liver section.[23–25] Previous studies on the interobserver variability among pathologists and the correlation between pathologists' and computerized evaluations are limited by the assessments of pathologists from the same institution and with different levels of experience.[25,26]

The aim of this study was to investigate the concordance among 4 internationally renowned pathologists from 4 different centers in Europe and North America regarding the quantitative and qualitative assessment of liver steatosis. Each pathologist had previously published experimental and clinical studies in the field of steatosis. In addition, the correlation between the pathologists' and the computerized evaluations was analyzed.

From the *Department of Surgery, Swiss HPB (Hepato-Pancreatico-Biliary) Center, University Hospital of Zurich, Zurich, Switzerland; †Department of Pathology, University Hospital of Zurich, Zurich, Switzerland; ‡Department of Pathology, Vanderbilt University Medical Centre, Nashville, TN; §Department of Pathology, Beaujon Hospital, Paris, France; ¶Department of Pathology, University Hospital of Geneva, Geneva, Switzerland; ‖Horten Centre for Patient-Oriented Research, University Hospital of Zurich, Zurich, Switzerland; and **Department of Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD.

Ashraf M. El-Badry and Stefan Breitenstein contributed equally as first authors.

Reprints: Pierre-Alain Clavien, MD, PhD, Department of Surgery, University Hospital of Zurich, Raemistrasse 100, 8091 Zürich, Switzerland. E-mail: clavien@chir.uzh.ch.

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MATERIALS AND METHODS

Study Design, Population

We conducted a cohort study on 46 consecutive patients undergoing any type of liver resection with liver steatosis of >5% (histologically assessed at the liver parenchyma on the resected liver specimen). Patients were treated between 2006 and 2007 in a single tertiary care center (Swiss Hepato-Pancreatico-Biliary Center, University Hospital of Zurich, Switzerland).

Four expert pathologists from 4 prominent centers in 3 countries across Europe and North America (Switzerland, France, and United States) were asked to participate in the study. The histologic evaluation was based on dynamically magnifiable digitalized images of liver tissue sections, which were uploaded to a server on the World Wide Web. At the same time, steatosis was quantified in the same sections by a computerized image analysis program developed in our center. Pathologists were blinded to the assessment of each other and to the computerized evaluation. The interobserver variability regarding the quantitative and qualitative assessments of steatosis and steatohepatitis and the agreement between the pathologists' and the computerized evaluations were analyzed.

Preoperative Patient Data

Demographic data included age, gender, body mass index (BMI), and diagnosis. Other relevant clinical conditions, such as diabetes and obesity (defined as BMI $\geq$30 kg/m^2), were also recorded. Preoperative chemotherapy was defined as chemotherapy used for downsizing or neoadjuvant treatment of liver tumors. The following biochemical blood variables were assessed preoperatively: prothrombin time, total bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, and creatinine. Cholestasis was defined as serum bilirubin levels $\geq$35 μmol/L ($\geq$2 mg/dL).

Histologic Evaluation

A slide scanner (Hamamatsu Nanozoomer) was used for the generation of virtual images from hematoxylin and eosin (H&E) stained sections (about 1 $\times$ 1 cm) of resected liver specimens with dynamic magnification up to 40$\times$ with 0.23 μm/pixel resolution. The images, available on the World Wide Web (available at: http://histodb2.usz.ch/dss), were then histologically assessed by the 4 blinded expert pathologists. Prior to conducting the study, each pathologist agreed on evaluation of 7 histologic features for steatosis and steatohepatitis per slide (Table 1). The extent of total hepatic steatosis was evaluated by estimation of the percentage of hepatocytes containing lipid droplets; irrespective of location of the hepatocyte nucleus or the number and size of lipid droplets. The qualitative assessment was concluded from the percentage of hepatocytes harboring micro- or macrosteatotic droplets. Microsteatosis indicates presence of small lipid droplets measuring less than 1 μm and filling the hepatocyte cytoplasm with centrally located nucleus while macrosteatosis refers to hepatocytes containing single lipid droplet, which displaces the nucleus to the periphery of the cell.[1]

TABLE 1. The Histological Criteria Used By Pathologists to Assess the Extent of Steatosis and Steatohepatitis

Histological Feature	Interpretation
Total steatosis	Percentage (no. hepatocytes containing lipid droplets/total no. hepatocytes)
Microsteatosis	Percentage (no. hepatocytes containing small lipid droplets with centrally located nucleus/ total no. hepatocytes)
Macrosteatosis	Percentage (no. hepatocytes containing single large lipid droplet with peripherally displaced nucleus/total no. hepatocytes)
Lobular inflammation	Present/absent
Portal inflammation	Present/absent
Hepatocyte ballooning	Present/absent
Mallory's hyaline	Present/absent

Quantitative data is given for total or subtype of steatosis. The other parameters are qualitatively assessed and judged as either present or absent.

