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BANFF CONSENSUS RECOMMENDATIONS FOR STEATOSIS ASSESSMENT IN DONOR LIVERS

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ABBRIEVIATIONS:
LDF: large droplet fat/steatosis
SDF: small droplet fat/steatosis
LP: low magnification
HP: higher magnification

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ABSTRACT: No consensus criteria or approach exist regarding assessment of steatosis in the setting of human donor liver suitability for transplantation. The Banff Working Group on Liver Allograft Pathology undertook a study to determine the consistency with which steatosis is assessed and reported in frozen sections of potential donor livers. A panel of 59 pathologists from 16 countries completed a questionnaire covering criteria used to assess steatosis in donor liver biopsies, including droplet size and magnification used; subsequently, steatosis severity was assessed in 18 whole slide images of donor liver frozen sections (n=59). Survey results (from 56/59) indicated a wide variation in definitions and approaches used to assess and report steatosis. Whole slide image assessment led to a broad range in the scores. Findings were discussed at a workshop held at the 15th Banff Conference on Allograft Pathology, September 2019. The aims of discussions were to (i) establish consensus criteria for defining "large droplet fat" (LDF) that predisposes to increased risk of initial poor graft function; and (ii) develop an algorithmic approach to determine fat droplet size, and the percentage of hepatocytes involved. Large droplet fat was defined as typically a single fat droplet that expands the involved hepatocyte and is larger than adjacent non-steatotic hepatocytes. Estimating severity of steatosis involves (i) low magnification estimate of the approximate surface area of the biopsy occupied by fat, (ii) higher magnification determination of the percentage of hepatocytes within the fatty area with large droplet fat and (iii) final score calculation. The proposed guidelines herein are intended to improve standardization in steatosis assessment of donor liver biopsies. The calculated percent LDF should be provided to the surgeon.

Marked/severe macrovesicular steatosis of donor livers is associated with early allograft dysfunction, primary nonfunction and post-reperfusion syndrome (1-4). The phenomenon was first identified in Cambridge and Pittsburgh in the late 1980's (2, 4). The steatosis was described in the first cases from Cambridge as "severe large droplet fatty change" without further clarification (4) and in the 2 cases from Pittsburgh as "severe diffuse macrovesicular steatosis, characterized by large intracytoplasmic globules, which pushed the nucleus toward the periphery" (2). Since then, assessment of steatosis in frozen sections of potential donors with a grossly fatty appearance was recommended (2) – a practice that continues to today.

Unfortunately, various centers throughout the world use different definitions and methodologies to estimate or quantify donor steatosis. After the three original case reports resulting in primary non-function, several centers analyzed their early experience with steatosis and outcome in protocol donor liver biopsies (3, 5, 6). In Pittsburgh, the terminology microvesicular and macrovesicular steatosis was used without further clarification and the percent was determined on the basis of the percent of hepatocytes involved (5). At the University of Wisconsin biopsies were graded as minimal, moderate or severe steatosis based on thirds of hepatocytes involved; however, there are no details regarding the size of fat droplets assessed except in the figure legends in which the term "macrovesicular" is used (6). Pathologists at the University of Nebraska expressed the fat content as a percentage, based on the approximate area of the liver lobule occupied by round non-staining vacuoles excluding the sinusoids and vessel lumens (3). They further subdivided the fat into microvesicular fat defined as multiple small vacuoles within a hepatocyte and macrovesicular fat defined as a single vacuole, usually larger than a hepatocyte nucleus (3). Reviews on donor liver steatosis assessment emphasize a lack of standardized definitions and criteria resulting in an inability to compare results from different centers (7, 8). More importantly, mis-assessment of steatosis has the potential to waste valuable donor organs that otherwise might have been successfully transplanted.

The biennial Banff Conferences on Allograft Pathology convenes interested pathologists, physicians, surgeons and immunologists with the goal of producing consensus guidelines for standardizing the assessment of allograft biopsies. Since the Banff Working Group on Liver Allograft Pathology first met in 1995, the group has produced guideline documents relating to acute rejection (9), chronic rejection(10), late post-transplant biopsies (11, 12), immunosuppression adjustments (12) and antibody-mediated rejection (13). These documents are widely used and have increased the uniformity with which histopathologic changes in liver allograft biopsies are reported across the world.

The lack of standardized criteria for the assessment of donor liver biopsies has been overlooked. This shortcoming is at least partially attributable to the enormity of the task to standardize assessment of rejection and post-transplant monitoring that were more pressing at a time of relative abundance of good quality donor organs and the high frequency of rejection in the early days of transplantation. The standardized grading was needed to facilitate trials focused on development and introduction of improved immunosuppressants. However, the tides have now turned, and the field is now faced with a relative shortage of donors, necessitating critical appraisal of the expansion of the donor pool using extended criteria donors. Since the obesity epidemic is now worldwide, donor organs become scarcer and donor steatosis has become more frequent.

The introduction of extra-corporeal machine perfusion clinical trials exposed the problem with a lack of uniformity in the assessment of donor steatosis. Donor livers discarded as being unsuitable for transplantation, based on liver biopsy assessment of an unacceptable degree of steatosis at one center, were considered to have only minimal or mild large droplet steatosis when subsequently assessed at other centers by different pathologists. This led to a preliminary study by the UK Liver Transplant Pathology Group (D. Neil, unpublished data) that evolved into the present expanded study involving pathologists throughout the world via the Banff Working Group on Liver Allograft Pathology. These findings served as the foundation of discussions at the 15th Banff Conference held in Pittsburgh USA September 2019.

This manuscript documents: a) the range of definitions and criteria used and the variability in the pathologists assessment of steatosis; and b) the need to develop consensus guidelines for assessment of frozen sections of donor liver biopsies for steatosis.

