Noninvasive assessment of liver steatosis in deceased donors: a pilot study

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Introduction

The growing imbalance between the need and the availability of liver donors has resulted in an increasing use of extended liver criteria donors, which are more likely to have hepatic steatosis (HS) with a risk of early graft dysfunction[1] in case of severe (≥ 67%) macro-vesicular steatosis. While accurate evaluation of HS is crucial for optimal donor selection, the diagnostic performance of noninvasive criteria for liver steatosis using clinical, biological, and morphological criteria remains limited and prevents a reliable assessment in daily practice. On the opposite, frozen section analysis of liver biopsy remains the gold standard to evaluate HS but its systematic use is not realistic in the very specific organizational setting of organ procurement. Altogether, the assessment of the degree of HS currently relies on visual inspection of the liver by an often junior surgeon, and may lead to inappropriate liver grafts selection.

In this context the development of an easy-to-use, handy and accurate device for non-invasive quantification of HS would obviously be most desirable. As smartphones have become ubiquitous in modern culture and now provide high quality pictures, a relevant use would be to obtain standardized images with instant processing by a dedicated application that would automatically evaluate liver steatosis. However, the feasibility of correlating imaging data obtained by smartphone cameras during liver procurement with HS as assessed by histological examination still remains to be assessed.

Methods

In the present pilot study, all grafts procured by the Beaujon Hospital Liver procurement team between January 2017 and February 2017 were included. These grafts all underwent biopsy and pictures. First, HS was estimated on triangular frozen section biopsies harvested on the left lobe during liver procurement (in case of suspected severe HS) or after reperfusion during LT as a routine procedure at our Institution. Steatosis was assessed based on the percentage of hepatocytes with macrovesicular steatosis using a quantitative scale up to 100%. According to the widely used scoring approach, HS was categorized as: Normal (grade 0) macrovacuolar steatosis from 0% to 5%; Mild (grade 1) macrovacuolar steatosis from 6% to 33%; Moderate (grade 2) macrovacuolar steatosis from 34% to 66%; Severe (grade 3) macrovacuolar steatosis >67% [2]. Second, digital pictures of the livers were taken during liver procurement using the same commercially available smartphone (Iphone 6S- Apple Inc, Cupertino, CA, USA). Exposure, white-balancing and focus area selection modes were commonly nonadjustable. Pictures of the livers were taken in situ before aortic cross-clamp with the highest resolution. The graft was photographed from a distance of approximately 30 cm with automatic flash light; the operating lights were turned away from the graft and maximal externalization of the liver from the abdominal cavity was applied. A set of images was created by manually cropping the original photos so the target organ occupies 85-100% of the frame. A team of dedicated engineers then processed the images using the CaptureProof software (ComputerVision Technology). The cropped frames were converted from Red Green Blue (RGB) to Hue Saturation and Value (HSV). The Hue (one of the main properties of a color, defined technically as "the degree to which a stimulus can be described as similar to or different from stimuli that are described as red, green, blue, and yellow) channel of the image was isolated and used to create a frequency histogram of all Hues in the image. The dominant Hue of the image was the red. The peaks of the reds Hues histograms were characterized as an asymmetric Gaussian (**Figure 1**) and the results values were characterized as follows: **μ** : Peak location of the most frequent colour; **σ** : Width of the peak; and **r** : Asymmetry of the peak. Finally, histogram characteristics (*μ*, *σ* and *r*) were correlated to HS percentage of liver grafts using the Spearman and Pearson correlation coefficients as appropriate.