Computerized Imaging Assessment

Computerized image analysis programs, available in some centers, were introduced for the objective quantification of hepatic steatosis.[23,25] Using an automated software, Marsman et al[25] reported that the computerized quantification of hepatic steatosis correlates with the visual interpretation by pathologist. We developed a computerized program for estimation of total hepatic steatosis in exactly the same images that were assessed by the pathologists. About 50 nonoverlapping and randomly chosen regions were used per slide. The images of the selected regions were stored in tagged image file format (tiff) with no compression. A 2-step algorithm was applied to eliminate the confusion between lipid droplets and the background in H&E stained liver sections. At the outset, all background pixels were regarded as fatty areas. An image consisted of red, green, and blue pixels. Only the green channel was taken, magenta parts of the image appeared dark and could easily be distinguished from the white parts of the fat. In the second step, lipid droplets were distinguished by their shape and color characteristics from nonfatty tissues such as vessels and other possible artifacts, eg, tissue cracks. A given area was considered lipid droplet if disclosing bright light information and rounded shape (matches with the minimal circle which covers all pixels of the selected region, or the arc length in the case of large areas). Steatosis was defined for each slide as the percentage of surface areas considered as lipid droplets, divided by the total surface areas of the selected 50 regions. Finally, images of the selected regions were stored as joint photographic expert group (jpeg) in 20$\times$ magnification (width and height are 1000 pixels).

Statistical Analysis

To assess interobserver agreement on the extent of hepatic steatosis (expressed in %), we used intraclass correlation coefficients (ICCs). Variability within pathologists was in the numerator, whereas variability among and between pathologists (noise) was in the denominator. Values for ICCs are between 0 and 1 and values $\geq$0.7 represent acceptable agreement. For the correlation between the pathologists' and computerized assessments, we used Spearman rank correlation coefficients. Finally, to assess agreement for the presence of specific histopathological features (eg, Mallory bodies), we used descriptive statistics to describe variability across pathologists. We conducted all analyses using STATA (version 10, Stata Corp., College Station, TX).

RESULTS

Was the Patient Collective Representative?

A total of 46 consecutive patients who underwent surgical resection of benign or malignant hepatic neoplasm were enrolled in the study. Thirty-one patients (67%) were men, the mean age was 59 (range: 26–81) years. As expected, the BMI was relatively high (28 $\pm$ 4), 11 patients (24%) were obese (BMI, $\geq$30 kg/m^2). Other clinical conditions such as preoperative diabetes, cholestasis, and previous chemotherapy are summarized in Table 2.

Did the Pathologists Agree on the Assessment of Liver Steatosis?

The pathologists were firstly asked to indicate the total number (and percentage) of hepatocytes containing lipid droplets. This quanti-

TABLE 2. Demographic, Clinical and Preoperative Data of the Patients

Preoperative Characteristics	Steatosis (n = 46)
Age, mean ± SD	59 ± 12
Age, range	26–81
Gender, male/female, number (%)	31/15 (67/32)
Benign/Malign disease, number (%)	10/36 (22/78)
Body mass index (kg/m^2), mean ± SD	28 ± 4
Obesity (>30 kg/m^2) (%)	11 (24)
Diabetes mellitus, number (%)	6 (13)
Cholestasis (≥35 μmol/L) (%)	3 (7)
Preoperative chemotherapy, number (%)	18 (39)
Oxaliplatin and/or Irinotecan (%)	7 (15)
Fluorouracil	9 (20)
Capecitabine	2 (4)
Bilirubin (μmol/L), mean ± SD	14.1 ± 11.9
Prothrombin time (sec), mean ± SD	96.0 ± 8.1
Creatinine (μmol/L), mean ± SD	84.9 ± 19.7

SD indicates standard deviation.