METHODS:

A survey was circulated among among 59 pathologists worldwide who routinely assess donor liver frozen sections. It consisted of 13 questions detailing their practices of donor fat droplet assessment, terminology used, the manner in which they arrived at a final assessment of steatosis severity, and the severity of steatosis considered “safe” under optimal conditions within their centers (supplementary information 1). The same participating pathologists were then asked to: 1) assess 18 whole slide images (scanned at 40X using an Aperio AT2 scanner) of frozen sections of donor liver biopsies from the archives of St James Hospital Leeds, UK; and 2) grade the severity using their standard practice as if they were reporting to the implanting surgeon at their center. A Surveymonkey proforma was used for responses to

the questionnaire and the assessment of steatosis. Information on whether the liver was transplanted, and how it functioned immediately post-transplant were obtained.

Data normalization was performed as follows; cases for which a percent range was given (eg.10-20%), the middle value (15%) was used. When < x% was given the midpoint between 0 and x was taken. When >66% was given 70% was used. When steatosis severity was graded with the NASH Clinical Research Network grade (14): cut offs of 0. none/minimal <5%; 1. mild 5-33%; 2. moderate 34-66% and 3. severe >66% were used. For each case, the individual pathologists steatosis percent parenchymal involvement, as provided to the clinical team as the clinically relevant amount of steatosis, were compared and plotted from low to high to reveal the distribution.

To compare the impact of different definitions and methodologies the pathologists were broken into 4 groups based on fat droplet size assessment and magnification used for assessment. Pathologists either assessed large droplet fat only or combined large and small/medium droplet fat to estimate the amount of steatosis. Pathologists used either a low magnification approach (to be able to assess the whole biopsy in 1-2 fields, usually 2 or 4x on a microscope) or high magnification (approximately 20x, when individual hepatocytes and fat droplets are readily visible) approach. These two variables resulted in 4 groups of pathologists with different assessment techniques: 1) low magnification large droplet fat only; 2) higher magnification large droplet fat only; 3) low magnification combined small/medium and large droplet fat and 4) higher magnification combined small/medium and large droplet fat.

The survey results were presented at the 15TH Banff meeting in Pittsburgh October 2019. This was followed by a discussion of optimal definitions of large and small droplet fat and a proposal for standardized definitions and algorithmic approach to scoring. Additional discussions centered on the whole slide image scores provided by 3 experienced liver pathologists (AJD, SGH, EB), whose scores were considered the "gold standard" based on 1) their collective experience, 2) the finding that their assessments were relatively similar and 3) the livers they considered as having minimal/mild steatosis that were transplanted all functioned well.

Annotated photomicrographs and diagrams were used to aid the discussion: 1) assessment of steatosis when the slide was viewed at low versus higher magnification (Figure 1A); 2) variably sized fat droplets in relation to the hepatocyte nucleus and adjacent non-fatty hepatocytes (Figure 1B); 3) comparison of percent steatosis using fat droplets of varying size and involving varying numbers of hepatocytes (Figure 1C).

Image J (http://imagej.nih.gov/ij) was used to morphometrically determine the percent steatosis in the annotated images and on a subset of the whole slide images following the consensus algorithmic approach, using pixels for the determination of area, to compare with the gold standard scores.

Nonparametric statistics were performed using SPSS version 26 to assess the impact of methodology: A Mann-Whitney U test was used to assess if there was any difference in the percent steatosis for each WSI and the total score between 1. high and low magnification 2. Large droplet fat only and combined large and small droplet fat. A Kruskal-Wallis test was used to test if there was any significant difference between the 4 methods.

ETHICS: The whole slide images are stored in an anonymized form on the Leeds Virtual Pathology Website, used for pathology External Quality Assurance: No specific ethics applies to these slides/EQA. Human subjects per se were not used for research purposes. No transplants performed in the UK are from executed prisoners or institutionalized individuals.

RESULTS:

Survey Results:

Of the 59 pathologists surveyed, 44 were subspecialized in liver pathology and the other 15 pathologists reported donor liver frozen sections as part of a general on-call system. Twelve pathologists each were from the USA and Italy, 10 from the UK, 5 from Australia, 4 from Turkey, 2 from Brazil, France, Germany and Norway and 1 each from Belgium, Canada, India, Ireland, Japan, Poland, Switzerland and the Netherlands. The responses to the questions were provided by 56 pathologists. All (n=59) assessed the whole slide images.

Definitions and terminology

All pathologists agreed that the amount of macrovesicular steatosis should be assessed to determine suitability of a donor liver for transplantation, however the definition of macrovesicular steatosis varied. A majority of pathologists 43/56 (77%) defined macrovesicular steatosis (in the transplant setting) as the large droplet subset of macrovesicular steatosis as defined by Brunt (15). The remainder (n=13) combined large and small droplet components of macrovesicular steatosis (15). Figure 2A illustrates the type of fat the pathologists consider important for the assessment of donor livers.

Amongst those pathologists who used the large droplet subset of macrovesicular steatosis to determine steatosis severity (n=43), the definition of large droplet varied (Figure 2B). The majority 24/43 (55.8%) defined it as a large droplet that distends the hepatocyte and displaces the nucleus, while 9/43 (20.9%)

defined as fat droplets greater than 2-3 times the diameter of a hepatocyte nucleus, and 5/43 (11.6%) had other definitions (including fat droplets occupying more than half the cell cytoplasm or fat droplet(s) larger than the nucleus). Five did not provide a precise definition. Over half 30/56 (53.6%) reported not using the term "microvesicular steatosis" to refer to the small or medium droplet fat, while 19/56 (33.9%) always did and 7/55 (12.7%) sometimes did (Figure 2C). There was country variation (Figure 2C) with most pathologists in Europe (excluding the UK) 15/24 (62.5%), using the term "microvesicular steatosis" for small and medium droplet fat, whilst in the rest of the world, including the UK, most said that they would never use the term 24/32 (75%) (range for different countries: 63.6-100%).

