Liver biopsy may facilitate pancreatic graft evaluation: Positive association between liver steatosis and pancreatic graft adipose infiltration

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OBJECTIVES: The number of pancreatic transplants has decreased in recent years. Pancreatic grafts have been underutilized compared to other solid grafts. One cause of discard is the macroscopic appearance of the pancreas, especially the presence of fatty infiltration. The current research is aimed at understanding any graft-related association between fatty tissue infiltration of the pancreas and liver steatosis.

METHODS: From August 2013 to August 2014, a prospective cross-sectional clinical study using data from 54 multiple deceased donor organs was performed.

RESULTS: Micro- and macroscopic liver steatosis were significantly correlated with the donor body mass index ([BMI]; \(p=0.029\) and \(p=0.006\), respectively). Positive gamma associations between pancreatic and liver macroscopic and microscopic findings (0.98; confidence interval [CI]: 0.95–1 and 0.52; CI 0.04–1, respectively) were observed. Furthermore, comparisons of liver microscopic findings showed significant differences between severe versus absent (\(p<0.001\)), severe versus mild (\(p<0.001\)), and severe versus moderate classifications (\(p<0.001\)). The area under the receiver operating curve was 0.94 for the diagnosis of steatosis by BMI evaluation using a cut-off BMI of 27.5 kg/m², which yielded 100% sensitivity, 87% specificity, and 100% negative predictive value.

CONCLUSIONS: We observed a positive association of macroscopic and microscopic histopathological findings in steatotic livers with adipose infiltration of pancreatic grafts.

KEYWORDS: Liver Transplant; Pancreas Transplant; Graft; Liver Steatosis; Visceral Fat.

INTRODUCTION

The total number of pancreatic transplants performed in the United States has decreased since 2004 (1,2). Pancreatic grafts have been underutilized compared to other solid organs, and the rates of pancreatic discard and non-procurement have increased (3). One cause of discard is a poor macroscopic appearance of the pancreas, mainly due to fatty infiltration of the organ.

Pancreatic graft adipose infiltration (PGAI) can be hazardous for whole pancreas transplant recipients. Since PGAI can increase postoperative complications, such as pancreatitis and pancreatic graft thrombosis, and cause a decrease in graft survival, many transplant teams discard the organ following macroscopic evaluation. However, macroscopic pancreatic examinations can be difficult due to the presence of peripancreatic adipose tissue, which leads to inaccurate evaluation of adipose infiltration into the pancreas (2,4,5). Pancreatic biopsy can be hazardous for pancreatic patients due to postoperative pancreatitis and fistula.

In contrast, liver graft steatosis is easily analyzed from both the macroscopic and microscopic points of view. Liver biopsy is very accurate (6), and steatosis can be classified as micro- or macrovesicular. Macrovesicular steatosis has a greater influence on ischemia/reperfusion injury and poor graft function than microvesicular steatosis, which occasionally occurs in isolated cases (5,6). During the organ recovery period, macrovesicular steatosis is generally suspected after the initial inspection. However, biopsy is the gold standard used to obtain an objective assessment (7). Furthermore, fatty tissue infiltration into pancreatic grafts does not undergo this type of evaluation.

Ninety percent of the organs used for whole organ pancreatic transplant are obtained from donors <50 years old with a body mass index (BMI) <30 kg/m², while organs from older, more obese donors are more often recovered...
Liver biopsy may facilitate pancreatic graft evaluation

Nacif LS et al.

Study design

Histopathology

Donor technique definition

Inclusion and exclusion criteria

Donor technique definition

In all cases, we performed total hepatectomy en bloc in conjunction with the pancreas as the conventional organ recovery donor surgery. Preservation was performed using the University of Wisconsin solution (UW). We performed a liver and pancreatic biopsy, which was sent to the pathology department for microscopic study. A board-certified expert surgeon with extensive experience in pancreatic and liver transplant performed a macroscopic evaluation and classified the degree of liver steatosis and pancreatic liposubstitution as absent, mild (0%–30%), moderate (31%–70%), or severe (>70%). The associations between these factors were determined.