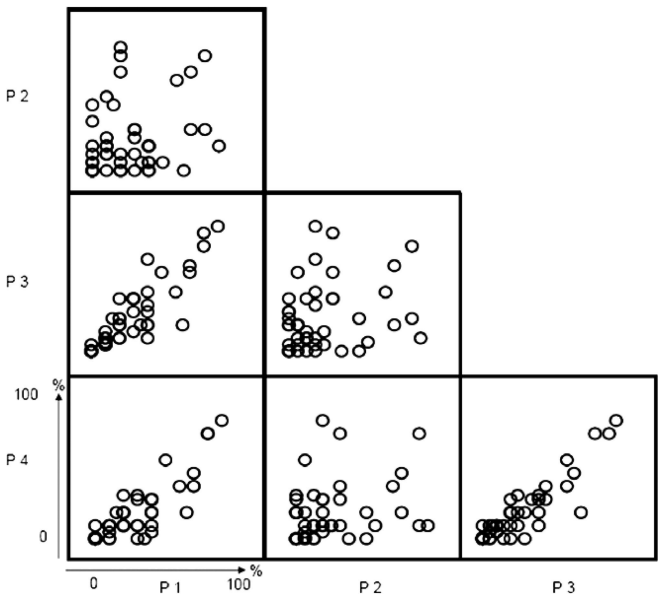

FIGURE 1. Correlation among pathologists' estimation of total steatosis. Individual pathologists' results were blotted against each other. Each axis (per square) represents the extent of steatosis in percent. The pathologist' identity was blinded and indicated by a number (P1, 2, 3, and 4). Intraclass correlation coefficient was poor (0.57).

tative assessment is assumed to be correlated with postoperative complications after major hepatectomy and OLT. Surprisingly, no significant correlation could be demonstrated among pathologists. The intraclass correlation coefficient (0.57) indicates poor agreement (Fig. 1). The percentages of hepatocytes containing lipid droplets are given for each pathologist as summarized in Table 3. There was also a wide range of variation among the pathologists with regard to the semiquantitative assessment of steatosis. The discrepancy among pathologists was more evident in the diagnosis of high (moderate and sever) grades of steatosis (≥30%). For instance, high-grade steatosis was identified in 46% of the cases compared with only 22% by pathologists 1 and 2, respectively, (Table 4).

TABLE 3. The Pathologists' Quantitative Estimation of Total, Micro- and Macrosteatosis

	Pathologist 1	Pathologist 2	Pathologist 3	Pathologist 4	ICC
Total steatosis (%)	20	12.5	20	10	0.57
Microsteatosis (%)	10	5	0	0	0.22
Macrosteatosis (%)	10	5	12.5	10	0.55

Median values of the assessment of total, micro- and macrosteatosis hepatocytes were collected. The intra-class correlation coefficients (ICCs) indicate poor agreement among the pathologists regarding the quantitative (total steatosis) and qualitative (micro- and macrosteatosis) evaluations.

TABLE 4. Semiquantitative Assessment of Steatosis*

	Pathologist 1	Pathologist 2	Pathologist 3	Pathologist 4
High grade (≥30% steatosis)	21 (46%)	10 (22%)	16 (35%)	13 (28%)
Low grade (<30% steatosis)	25 (54%)	36 (78%)	30 (65%)	33 (72%)

*Absolute number of patients (%) identified as high grade (≥30%) or low grade (<30%) steatosis.

How Accurate Was the Agreement Among Pathologists on the Qualitative Assessment of Hepatic Steatosis?

Most pathologists consider micro- and macrosteatosis as separate entities.[20] They assess both types separately assuming that each form has a potentially different impact on the clinical outcome after liver resection and OLT. Again, there was no agreement among the pathologists for both micro and macrosteatosis (ICCs: 0.22 and 0.55, respectively) (Figs. 2, 3; Table 3). This disagreement was more pronounced than for total steatosis and suggests that these forms are even harder to differentiate.

Was the Interpretation of the Histologic Features of Steatohepatitis Consistent Among Pathologists?