Most 50/56 (89%) pathologists provide the surgeon with an estimated steatosis percentage, the remaining 6 pathologists give an indication of whether steatosis was mild, moderate or severe (Figure 2D). Of the 6 pathologists that gave an overall grade, 5 use the NASH Clinical Research Network cut-offs of thirds (33% and 66%) with <5% considered nil (14). Sixty-seven percent (29/43) of liver pathologists and 53.8% (7/13) of general pathologists give both percent and grade of steatosis. Overall, the cut-offs between grades used by the 33 pathologists that gave an indication of what they considered them to be were similar: 28 used the NASH Clinical Research Network cut off with the remaining 5 using 30 and 60%. The amount of steatosis considered safe to transplant under optimal conditions varied widely from 20% to 66% (Figure 2E). Overall, 95% of the pathologists stated that if the steatosis was graded at no more than 33% then their center would consider the liver safe to transplant under optimal conditions.

A similar percentage of pathologists used low magnification assessment 29/56 (51.8%) and high magnification assessment 27/56 (48.2%) in estimating macrovesicular steatosis severity (Figure 2F). Most pathologists did not use aids when assessing steatosis 47/56 (83.9%); 5 relied on comparisons to either a) representative photomicrographs or b) diagrams (n=3) and 2 used an oil red 0 stain (1 used diagram + oil red O).

The specialist liver pathologists and general surgical pathologists were similar in their responses to the questionnaire and no pattern was apparent in the distribution of the steatosis assessment between the groups.

The wide spectrum of responses to the survey underscores the lack of a uniform definition of what type of steatosis should be assesed, how the assessment should be approached, and what terminology should be used.

Assessment of amount of steatosis in whole slide images

Individual pathologist's assessment of the percent steatosis (not otherwise specified: as provided to the surgeon, relevant to transplantation) for each whole slide image is shown in Figure 3. Twelve of the 18 cases (#1, 2, 3, 4, 6, 7, 11, 13, 14, 15, 17 and 18) were considered by the three experienced pathologists to have at most mild steatosis (maximum of 33%), with 9 of these assessed as having no relevant steatosis (<5%) (#2, 3, 6, 7, 11, 13, 14, 15 and 17) (Figure 3). In 5 of these 12 cases (#11, 13, 14 17 and 18), all the other pathologists agreed there was at most mild steatosis. In 7 of the 9 cases considered to have no relevant steatosis by the three experienced pathologists, 96% of the remaining pathologists also agreed. There were 2 cases considered by the three experienced pathologists to contain moderate steatosis (>33% to <67%) (#5 and 8), and most of the other pathologists agreed (42/56 (75%)). There were 9 cases with a wide range of opinions (#1, 3, 4, 5, 9, 10, 12, 15 and 16).

Of the whole cohort, 10 of the 18 cases were transplanted (#1, 2, 4, 6, 7, 11, 13, 14, 17, 18) and all functioned well. The steatosis grading by the three experienced pathologists in these cases ranged from 0% to 17.5%. From the cases with a wide range of opinions, 2 were transplanted (#1 and 4) and functioned well from the start and continue to function well > 1-year post-transplant. One (#16) was originally graded as >50% steatosis by the reporting pathologist and was turned down for standard transplantation based on this biopsy. It entered a machine perfusion clinical trial where a repeat biopsy, prior to machine perfusion, at the research center was assessed as 15-20% large droplet steatosis, functioned well during machine perfusion, and was successfully transplanted.

Influence of method on score

The median value and ranges, based on method of assessment, for each whole slide image and the total score of individual pathologists for all the cases are shown in supplementary table 1. Graphs of the median values of the total score for all 18 cases based on method grouping of the pathologists are shown in Supplementary Figure 1.

As expected, the reported steatosis percent is higher if all fat droplets are assessed as opposed to just the large fat droplets. However, this approach reached statistical significance in only 3/18 cases. The impact of magnification seems minimal: There was little difference between the median values overall and only a single case (#4) showed statistical significance between high and low magnification assessment, despite the median values being the same. When the 4 overall approaches to steatosis scoring are compared, assessment of combined small and large droplet fat at higher magnification tends to lead to higher scores. However, a correlation is not seen between the methods and in only 3 cases is statistical significance seen between the scores across the 4 methods.

The spread of values of total percentages and grades for each pathologist is shown in Supplementary Figure 1B. The 3 experienced pathologists fall in the lower quartile of the spread of scores.

Consensus algorithm on how to assess and grade steatosis

With the aid of the annotated photomicrographs and diagrams (Figure 1) for the discussions, there was clear consensus that only large droplet fat (LDF) should be used for determination of liver suitability for transplantation***. Large droplet fat was defined as typically a single droplet distending the cell and therefore the fat droplet should be at least slightly larger than adjacent hepatocytes, which contained no fat or small fat droplets.***

It was agreed that the most accurate estimate of the percentage of large droplet steatosis in a donor liver biopsy could be achieved using a **3-step process** (Figure 4):

1. Low magnification (LP) assessment of overall percent of the biopsy affected by steatosis (stands out white)
2. Higher magnification (HP) assessment of the fatty areas to see what proportion of the cells in these areas contain large droplet fat as defined above.
3. Adjust the low magnification % accordingly: HP% of LP% = Total %LDF

When no areas of steatosis are obvious on LP giving a LP% of 0, the total %LDF calculation will also be 0%; therefore, no HP assessment is required. In this situation, HP assessment may show a few hepatocytes containing LDF which would be within the <5% steatosis range considered negligible so a recording of 0% is acceptable.