Histopathology

Fifty-four segments of pancreatic tissue between 3.0 and 5.0 cm in diameter (with no transplanted pancreatic graft) and liver biopsy (with transplanted hepatic grafts) specimens were obtained using a Tru-Cut biopsy needle 14G (Langeskov, Denmark). The segments were fixed in 10% formalin and sent to the pathology division for macroscopic evaluation. Three fragments were chosen, thereby generating three blocks each per case. The material was processed and embedded in a paraffin mold until it had completely solidified. Four-micrometer sections were cut, placed on a slide, and stained with hematoxylin and eosin. The evaluation was performed by the same pathologist (a board-certified expert pathologist with extensive experience in hepatobiliary and liver transplant) without knowledge of clinical information and prior macroscopic evaluation of formaldehyde-fixed sections. Fatty replacements of the pancreatic parenchyma and liver steatosis were evaluated as pathological parameters (Figure 1).

Statistical analysis

Median (25%–75% quantile) or mean and standard deviation values are presented for quantitative variables, and percentages are presented for categorical variables. Gamma ordinal association (strong > 0.7, moderate < 0.7 and > 0.4, and low < 0.4) was performed between categorical variables (9). Analysis of variance followed by the Tukey or Kruskal-Wallis test followed by the non-parametric Tukey test were applied to analyze differences among groups (10). Finally, a receiver operating characteristic (ROC) curve was used to determine cutoff points for examination values. All analyses were conducted with the R statistical program, version 2.15.1 (11). A value of $p < 0.05$ was considered statistically significant in the final analysis.

Ethical aspects

The study was approved by the Institutional Review Ethics and Research (Cappesq) committee of the Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, number 399.857, on 18/07/2013, fulfilling all requirements for studies on humans according to the guidelines of the 1975 Helsinki Declaration.

RESULTS

Clinical and demographic profile of the population

Clinical data from 54 deceased donors from August 2013 to August 2014 were studied. The mean donor age was 40.82 ± 17.24 years, and the median age was 43 (ranging from 5 to 71) years. The mean donor BMI was 24.38 ± 4.38, and the median was 24 (ranging from 15.3 to 31). Moreover, the surgeon evaluated macroscopic steatosis and adipose infiltration into the pancreas for the classification of subtypes as shown in Table 1.

The macroscopic evaluation of liver steatosis cases showed that steatosis was absent in 42.86%, mild in 39.29%, moderate in 14.29%, and severe in 5.71% of cases. Pancreatic macroscopic liposubstitution was absent in 36.36%, mild in 30.33%, moderate in 27.27%, and severe in 6.06% of cases.

The microscopic evaluation indicated no steatosis in 40%, mild steatosis in 42%, moderate steatosis in 10%, and severe in 8% of cases. Pancreatic microscopic liposubstitution was absent in 13.33%, mild in 80%, moderate in 10%, and severe in 8% of cases.

The demographic associations between the liver and pancreatic macroscopic and microscopic evaluations are shown in Figure 1, and multiple comparisons and analyses are shown in Table 2.

Group comparisons and associations

Gamma ordinal association was performed between categorical variables. Table 3 shows a significant association between pancreatic and liver macroscopic findings (0.98; CI 0.95–1) and between pancreatic and liver microscopic findings (0.52; CI 0.04–1). Tests were applied to determine differences among groups, as shown in Table 3. The results in Table 2 demonstrate statistically significant associations of microscopic and macroscopic liver steatosis with donor BMI (0.029 and 0.006, respectively). Multiple comparison tests for variable donor BMI are shown in Table 4, which demonstrates a significant association of macroscopic liver steatosis between the moderate and absent groups ($p=0.030$).
Furthermore, a comparison of liver microscopic findings showed significant differences between the severe versus absent ($p < 0.001$), severe versus mild ($p < 0.001$), and severe versus moderate groups ($p < 0.001$), as shown in Table 4.

Table 1 - Demographic parameters for all deceased donors evaluated.

| Parameters                        | Deceased donor (n=54) |
|-----------------------------------|-----------------------|
| Sex (n/%)                         | M=34 (62.9%) / F=20 (37.1) |
| Mean age (years)                  | 40.82 ± 17.24         |
| Median age (years)                | 43 (range, 5-71)      |
| Mean donor BMI                    | 24.38 ± 4.38          |
| Median donor BMI                  | 24 (range, 15.3-31)   |
| Macroscopic evaluation of liver steatosis (%/ n) | Absent 42.86% (N=23) Mild 39.29% (N=21) Moderate 14.29% (N=8) Severe 3.57% (N=2) |
| Pancreatic macroscopic liposubstitution (%/ n) | Absent 36.36% (N=20) Mild 30.3% (N=17) Moderate 27.27% (N=14) Severe 6.06% (N=3) |
| Microscopic evaluation of liver steatosis (%/ n) | Absent 40% (N=22) Mild 42% (N=23) Moderate 10% (N=5) Severe 8% (N=4) |
| Pancreatic microscopic liposubstitution (%/ n) | Absent 13.33% (N=5) Mild 80% (N=43) Moderate 10% (N=4) Severe 8% (N=2) |

Note: BMI, Body mass index; Surgeon, macroscopy; Pathology, microscopy.