Four features of steatohepatitis (lobular and portal inflammation, hepatocyte ballooning, and Mallory's hyaline) were evaluated and interpreted as absent or present. Additionally, the pathologists were asked to provide an overall diagnosis of steatohepatitis. A disagreement among pathologists was evident with regard to the assessment of all of the parameters as well as the final diagnosis of steatohepatitis (Table 5).

How did the Pathologists' Evaluation Correlate With the Computerized Assessment of Hepatic Steatosis?

Our computerized image analysis program was used for evaluation of hepatic steatosis by estimation of the surface area occupied by lipid droplets in a given field of liver section (Fig. 4). In 33 of 46 patients, less than 5% of the surface area of the randomly selected regions of each liver section was occupied by lipid droplets. The remaining 13 disclosed steatosis ranging from >5% to 20%. The computerized evaluation correlated significantly only with 1

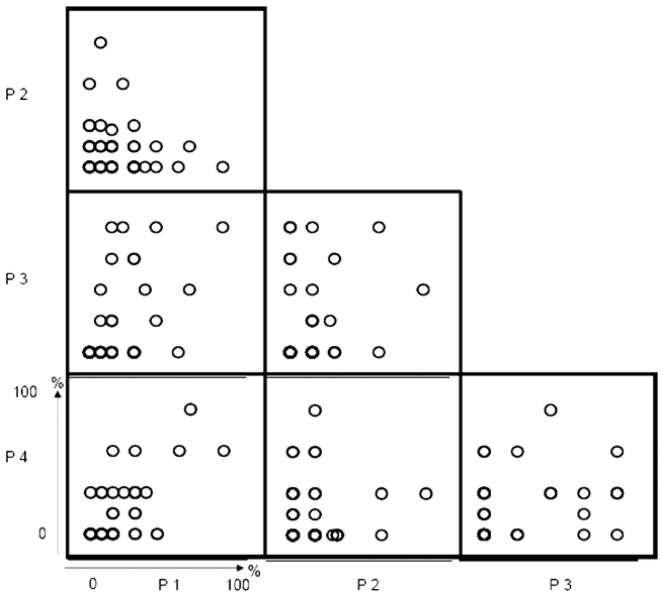

FIGURE 2. Correlation among pathologists' estimation of microsteatosis. Intraclass correlation coefficient was poor (0.22), (further details are presented in legend of Fig. 1).

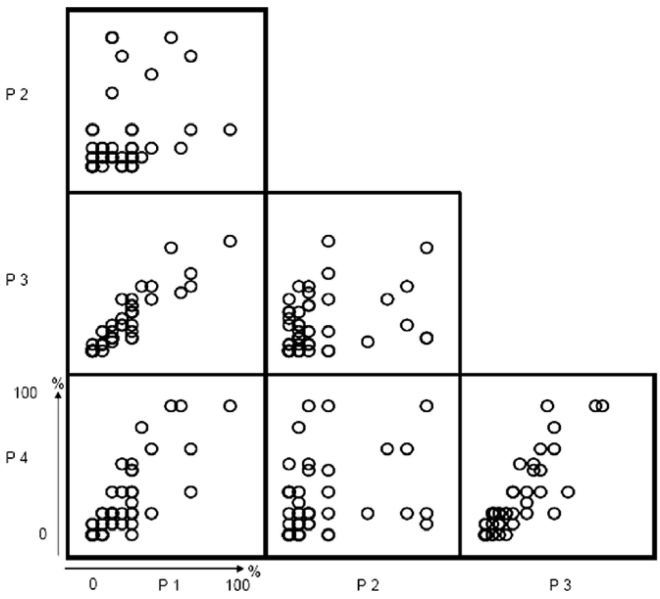

FIGURE 3. The correlation among pathologists' estimation of macrosteatosis. Intraclass correlation coefficient was poor (0.55), (further details are presented in legend of Fig. 1).

pathologist (Spearman rank correlation coefficient: 0.82), whereas poor conformity was documented with the other 3 (Spearman rank correlation coefficient: 0.22, 0.28, and 0.38) (Figs. 5 A–D).