Two illustrative cases showing the application of the 3-step process to determine the amount of large droplet fat using morphometry to demonstrate the assessment of the percent steatosis at both step 1 and 2. The step 3 adjusted score agrees with the 3 experienced pathologists' assessment as safe to transplant. These livers were transplanted and did well. The impact of the current non-standardized grading on organ utilization is demonstrated at 33% LDF being considered safe to transplant.

Case #1 (Supplementary Example case 1): Reported percent steatosis (not otherwise specified as provided to the surgeon), ranged from 2.5% to 70%. The 3 experienced pathologists all considered this liver safe to transplant, their assessment of steatosis ranged from <5% to 15-20%, none to mild. The liver was transplanted with a peak post-operative aspartate transaminase of 1796 IU which fell rapidly and there were no postoperative issues.

Case #4 (Supplementary Figure 3): The estimates of percent steatosis ranged from 0% to 70%: ranging from none to severe steatosis. The 3 experienced pathologists all considered this liver safe to transplant, their assessment of % steatosis ranged from <1 to 20%, none to mild. This liver was transplanted with a peak postoperative alanine transaminase of 860 IU and no postoperative issues.

Potential Impact on organ utilization

Figure 5A shows the potential impact of the pathologists' score on donor liver utilization. Of the 12 cases the three experienced pathologists all consider at most mild steatosis (≤33%), in only 5 is there is complete agreement by all the remaining pathologists. As the three experienced pathologists fell into the 1st quartile for the spread of scores, it can be seen the utilization rate is almost identical between the three experienced pathologists and the 1st quartile. The impact of a lack of standardized grading system can be seen in the pathologists that fell into the higher 3 quartiles of the spread of scores. The majority 40/59 (68%) of pathologists participating in this study fall into the higher 3 quartiles. The impact is negligible in grafts with minimal steatosis.

Where the safety cut off for a center is 50% steatosis, many more livers are considered safe to transplant (Figure 5B). This is especially evident in cases #5, 8, 9, 10 and 16, sometimes with much less difference between the low scorers and the rest (eg #5). Case 8 highlights the impact of the safety level where almost all pathologists considered this liver to have more than mild steatosis, but with the safety cut off raised to 50% all three experienced pathologists and 80% of the lower quartile consider this liver to fall within this safety cut-off, whilst less than a third of the pathologists in the upper 3 quartiles would assess this liver as having at most 50% steatosis. This liver was not transplanted so there is no outcome data.

DISCUSSION

Standardization of terminology

Our questionnaire shows that pathologists know that "macrovesicular steatosis" is the type of steatosis associated with primary graft dysfunction but identified that pathologists use different definitions. The minority use the NASH Clinical Research Network definition of macrovesicular steatosis being all steatosis that is not "true" microvesicular steatosis, whilst the majority use a large droplet subset with no uniformity in what is considered large droplet. This mirrors the early literature, and the ongoing lack of standardization is a major contributor to confusion in practice and in the literature. To prevent confusion in terminology we propose that for the assessment of steatosis in donor livers the terms large droplet fat (LDF) and small droplet fat (SDF) be used; both represent a subset of macrovesicular steatosis, as opposed to the steatosis formed by acute onset mitochondrial dysfunction, referred to as microvesicular steatosis

(Table 1). If true microvesicular steatosis is present, then it should be referred to using the correct definition and term.

In the original manuscripts warning of the danger of transplanting severely steatotic livers, the term microvesicular steatosis was used for what we have defined as SDF (3, 5). It has continued to be used in the transplant literature for what became known as small droplet macrovesicular steatosis in more general liver pathology literature (14, 16). Based on the survey results, pathologists in most of the world are no longer using the term "microvesicular" in their donor liver biopsy reports.

A recent review identified an inability to interpret the literature for outcome/safety factors due to lack of standardized definitions, terminology and descriptions for both clinical end points and pathological assessment (7). The confusion documented in this study further highlights the need for guidelines on definitions and how to grade steatosis to assess suitability of donor organs to standardize the process and allow outcome data to determine suitable safe levels of steatosis for individual recipient and donor pairs.

Overcoming a paucity of outcome data

In view of the paucity of reliable outcome data with clear definitions of pathology, we have used the combined experience of 3 expert liver transplant pathologists, one of whom (AJD) described 2 of the original cases recognizing the link between steatosis and primary nonfunction (2), as the basis of our definitions. All recipients of livers deemed suitable for transplantation and were subsequently transplanted did well following transplantation supporting the safety of this approach. Images from the original 2 reports (2, 4) appear to agree with the consensus definition of LDF as being the relevant fat droplets to assess (Suppl Figure 2). Nonetheless, the liver pathology community should continue to standardize their approach and terminology, using these guidelines, in order to produce more robust outcome data in the future. We are not recommending a defined %LDF cutoff for organ usage as both donor and recipient factors influence outcome. Nevertheless, it is generally accepted that a direct correlation exists between the LDF severity and development of a reperfusion syndrome after transplantation with most patients suffering from this complication when a donor liver with more than moderate (>2/3, 60-66%) LDF is used.

What are fat droplets and why should size matter?

Fat droplets, or lipid droplets, are ubiquitous highly dynamic organelles found in all cell types which store neutral lipids for energy metabolism and other metabolic roles (17-19). Lipid droplets are actively synthetized within the endoplasmic reticulum membrane and there is a continuum in size from very small (true microvesicular) through to giant, which are only found in hepatocytes and adipocytes (17, 19).

Small droplet fat increases during preservation and reperfusion, an indication that it is an acute, "short-lived" process and either a sign of liver stress/injury (5, 20) or repair/regeneration (21, 22). Preimplantation donor small droplet fat has not been found to be a risk factor for primary non-function and has no impact on long term graft survival (23), although its presence is associated with increased rejection post-transplantation (24). Rejection risk is increased with more severe preservation-reperfusion injury to the liver (25) and other organs (26) related to upregulation of inflammatory pathways (27, 28).