Figure 1 - Illustration of microscopic pathologic findings: (A) mild and (B) moderate pancreatic graft adipose infiltration and (C) moderate and (D) severe steatotic liver.

ROC curve analysis

The area under the ROC curve (AUROC) of 0.94 demonstrates an excellent fit, as shown in Figure 2. This finding indicates an improved steatosis diagnosis corresponding to BMI evaluation. The ROC curve revealed an optimal BMI cut-off value of 27.5 kg/m², with a sensitivity of 100%, specificity of 87%, negative predictive value 100%, and positive predictive value of 40% (Figure 2).

Discussion

In this study, we observed a positive association between macroscopic evaluation by a surgeon and microscopic pathologic findings in relation to steatotic liver and adipose infiltration of pancreatic grafts. Furthermore, we determined the optimal donor BMI value for predicting steatosis and adipose infiltration of the pancreatic graft.

Stratta et al. discussed several methods to alleviate the decrease in pancreatic transplants (2), including expanding the number of acceptable donors (2). The present study may facilitate the process of pancreatic transplant by improving the evaluation of the association between pancreatic graft and liver biopsy. We found a positive association between macroscopic and microscopic findings in relation to steatotic liver and adipose infiltration of the pancreatic graft. This finding indicates that macroscopic findings should be compared with the microscopic findings. Furthermore, pancreatic grafts do not have to be discarded based on possible mistakes by the surgeon who interprets the macro/microscopic results.
In this situation, we suggest confirming the association of the pancreatic histopathology analysis with the liver biopsy. A growing obesity epidemic coupled with a high diabetes prevalence in the general population can cause hepatic steatosis and likely causes fatty tissue infiltration of the pancreas (4). This is one of the donor characteristics that is termed “marginal.” This situation is increasingly common and interferes with graft procurement (4). Additional donors, based on recently expanded criteria, are desired due to organ shortages and a constant imbalance between available organs and transplant candidates. The literature shows that variations occur in the definitions, selection criteria, and use of expanded criteria donors according to different geographical areas and centers based on the acceptable risk of graft failure (4,5).

Cucchetti A et al. analyzed data from 374 deceased liver donors from whom a liver biopsy had been obtained to identify variables that could predict the degree of macrovesicular steatosis. Steatosis could be identified accurately to a level of >30% with an AUC of 0.86 (95% CI=0.81–0.91) in combination with BMI, an elevation of alanine aminotransferase, the presence of type II diabetes, a history of heavy alcohol consumption, and ultrasonographic steatosis signs (12). In the present study, we found that an AUROC curve of 0.94 provided an excellent tool for BMI-associated steatotic diagnosis. The AUROC curve area revealed an optimal BMI cut-off value of 27.5 kg/m² with a sensitivity of 100%, specificity of 87%, and negative predictive value of 100%.

During organ recovery surgery, inspection at procurement facilitates detection of the degree of liver steatosis. However, a poor correlation exists between surgical assessment and the degree of steatosis when the degree of steatosis is >35%. A biopsy should then be systematically performed (5). In this study, difficulties were encountered in evaluating liver steatosis at advanced stages and performing correlations with severe fatty infiltration of the pancreas.