DISCUSSION

In this study, we demonstrated a highly significant interobserver variability regarding both quantitative and qualitative assessments of the histologic features of liver steatosis as well as steatohepatitis. Highly experienced, internationally established pathologists, experts for all aspects of hepatic steatosis, assessed biopsies of 46 patients. Obvious

TABLE 5. The Pathologists' Evaluation of the Histological Features of Steatohepatitis

	Pathologist 1	Pathologist 2	Pathologist 3	Pathologist 4
Lobular inflammation	0 (0)	15 (32)	20 (44)	24 (52)
Portal inflammation	15 (32)	19 (41)	18 (40)	25 (54)
Hepatocyte ballooning	1 (2)	14 (30)	16 (35)	8 (17)
Mallory hyaline	1 (2)	8 (17)	11 (25)	7 (15)
Steatohepatitis (overall diagnosis)	0 (0)	9 (19)	12 (26)	2 (4)

Assessment of parameters indicative of hepatic inflammation by 4 pathologists (1–4) The number of patients positive for a certain parameter (and the percent of total) varies considerably and indicates a strong disagreement for all parameters including the overall diagnosis.

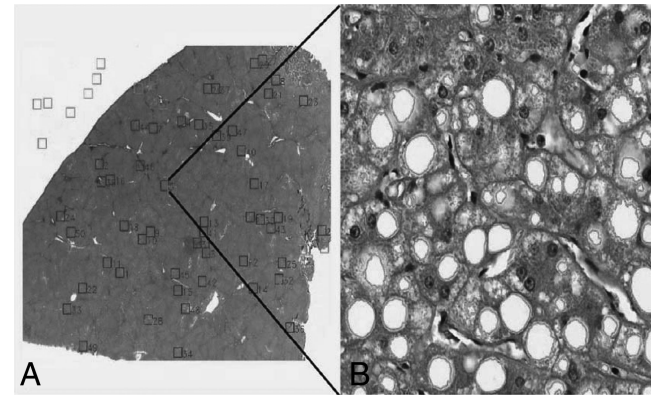

FIGURE 4. Analysis of fat content by computer assisted calculation. Fifty regions of tissue (A) were randomly selected with avoidance of vessels or nontissue areas. A higher magnification was used for examination of the regions which were considered for analysis. The computer algorithm selected white areas, but excluded vessels. The boundaries of the areas considered as lipid droplets are presented in B.

inconsistencies and disagreement were apparent among the pathologists. A computerized quantification of hepatic steatosis was also inconsistent with the pathologists' view.

To investigate the interobserver variability, we ensured that the assessment was performed on exactly the same high resolution images of liver specimens. H&E stained sections were used, which is the most commonly used method of staining in current surgical practice. The strategy to carry out histologic evaluation by 4 very experienced pathologists from 4 high volume centers across Europe and North America ensured that a mentor- or institution-related factor influencing the interpretation of hepatic steatosis was eliminated. In a single center, some concordance may exist among different pathologists as demonstrated by Younossi et al,[26] where the evaluation had been carried out by 2 senior and another 2 junior pathologists. However, as a precondition for clarifying the significance of fat in the liver for liver resection and liver transplantation, a reproducible observer- and institution-independent method is required to precisely assess hepatic steatosis.

The disagreement among blinded expert pathologists on the routine quantification of total liver steatosis, as shown in the present study, is very disturbing. A similar discrepancy was demonstrated with respect to micro- and macrosteatosis. Consequently, it seems plausible to assume that the contradiction among previous re-

