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Original article

Evaluation of Spleen Function in Renal Transplant Patients and Controls by Liver-Spleen Scanning Using Qualitative and Quantitative Methods

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Abstract

Objective: The purpose of this study was to compare renal transplant recipients healthy controls, in order to find scintigraphic signs of hyposplenism or hypersplenism by using qualitative and quantitative liver-spleen scan parameters. ***Material and Methods:*** Scanning parameters were evaluated in 88 renal transplant recipients and 59 controls after administration of 5-mCi of 99mTc-stannous colloid. Spleen uptake was characterized as normal, low, or high in comparison to the liver and bone marrow pattern uptake was described as normal or high. The images were drawn over the liver and spleen for counting scintillations and measuring the area. Spleen/liver ratio from controls was correlated with qualitative spleen uptake of renal transplant recipients. Immunosuppressive regimens consisted of combinations of azathioprine or mycophenolate mofetil and rapamycin, tacrolimus or cyclosporine. ***Results:*** renal transplant recipients took up more radiocolloid and had larger livers and spleens than controls. Cases of lower (hyposplenism) and higher (hypersplenism) uptake in the spleen were more frequent in renal transplant recipients than in controls. There was a good correlation between spleen uptake of renal transplant recipients and spleen/liver ratio of controls. ***Discussion:*** renal transplant recipients liver and spleen took up more radiocolloid consistent with their enlarged state, likely due to activation of the mononuclear phagocyte system, probably by repeated exposure to infection. Low and high splenic uptake were found in renal transplant recipients, these findings are consistent with diagnosis of hyposplenism and hypersplenism respectively. Quantitative methods validated visual assessment.

Keywords: ***renal transplant, liver-spleen scan, stannous colloid, hyposplenism, hypersplenism***

Introduction

Splenectomy has been associated with an increased risk of fulminant infection, overwhelming post-splenectomy infection (OPSI), caused by encapsulated bacteria [1]. However, OPSI has also been reported in functional hyposplenism (FH) [2], a pathological condition in which an anatomically present spleen fails to take up radiocolloid [3].

FH is associated with a variety of clinical conditions [4,5] including renal transplant [6]. Kidney transplant is considered the modality choice in renal replacement therapy for patients with end-stage chronic renal disease [7]. Following renal transplant immunosuppressive drugs used to prevent rejection make the patients more susceptible to infection. Although the explanation remains elusive, the potential of hyposplenism to contribute further to the risk of infection in renal transplant should prompt consideration of the role of this condition in this group of patients.

Diagnosis of hyposplenism in renal transplant recipients (RTRs) has been established based on the findings of Howell-Jolly bodies in blood smears [8] and qualitative assessment of liver-spleen scans [6], however no control group was included in these studies for comparison.

On the opposite side of the spectrum of spleen function, hypersplenism has also already been reported in RTRs [9].

Although there is no consensus on a gold-standard method for diagnosis of splenic function [10,11], quantitative measurement of parameters is necessary for estimating the degree of impairment to organ function. Moreover, to date no studies have been performed to compare spleen function in RTRs with control individuals. Therefore, the purpose of this study was to compare RTRs with

healthy controls, in order to find scintigraphic signs of hyposplenism or hypersplenism by using qualitative and quantitative liver-spleen scan parameters.

Material and Methods

This study evaluates qualitative and quantitative scanning parameters in 88 RTRs and 59 healthy controls. We included both variables related to the graft and those related to spleen and liver function. Accordingly, levels of serum creatinine, platelets, and liver enzyme (aspartate aminotransferase - AST; alanine aminotransferase - ALT) were compared between the groups. Variables related to the transplant were obtained from patient records.

Scintigraphy Protocol

Liver and spleen scintigraphy was performed with a gamma camera (Siemens E.CAM) using frames in 128 x 128 matrices. Digital planar images were achieved between 15 and 20 minutes after the intravenous administration of 5-mCi of 99mTc-stannous colloid (IPEN, São Paulo, Brazil); particles were in the nanometer range (taken from the manufacturer's package insert). Data were collected for a preset time of 5 min or preset counts of 500,000 per image, whichever came first.

Using posterior projections, properly exposed images for assessment of the relative distribution of colloid among liver, spleen, and bone marrow were qualitatively (visual assessment) interpreted by two researchers in a single separate session. Those patients whose scans presented equal density in the spleen and the liver were characterized as normal, patients who had lower uptake in the spleen compared with the liver were designated as hyposplenic, and patients who had higher uptake in the spleen compared with the liver were considered as hypersplenic (**Figure 1**). Bone marrow uptake was graded as normal or high (normal: no uptake or faint visualization of the lumbar spine; high: clear visualization of the lumbar spine; **Figure 2**). In cases of disagreement, differences were resolved by joint discussion.