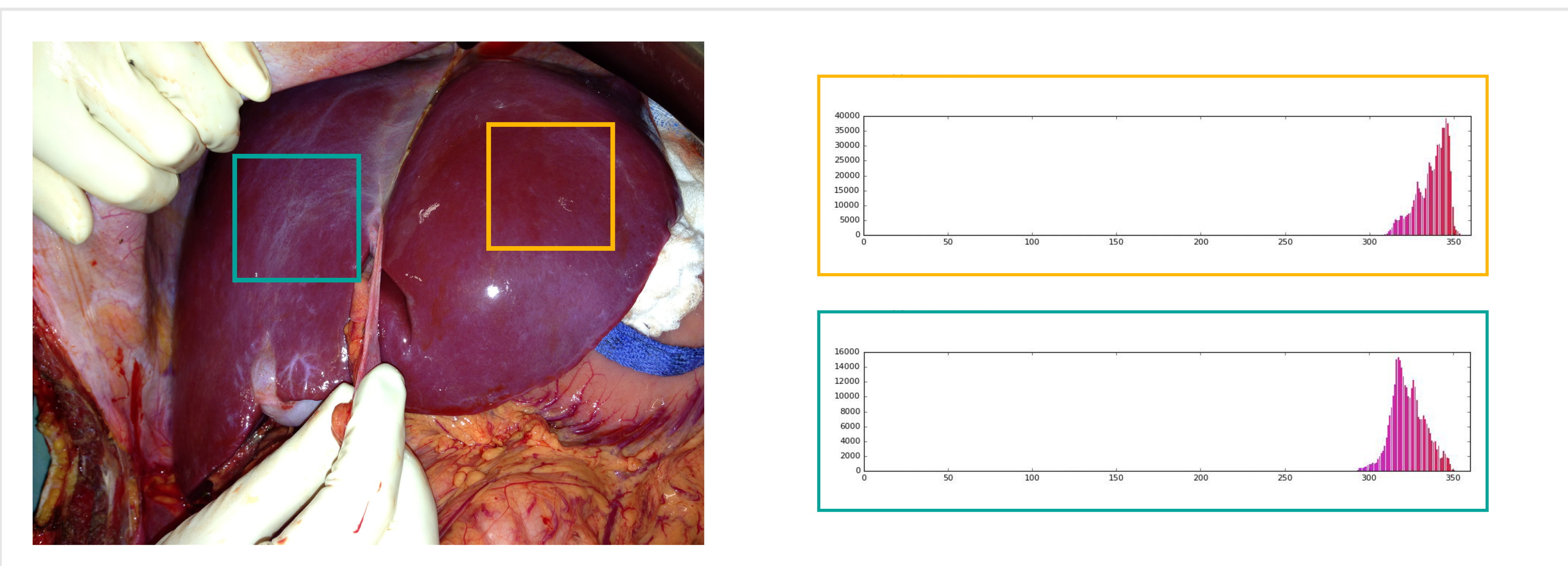

Figure 1. Color profile of two lobes. The Hue channel of the image was isolated and used to create a frequency histogram of all Hues in the image. For each Hue value in the histogram that meets minimum frequencies of 0.5% of total pixels in the image, an image slice was created that maps the location of all pixels of that hue. The cartesian centre of mass is calculated for each slice. Each centre-of-mass coordinate, and its associated Hue value, is stored in an array. The dominant Hue of the image is determined by identifying the range of slices and hues with the most consistent sequential positions. The results are delivered through the analysis of the HUE channel.