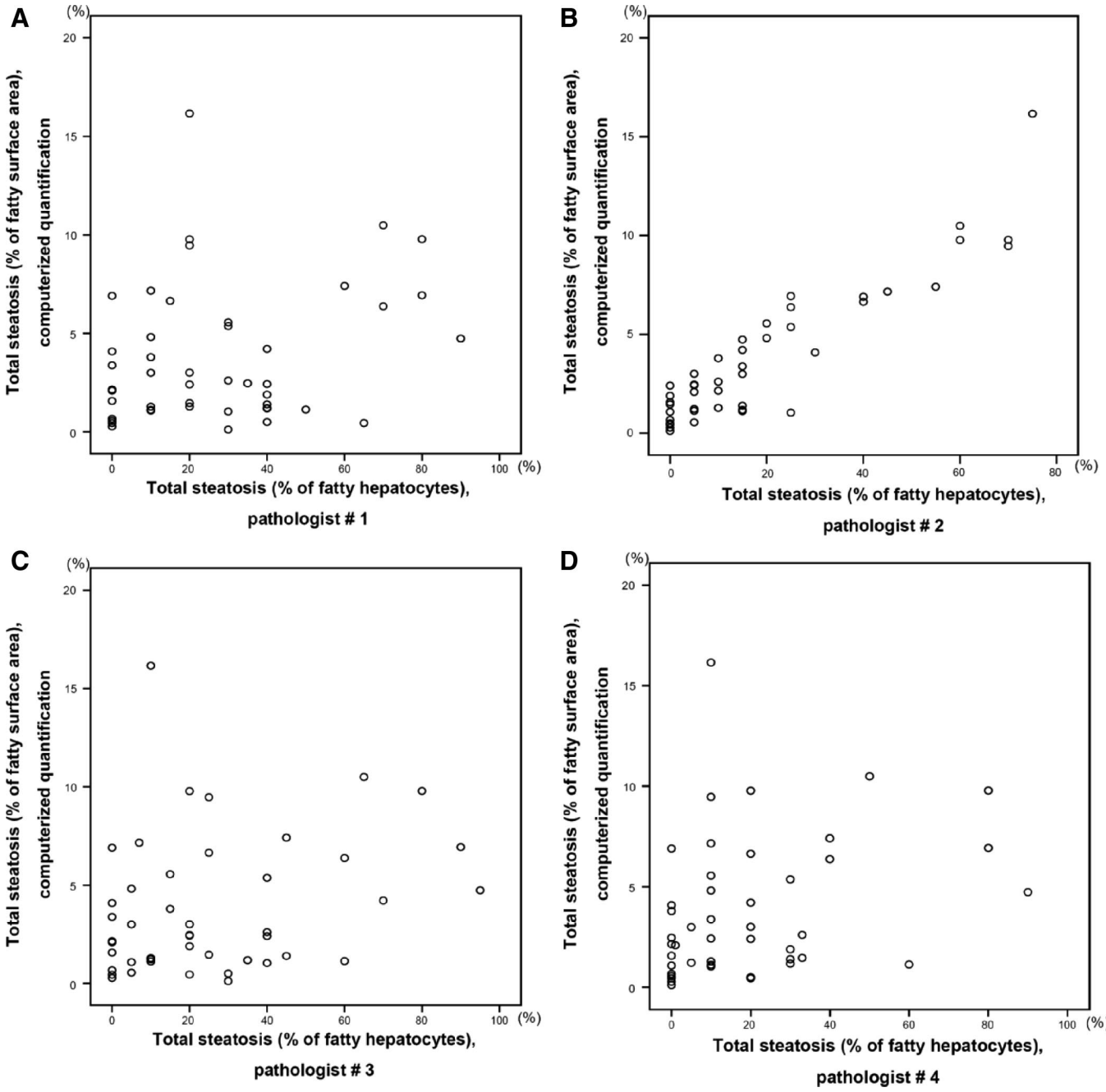

FIGURE 5. The correlation between pathologists' and computerized evaluation of hepatic steatosis. Spearman rank correlation coefficients were poor with 3 pathologists (A, 0.22), (C, 0.28), and (D, 0.38) and acceptable with 1 pathologist (B, 0.82).

ports[4–10] might be caused by the inconsistent interpretation of steatosis among various pathologists of different centers. The computerized quantification of liver steatosis is an unconventional tool to exclude observer-related biases. As expected, the rates of fat content of liver parenchyma assessed by the computerized method (surface of fat related to the whole surface of parenchyma) were in general lower than considered by the pathologists (rate of hepatocytes containing lipid droplets). However, the correlation of the computerized quantification with the pathologists' assessment was poor (with 3 of 4 pathologists). Currently, only little data exist comparing computerized steatosis quantification with the histologic assessments or clinical outcome. Marsman et al[25] reported a correlation between the visual interpretation of hepatic steatosis by 1 pathologist and automated software analysis.[25] Other studies reported an agreement between the traditional assessment of hepatic steatosis and the computerized evaluation in the settings of alcoholic steatosis and hepatitis virus infection.[27,28] Of note, none of those studies involved many expert pathologists from different international centers. Due to the lack of validation, further studies are warranted to investigate the relation between hepatic steatosis quantified by the computer-based method and the occurrence of postoperative complications after liver resection and OLT.