### Table 2 - Multiple comparison tests for all parameters and groups.

| Parameters | Organ          | Severity | Median (25%-75%) | p-value |
|------------|----------------|----------|------------------|---------|
|            | Pancreas       | Absent   | 40 (28 - 49.25)  | 33.5 (15.75 - 40) | 0.399   |
|            |                | Mild     | 50.5 (34.75 - 57.75) | 45 (34 - 54) | 0.565   |
|            |                | Moderate | 44 (31.5 - 58)   | 48 (35.5 - 57.5) | 0.999   |
|            |                | Severe   | 54.5 (51.25 - 57.75) |  - | - |
|            | Liver          | Absent   | 43.5 (28 - 50.5)  | 40 (18.25 - 52) | 0.999   |
|            |                | Mild     | 49 (39 - 59.5)   | 42.5 (36.25 - 56) | 0.517   |
|            |                | Moderate | 53 (40.5 - 63.25) | 48 (48 - 50) | 0.902   |
|            |                | Severe   | 38 (30.25 - 45)  |  - | - |
|            | Donor BMI      | Pancreas | Absent | 21.8 (19.45 - 26.02) | 21.6 (20.25 - 22.57) | 0.707   |
|            |                | Mild     | 25.15 (24.18 - 26.85) | 24.2 (22.7 - 27.2) | 0.353   |
|            |                | Moderate | 23.7 (21.08 - 24.65) | 24.7 (23.75 - 24.9) | 0.609   |
|            |                | Severe   | 25.6 (24.2 - 27)  | - | - |
|            | Liver          | Absent   | 21.8 (19.45 - 26.1) | 22.45 (19.73 - 24.88) | 0.885   |
|            |                | Mild     | 24.7 (23.95 - 26.55) | 24.2 (23.12 - 26.73) | 0.411   |
|            |                | Moderate | 27.2 (25.3 - 30.75) | 25.1 (25.1 - 25.7) | 0.268   |
|            |                | Severe   | 32.8 (27.73 - 37.85) | - | - |

Note: Median and interquartile range (25%-75%); BMI, Body mass index; Surgeon, macroscopy; Pathology, microscopy.

### Table 3 - Gamma ordinal association between categorical variables.

| Factor 01 | Factor 02 | Gamma | 95% CI      |
|-----------|-----------|-------|-------------|
| Pancreas, macro | Pancreas, micro | 0.04  | [-0.44; 0.52] |
| Liver, macro     | Liver, micro     | 0.18  | [-0.33; 0.7] |
| Pancreas, macro  | Liver, macro     | 0.98  | [0.95; 1]   |
| Pancreas, micro  | Liver, micro     | 0.52  | [0.04; 1]   |

Note: CI, confidence interval; Surgeon, macroscopy (macro); Pathology, microscopy (micro).

### Table 4 - Multiple comparison tests for donor body mass index variables.

| Factor          | Group comparison | %/n         | p-value    |
|-----------------|------------------|-------------|------------|
| Liver, surgeon  | Mild vs. Absent  | 39.2 (n=21) vs. 42.8 (n=23) | 0.189 |
|                 | Moderate vs. Absent | 14.2 (n=8) vs. 42.8 (n=23) | 0.030 |
|                 | Moderate vs. Mild | 14.2 (n=8) vs. 39.2 (n=21) | 0.349 |
|                 | Mild vs. Absent   | 39.2 (n=21) vs. 42.8 (n=23) | 0.313 |
|                 | Moderate vs. Absent | 14.2 (n=8) vs. 42.8 (n=23) | 0.238 |
| Liver, pathology| Severe vs. Absent | 8 (n=4) vs. 40 (n=22) | <0.001 |
|                 | Moderate vs. Mild | 10 (n=5) vs. 42 (n=23) | 0.999 |
|                 | Severe vs. Mild   | 8 (n=4) vs. 42 (n=23) | <0.001 |
|                 | Severe vs. Moderate | 8 (n=4) vs. 10 (n=5) | <0.001 |

Note: BMI, Body mass index; Surgeon, macroscopy; Pathology, microscopy.

In this situation, we suggest confirming the association of the pancreatic histopathology analysis with the liver biopsy.
Liver biopsy may facilitate pancreatic graft evaluation

In conclusion, positive associations of macroscopic and microscopic histopathological findings in steatotic livers and adipose infiltration of the pancreatic graft were observed.

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Author Contributions

All authors have approved the final version of the manuscript. Nacif LS was responsible for the study conception and design, data collection and analysis, manuscript writing and interpretation, and literature search. Rocha-Santos V, Pinheiro RS, Vintimilla A, and Ferreira LA were responsible for the study conception and design, data collection and analysis, interpretation and critical revision of the manuscript. Alves VA, Claro LC and Arantes RM were responsible for the study conceptions, interpretation and critical revision of the manuscript.

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