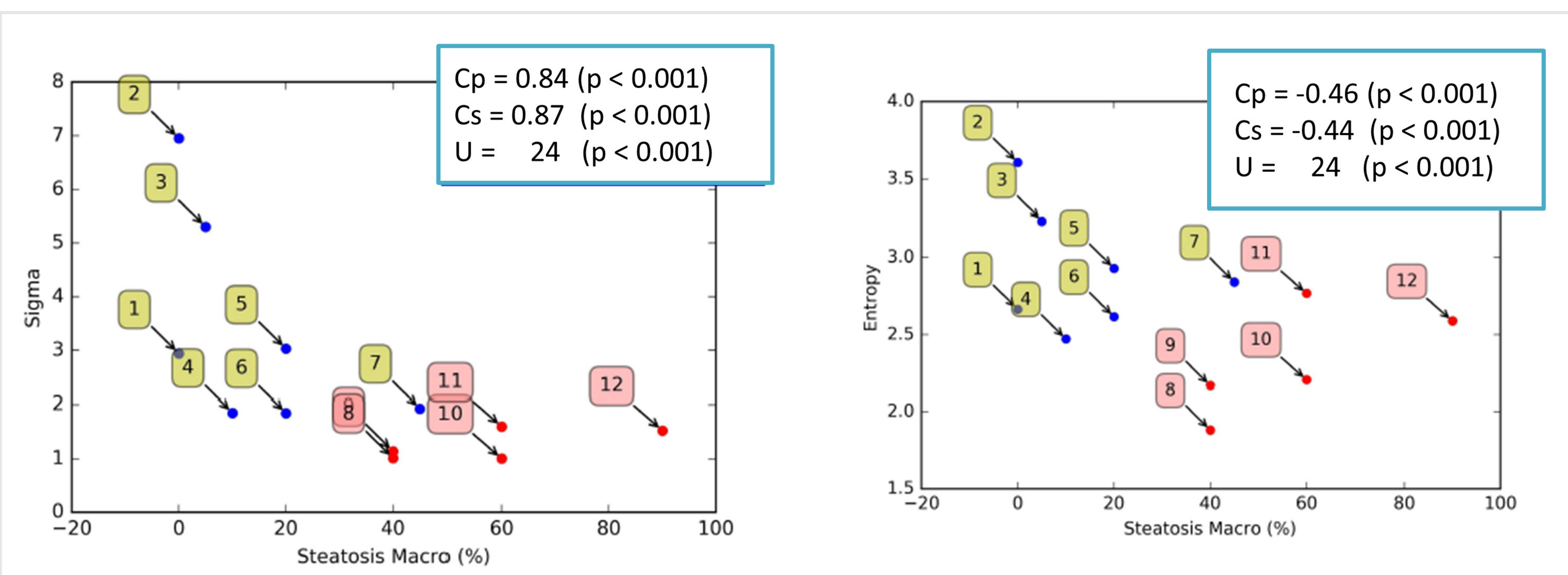

Figure 3. Sigma and image entropy of HUE. Sigma is the width of the color distribution, R is the asymmetry of the sigma. Entropy is similar to sigma (it is the disorder of the image HUE)

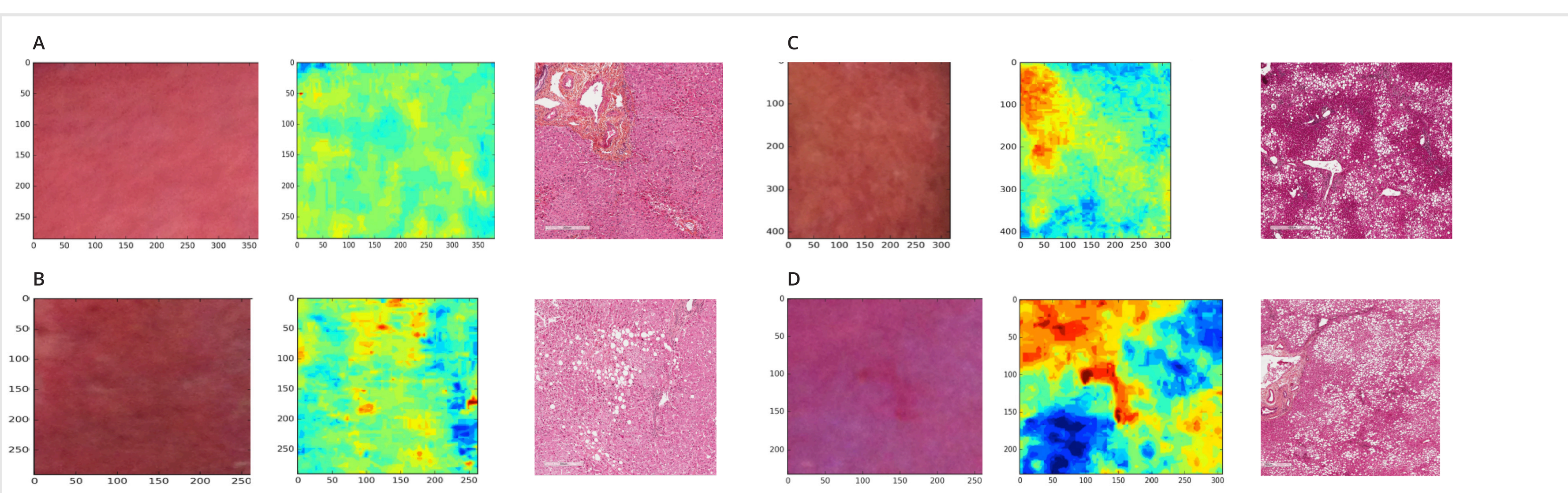

Figure 4. Example of intraoperative liver frame photo, histology and Hue spectral image for each of the 4 classifications of hepatic steatosis (a: Normal; b: Mild; c: Moderate; d: Severe)