Surprisingly, identification of steatohepatitis as well as the identification of its histologic features was also highly variable, challenging previous attempts to develop scoring systems for estimation of the degree of steatohepatitis.[29] Steatohepatitis is a highly relevant disorder which leads to cirrhosis in 20% to 30% of patients.[30] It appears to be the main cause for cryptogenic cirrhosis,[31] which indeed carries a grim prognosis.[32] Therefore, our results highlight the necessity to establish a reliable alternative approach to accurately identify patients with such a serious disease. Thus, even on the level of a qualitative assessment, there was low agreement that was highly surprising.

At the outset, we infact planned to test the correlation between the assessment of hepatic steatosis (pathologists' assessment and computerized imaging analysis) with the clinical outcome. Due to the discrepancies among pathologists in the quantitative, semiquantitative, and qualitative assessments of steatosis, it would be irrelevant and might be confusing to compare pathologists' evaluations with clinical outcome. Further studies are warranted to test the competence of the computerized analysis of steatosis to predict complications after major hepatectomy as well as after OLT.

In conclusion, inconsistent histologic assessment of hepatic steatosis among pathologists with the highest level of expertise from well-recognized European and American centers strongly must lead to perplexing interpretation of most previous literature on the topic, and explains the divergence among the unpublished reports. This study questions the current standards of hepatic steatosis quantification, and highlights the urgent need to develop novel tools for the assessment of liver steatosis and the relevance of hepatic steatosis to liver surgery and transplantation.

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Discussions

PROFESSOR R. ADAM (PARIS, FRANCE): I have 3 critical points about the manuscript. The first is methodological. You assessed by computerized imaging the percentage of surface area considered as lipid droplets, divided by the total surface area of the selected regions and you compared this to what appeared to be different data from the pathologists; the percentage of the hepatocytes containing lipid droplets. We may assume that large droplets in macrovascular steatosis could be more "surface-occupying" than microvascular steatosis in the computerized imaging. In this situation, however, the same percentage will be reported by pathologists, since it is the proportion of hepatocytes that is considered. My first question is: do you really think that we are comparing the same thing and have you subdivided your analysis between micro- and macrovascular steatosis.

The second point concerns the low rate of steatosis. I was surprised that only a 10% to 20% steatosis rate was reported in the

patients that you evaluated. Knowing that a small variation is admitted in the estimation of steatosis between different observers, I would like to draw attention to the fact that a 5% variation in 10% steatosis represents a 50% difference, whereas for a 50% steatosis, it is only a 10% difference. This could impact the statistical view of the results. Have you stratified the inter-observer variation according to the grade of steatosis? My third concern is the clinical relevance of the disagreement that you very nicely reported from the pathologists. Owing to the fact that for both liver resection and liver transplantation, the impact of steatosis is all the more important than the degree of infiltration is high, it is likely that for up to 30% steatosis the risk of complication is low even with variation from the pathologists. Although you clearly showed that the difference was statistically significant between the computerized method and the impression of the pathologists, do you really think that these differences would have impacted the patients' clinical complications?

DR. STEFAN BREITENSTEIN (ZURICH, SWITZERLAND): Beginning with your first question regarding the comparison of the pathologists and the computer assessments, during the traditional pathologist's evaluation of hepatic steatosis, each hepatocyte containing fat, whatever its quantity, is counted as fatty; leading possibly to overemphasis of steatosis. There are also concerns about the subjectivity of this method. We agree that the computer assessed fat content differently calculating the surface covered by fat. Therefore the estimations of the computer were in general lower than the evaluations made by the pathologists.' However, it is theoretically possible to get a correlation between the 2 methods, provided that the lower estimation of the computer is constant among the different cases. That is why we used the so called intra-class correlation coefficient to assess the agreement. The results showed that the computerized evaluation of steatosis did not correlate with 3 out of 4 pathologists regarding the overall fat content. Interestingly, there was good agreement with pathologist 2 who, in line with the computerized evaluation, did consider lower rates of steatosis compared with the remaining pathologists. Micro- and macrosteatosis were not considered by the computerized analysis and therefore they were not correlated with the pathologists' evaluation separately.