Cultured adipocytes, when placed in a refrigerator and cooled, will rupture (personal communication Dr Margaret Eggo). It is postulated that large droplet fat, as defined in this paper, causes mechanical injury to the hepatocyte due to the size of the droplet which solidifies during the cooling component of organ preservation. This results in the rupture of the cell membrane, death of the hepatocyte, and release of the fat droplet and other noxious intracellular substances into the sinusoids/circulation. The released fat globules may coalesce, a process called lipopeliosis (29) or more correctly pseudopeliotic steatosis as the fat globules are predominantly in the extravascular space (30), and cause physical obstruction to blood flow on reperfusion or perfusate during machine perfusion. The released intracellular substances from the dead hepatocytes, if the liver is not flushed adequately prior to transplantation, may contribute to postreperfusion syndrome. This mechanism of injury was postulated in one of the early papers (6) and the coalescence of the fat globules within the sinusoids was noted in the failed allografts in the early failed allografts (2). Fat globules of hepatocyte origin have been found in the pulmonary micro-circulation following transplantation of steatotic livers, supporting this hypothesis (31).

Standardization of approach

The assessment of fat droplet size is not reliable at low magnification and requires higher magnification assessment to determine the relevant subset of fat. A low magnification overview of the whole tissue is required, however, to determine how much of the biopsy is affected. To standardize the assessment a 3-step algorithm was developed based on experience/practice (Figure 4), as discussed above in overcoming a paucity of outcome data. It should be noted that this approach can be done by the pathologists viewing either glass slides or whole slide images, without image analysis, by making rough adjustments mentally. Formal morphometry is not required and has only been used to demonstrate that the 3-step process came up with similar values to the three experienced pathologists' scores providing evidence that this approach works. Morphometric techniques might become more widely available in the near future and their development will greatly benefit from standardized definitions and approaches.

The percent LDF is to be provided to the surgeons to add to the other factors involved in donor/recipient organ selection. An indication of the small droplet fat (SDF) may also be provided. However, small droplet

fat percentage or scoring should not be used by the surgeon in the decision on suitability for transplantation and should not be added to come up with a total steatosis percent. Assessment of small droplet fat is beyond the scope of this manuscript. As there are no descriptions of how to quantify or the importance of such factors as number of small droplets per hepatocyte or number of hepatocytes containing varying numbers of the small fat droplets, surgeons should be aware that this figure is unlikely to be reproducible between pathologists.

Inherent difficulties

Other factors that may explain some of the variation in the scoring of the steatosis are: 1) gradual variation in the size of fat globules making it difficult to score; 2) inherent difficulties in assessing steatosis in frozen sections and recognizing the cell membrane of non-steatotic hepatocytes to compare fat droplet size. This is particularly difficult when there are mixed medium and large droplets present. 3) The assessment of a percent of a subset of fat droplets within irregularly shaped areas in an irregularly shaped biopsy is not easy for the human brain to compute. 4) Variation in frozen section tissue thickness (not relevant for the pathologists variation in this study, but a problem worth highlighting). While more standardization is needed, we recommend 5 - 6 micron-thick sections because thinner sections tend to overestimate while thicker (> 8 -10 microns) tend to underestimate the severity of LDF.

Summary

This study documents a lack of uniformity in the assessment of steatosis in donor livers resulting in a wide range of steatosis severity being reported to the implanting surgeons with a potential impact on donor liver utilization. A variety of definitions and methods are used by pathologists with no clear correlation between these and the steatosis assessment. As a result, the Banff Liver Working Group has defined the relevant type of fat droplet to assess, namely LDF, and has proposed a 3 step approach of how to assess the LDF percent in the biopsy. Three experienced liver transplant pathologists were used as the “gold standard” and identified to fall within the lower quartile of all the pathologists total scores of all cases combined. The majority of pathologists were in comparison overscoring the extent of relevant steatosis in donor liver biopsies. Where available, outcome data following transplantation supported the three experienced pathologists and the other pathologists who fell within the lower quartile of scorers had been correct in their assessment of safety of the liver. The 3-step approach was “validated” using morphometry to show that the calculations are similar to the three experienced pathologists. The calculated percent LDF should be provided to the surgeon.

Future direction

Following training on this standardized grading system, further validation will be undertaken to confirm improved agreement between pathologists. A standardized approach will allow refinements on safe amounts of steatosis based on reliable outcome measures in the future, under varying clinical circumstances (eg. donation after cardiac death, prolonged storage times and the varying methods of machine perfusion and varying recipient characteristics).

Clear definitions are necessary for the development of digital algorithms for donor fat evaluation, including discrimination of fat droplet size. These algorithms will need to be developed with, calibrated and validated by expert pathologists. Once in use, these algorithms could play a significant role in overall donor liver assessment, as well as standardizing the assessment of donor livers, and importantly will be invaluable for providing standardized data to evaluate with the outcome data. Access to digital pathology is an unspoken necessity for their routine use, and its introduction will also importantly allow the surgeon to remotely view the sections with the pathologist.

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AUTHOR CONTRIBUTIONS:

DAHN was involved in the initial UK study design; DAHN, MM, MS and AJD were involved with expanding the study to the rest of the world; All were involved with the assessment of the whole slide images. DAHN analyzed the data and performed the image analysis of % steatosis; DAHN and MS led on the consensus discussions with the Banff participants; DAHN, MM, MS, SGH, EB, AJD compiled the consensus information to draw up the guideline; SGH,EB,AJD were the experienced pathologists whose assessment of the biopsies was used as a basis for the consensus development and used to test the 3-step process; DAHN, MM, MS, SGH, EB, AJD assessed the 3-step process using the image analysis data to confirm correlated with the experienced pathologists interpretation; All critically appraised the draft document and approved the final draft.