Results

Overall, 12 consecutive livers from deceased donors procured with the intention to be transplanted were included. A total of 34 photos of liver grafts taken in situ before aortic cross-clamping, were analyzed. The grafts were anonymously linked to their respective French Network for Organ Sharing number (number CRISTAL), to ensure patients privacy and blinding for CaptureProof Engineers. Liver biopsy was performed event-free in all cases. The median length of biopsies was 20 mm (range: 18-22mm). Five liver biopsies were performed during donor surgery because of gross appearance of severe HS and were discarded while the remaining seven were considered suitable for transplantation and underwent reperfusion biopsy. The characteristics of the donors of discarded and transplanted grafts are detailed in **Table 1** and **Table 2**.

Image analysis of manually selected areas of both liver lobes (avoiding specular reflection) was assessed in less than 60 seconds. Redness Hue histogram distribution showed a significant correlation between Hue histogram distribution and HS% (Cs: 0.86, p<0.001; Pearson correlation coefficient: 0.83, p<0.001 for *r* and Cs: 0.87, p<0.001; Pearson correlation coefficient: 0.84, p = 0.001 for *Ω*) (**Figure 2-3**). Examples of liver frame, histology and Hue spectral image for each of the 4 classifications of hepatic steatosis are reported in **Figure 4**.

Up to now, while several alternatives to histopathology for the assessment of HS have been proposed (MRI, Spectrometry, Bioelectrical impedance analysis)[4], none of them has to date shown practical in the very specific setting of organ procurement. To the best of our knowledge, this is the first report evaluating the performance of a smartphone camera to assess macrovesicular steatosis at procurement site with liver biopsy as the reference. This result could only be achieved using a standardized protocol in photo-shooting, which supports previous findings regarding how to optimize intraoperative photography[3]. Currently, there are over 90,000 mobile apps directed towards healthcare and there is tremendous enthusiasm for the potential of these apps demonstrated by the newborns mobile health, or m-Health, technologies. Hence, the next logical step will be the development of a dedicated mobile app, allowing for real time assessment of HS in an organ procurement setting. In this context, the adjunction of clinical, biological and imaging pre-procurement data such as age, BMI, gGT and Housefield Unit may also allow for the optimization of the algorithm in order to improve both detection and quantification of HS.

	Transplanted liver (n=7)	Discarded liver (n=5)
Age (years)*	52 (35 - 79)	58 (50 - 72)
Male sex (%)	4 (57.2)	4 (80.0)
BMI (kg/m²)	26 (19 - 38)	31 (28 - 38)
Cause of death		
Anoxia (%)	2 (28.6)	2 (40.0)
Trauma (%)	1 (14.3)	1 (20.0)
Cerebro-vascular (%)	4 (57.2)	2 (40.0)
Cardiac arrest (%)	4 (57.2)	2 (40.0)
ICU (days)*	2.7 (1 - 8)	2.4 (2 - 3)
Blood Liver Test (UI/l)		
AST*	83 (29 - 256)	61 (18 - 194)
ALT*	159 (11 - 430)	64 (19 - 132)
gGT*	56 (20 - 124)	98 (47 - 245)
Bilirubin (μmol/l)*	8.7 (5 - 14)	11.6 (5 - 21)
Lactate (mmol/l)*	1.2 (0.5 - 1.9)	1.2 (0.7 - 1.8)
Comorbidities		
Diabetes (%)	1 (14.3)	0 (0.0)
Alcohol (%)	1 (14.3)	1 (20.0)
Arterial hypertension (%)	3 (42.9)	2 (40.0)
Dyslipidemia (%)	0 (0.0)	1 (20.0)
Hepatic Steatosis		
Normal (≤5%) (%)	3 (42.9)	0 (0.0)
Mild (6-33%) (%)	3 (42.9)	0 (0.0)
Moderate (34-66%) (%)	1 (14.3)	4 (80.0)
Severe (≥67%) (%)	0 (0.0)	1 (20.0)