Regarding your second question, the 4 pathologists identified up to 46% of the cases with a steatosis of ≥30%. The agreement among pathologists was poor independent of the degree of steatosis. With respect to your comment on the clinical relevance, we indeed believe that liver steatosis affects clinical outcome. However, the disagreement among pathologists emphasizes the need for an objective method for fat quantification, which is likely to be the computerized analysis. The clinical correlation of such an approach needs to be evaluated through further study involving large numbers of patients.

PROFESSOR D. CHERQUI (PARIS, FRANCE): In transplantation, we talk about frozen sections, which are even more difficult than this and you have not included any study of frozen sections here.

DR. STEFAN BREITENSTEIN (ZURICH, SWITZERLAND): Yes, we have not used frozen sections in this study. However, since the evaluation of hepatic steatosis is more difficult in frozen compared with paraffin embedded sections, it might be plausible to expect more disagreement among pathologists when evaluating frozen sections, which conforms to our view.

PROFESSOR D. CHERQUI (PARIS, FRANCE): There are other important factors such as the importance of a surgeon's eye and his or her experience, and it is not always the most senior surgeon that performs the harvesting; usually, we try to use a senior surgeon and we always use pathology. I am surprised by your data because we find a very good correlation between the pathology evaluation and the outcome of the grafts. My main question is; what next? What do you propose? Should we replace the pathologist with computers?

DR. STEFAN BREITENSTEIN (ZURICH, SWITZERLAND): Concerning the correlation between fat assessment by your pathologists and the outcome, we think that without a reproducible assessment, a possible association of 1 pathologist's interpretation with the clinical outcome could occur by chance while the association might still be nonexistent or weak if another pathologist, particularly from a different center, carries out the evaluation. This may be the likely reason behind the inconsistency among a number of studies with regard to the relevant amount of fat necessary to cause injury. We believe that our study challenges the current standards of the assessment and the relevance of liver steatosis and draws much attention to the need for further investigation. We think the correlation between hepatic steatosis evaluated by the computerized software and the incidence of complications should be investigated in larger studies. The same applies for the radiological (computerized tomography and magnetic resonance imaging) assessment of hepatic steatosis. Of note, the clinical impact of fat composition, particularly the polyunsaturated fatty acid content needs further investigation.

PROFESSOR K. G. TRANBERG (LUND, SWITZERLAND): You can use the computer in even more sophisticated ways. I think you should look at the correlation with the clinical outcome. I know the numbers are small, but that would be a good way to persuade other people to use the new computerized method.

DR. STEFAN BREITENSTEIN (ZURICH, SWITZERLAND): We completely agree. A larger study is needed to examine the correlation between the degree of steatosis measured by the computerized software and the incidence of postoperative complications.

PROFESSOR P. A. CLAVIEN (ZURICH, SWITZERLAND): Let me add a few comments. The computer system has not been validated yet and thus cannot be recommended at this point as a valuable tool to assess the impact of steatosis on the outcome of surgery or transplantation. Next, this provocative study may create serious anxiety in all those who must make a decision to use a fatty graft for transplantation or to perform major surgery on a yellow liver. The data show convincingly that histological evaluation of steatosis, even performed on a large wedge biopsy and assessed electively by experts, is unreliable. Worse yet, the reality in daily practice is certainly more worrisome than disclosed in this study, where the methodology was designed to eliminate bias related to the type of fat staining, the use of frozen vs. paraffin embedded sections, or the experience of the pathologists.

René Adam highlighted a critical point regarding the total amount of steatosis in our population of patients, and thereby the relevance of the findings. According to one pathologist about half of the patients had severe steatosis, while another diagnosed it in only one-quarter. Thus, the limited number of patients with a high degree of steatosis does not explain the discrepancies. Knowing from many experimental and clinical studies that steatosis is a major risk factor for postoperative morbidity and mortality, the real question now is: how to assess the risk? In other words, which fat and how much fat can we accept for surgery? I see 2 new directions; first, to develop a computer system to assess fat, which must be convincingly validated by correlating the data with outcome after surgery, and transplantation. The second solution would be, more elegantly, to develop a simple assay to measure the various types of fat in the liver to identify the good and bad ones, which would provide predictive information about the outcome after surgery.