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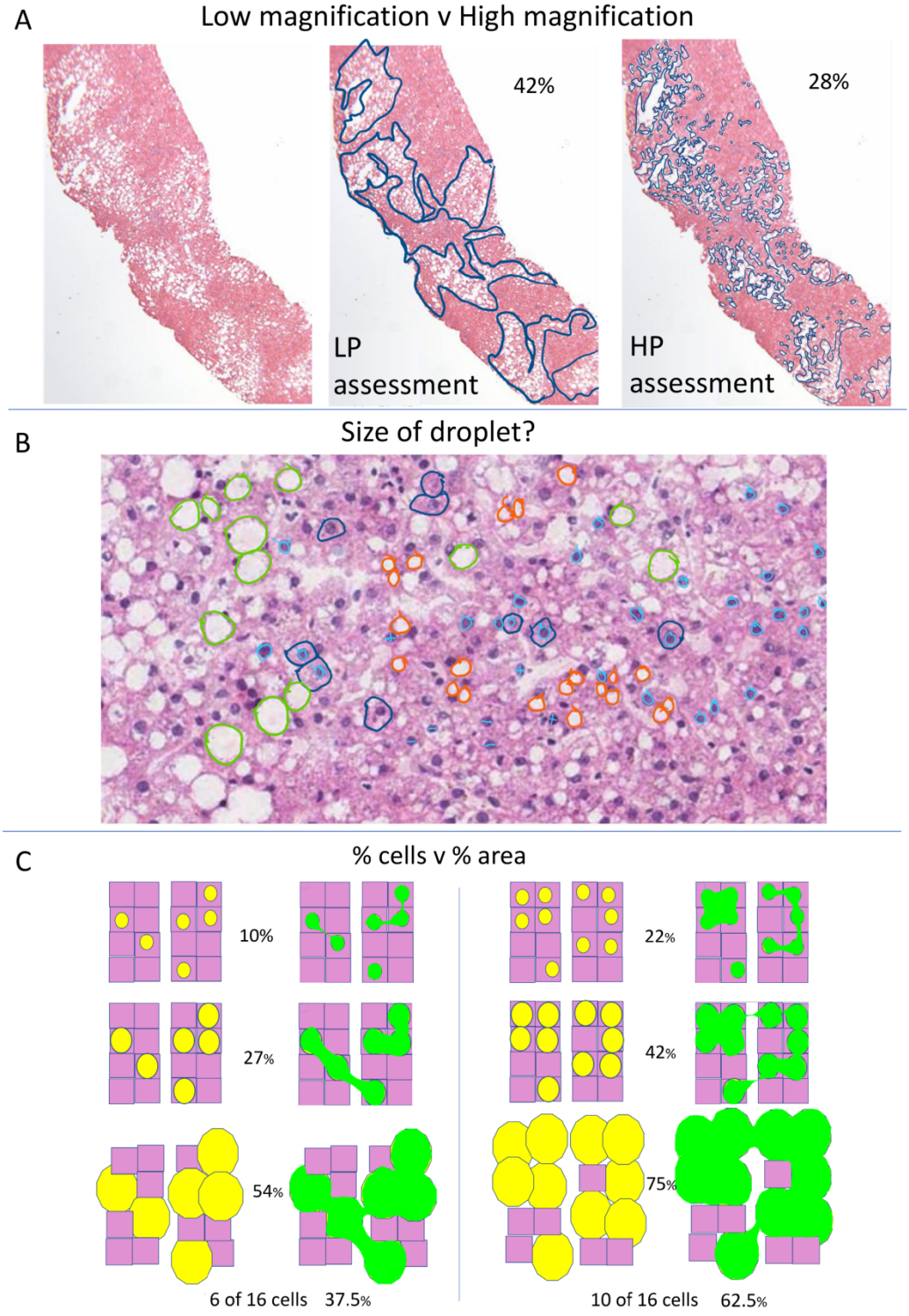
TABLE 1: TERMINOLOGY FOR USE IN ASSESSING STEATOSIS IN DONOR LIVER BIOPSIES

TERM	ABBREVIATION	DEFINITION	NASH CLINICAL RESEARCH NETWORK TERM
Large droplet fat	LDF	Typically a single droplet distending the cell, which thus is larger than adjacent non steatosis/minimally steatotic hepatocytes and the nucleus if present in the section is displaced to the periphery of the hepatocyte.	Macrovesicular steatosis
Smaller droplet fat	SDF	All fat droplets that are not LDF or "true" microvesicular steatosis.	Macrovesicular steatosis
"true" microvesicular steatosis		Tiny droplets distending and filling hepatocytes producing a foamy appearance. They are often not discernible as discrete vacuoles and usually require a fat stain to confirm. They occur as non-zonal aggregates or diffuse involvement of the liver. They occur in the setting of acute liver failure and are therefore not likely to be present in a liver being considered for transplantation.	Microvesicular steatosis