Table 1. Characteristics of the donors of transplanted and discarded liver grafts.
*expressed as median (range); BMI: body mass index; ICU: intensive care unit

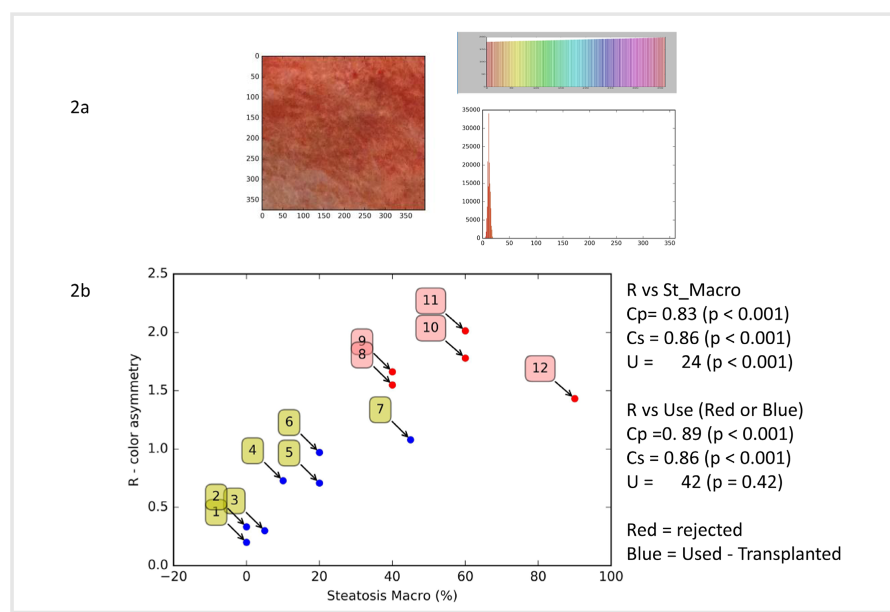

Figure 2a. Liver sample with color texture. Visually this appears to be a red-yellow texture but histogram shows that the color is uniform (no yellow). The variation is due to brightness. **2b.** Images exhibit a relation between liver steatosis and images parameters. The trend is the distribution of color. Higher steatosis tends to have a narrower distribution but asymmetric and shifted to the yellow.

	Macrovesicular steatosis	Microvesicular steatosis
Patient 1 (%)	0	0
Patient 2 (%)	0	0
Patient 3 (%)	5	0
Patient 4 (%)	10	90
Patient 5 (%)	20	10
Patient 6 (%)	20	20
Patient 7 (%)	45	20
Patient 8 (%)*	40	30
Patient 9 (%)*	40	20
Patient 10 (%)**	60	70
Patient 11 (%)**	60	90
Patient 12 (%)**	90	60

Table 2. The actual pathologic evaluation of both microvesicular and macrovesicular steatosis for each biopsy grafts.
*These two grafts were rejected because of bowel necrosis at the time of procurement;
** These grafts were discarded because of steatosis rate ≥60%

Conclusions

There are several limitations to this small size preliminary report. Importantly, the software is currently unable to differentiate macro- and microsteatosis, whereas only macrosteatosis is predictive of liver graft dysfunction. Yet, our image analysis is not a substitute of liver biopsy as it does not predict others pathologies (balloon degeneration, centrilobular necrosis, and chronic hepatitis) which can also adversely affect graft outcome. In conclusion, our study proves for the first time, the feasibility of analysis of smartphone image parameters as technique for steatosis assessment in liver graft, but as long as image capture is performed rigorously. Future larger studies will have to aim at simplifying this analysis in a dedicated smartphone application in order to make it clinically relevant in a setting of organ procurement.

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