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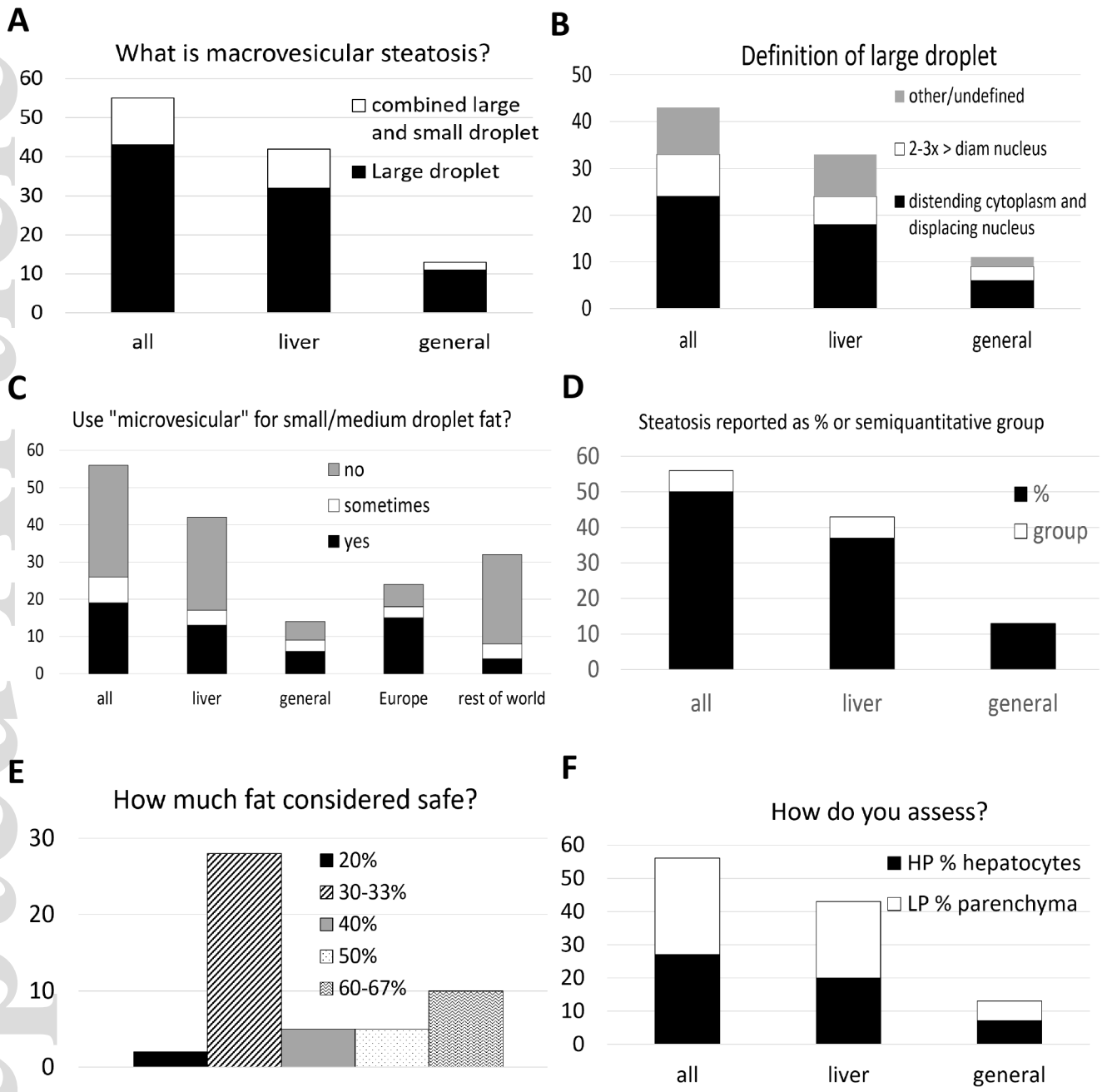

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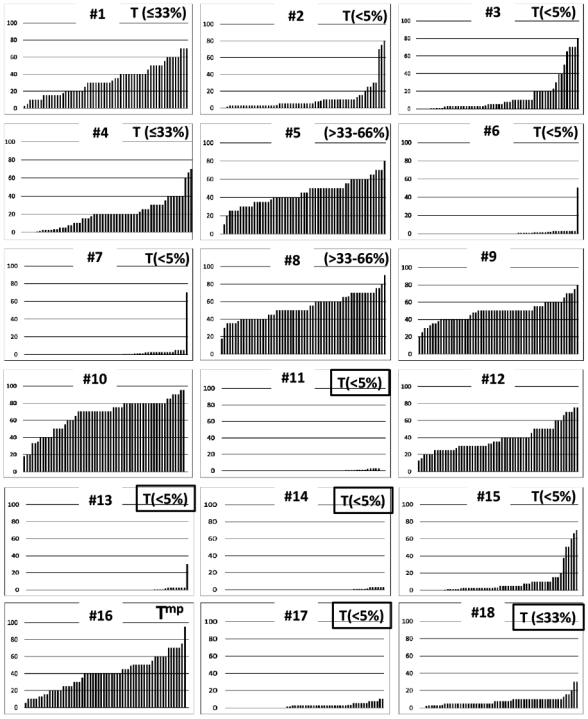

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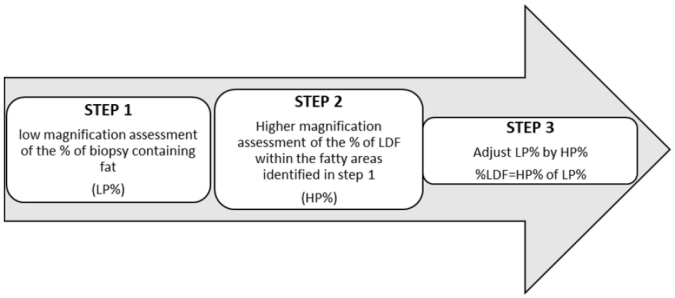

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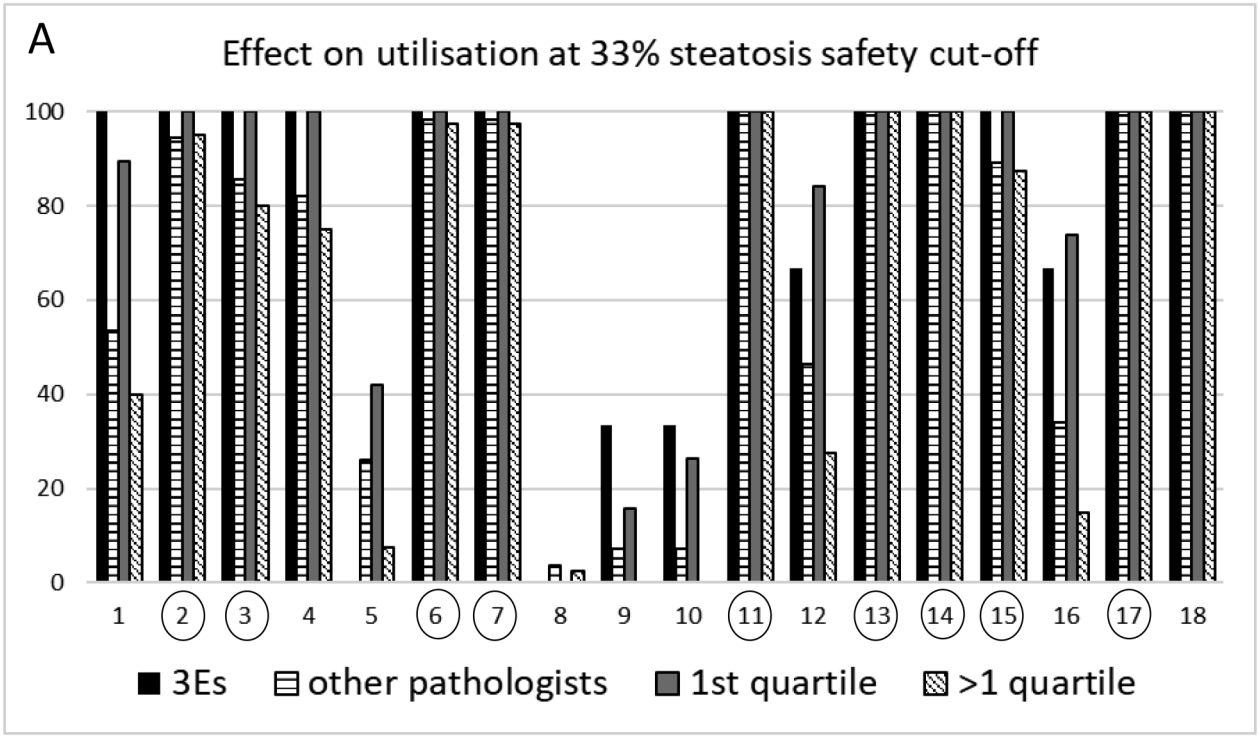

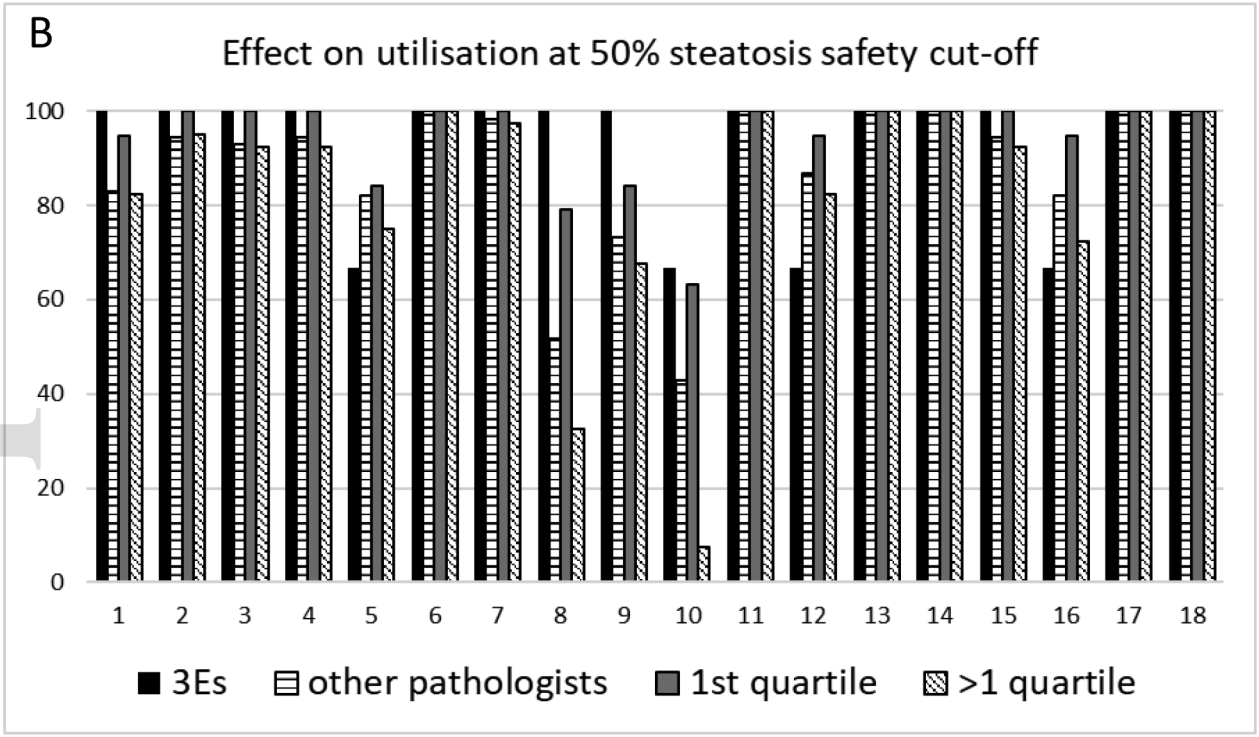

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