Using both posterior and anterior projections, the images of the liver and spleen produced by radiation activity were manually drawn over the entire organ for the purposes of counting scintillations and measuring the area (**Figure 2**). The arithmetic average value was used in the analysis of the data. In posterior projection, the background activity was measured over a square region of interest (ROI) of 827.8 mm2 placed in the left quadrant of the abdomen below the spleen (**Figure 2**). In addition to the analysis of the whole radioactivity and area, we used two further parameters of organ activity: activity divided by the area of spleen and liver, yielding the mean count rate; and the S/L ratio, mean count rate of the spleen divided by the corresponding values in the liver. Additionally, the sum of the activities in the liver and spleen was used as an estimation of total uptake. Quantitative assessment of bone marrow activity or area was not performed. S/L ratio values from controls were divided into quartiles. The groups (quartiles) were defined by cutoff values reflecting the ≤25th, >25th and ≤50th, >50th and ≤75th, and >75th percentiles of S/L ratio distribution. We correlated qualitative spleen scan assessments from RTRs with S/L ratio quartiles from the controls to predict correlation between the visual estimate and quantitative methods.

Statistical analysis

Continuous variables were expressed in mean ± standard deviation and categorical variables in percentages. Normal distribution was assessed by means of the Shapiro-Wilks test. Differences in non-normal variables between groups were analyzed using the Mann-Whitney test. Chi-Square or Fisher's exact test were used for categorical variables. Linear correlation was evaluated using Pearson's test. The significance of the difference between the correlation coefficients was assessed using the Fisher r-to-z transformation. Data were analyzed using SPSS software (version 17.0, Chicago, Illinois, USA). Significant differences between groups were indicated by a p-value less than 0.05.

The study protocol was approved by the institutional ethics committee. All subjects provided written informed consent for participation in the study.

Results

We enrolled, prospectively, 88 unselected patients (52 males, 36 females; 54 kidneys from deceased donors, 34 kidneys from living donors - related, 30, unrelated, 4). Mean time after transplant was 2243 days (range from 2 to 8978).

All immunosuppressant regimens basically consisted of antimetabolite (azathioprine or mycophenolate mofetil) and corticosteroid. In only fourteen patients was the double regimen used, whereas a triple-therapy immunosuppression regimen included the use of rapamycin in 22, tacrolimus in 42, and cyclosporine in 10 patients.

Renal transplant recipients and controls were sex (female, 41.4% vs. 52.5%; p = 0.184) and age matched (**Table 1**).

Although serum creatinine was higher and platelet counts were lower in RTRs, there was no difference between groups with respect to liver enzymes (AST and ALT) (**Table 1**).

Quantitative data analysis showed that RTRs took up more radiocolloid than controls in liver and spleen. Moreover, the average planar area of liver and spleen was larger in RTRs. However, radioactivity by area in liver and spleen and the S/L ratio were similar to the controls. On the other hand, background activity was higher in controls (**Table 1**).

According to the visual assessment of spleen uptake, hyposplenism and hypersplenism were more frequent in RTRs; indeed 28 patients (31.8%) were classified as hyposplenic and 21 as hypersplenic (23.9%), whereas only 4 controls (6.8%) were classified as hyposplenic and none as hypersplenic (Spearman'rho = 0.504; p < 0.001). There was no difference in frequency of bone marrow uptake between groups (Controls = 5.3% and RTRs = 9.1%, Fisher exact test, p = 0.285).

Good association was found between qualitative assessment of spleen uptake of RTRs (hyposplenic and hypersplenic) and the quartiles (lowest and highest) of S/L ratio of controls (**Figure 3**).

The RTRs showed many differences in mean values of the quantitative parameters analyzed according to spleen uptake, as characterized by visual assessment. Indeed, hyposplenic patients were older, their livers were smaller and took up more radiocolloid, and their spleens were smaller and took up less radiocolloid than hypersplenic patients (Table 2). In relation to radioactivity per area, patients with hyposplenism in comparison to patients with hypersplenism took up more in the liver and less in the spleen (Table 2). Additionally, patients with hyposplenism had a lower S/L ratio than patients with hypersplenism. Total and background counts were similar in both groups (**Table 2**).

The Fisher r-to-z transformation showed that the correlation coefficients between total and background activity found in controls and RTRs were different (**Figure 4**).

Table 1: Significance of the differences between controls and renal transplant recipients (RTRs)*

Variable	Controls (n=59)	RTRs (n=88)	p
Age,years	50.76±11.22	46.88±11.71	0.067
Creatinine, mg/dL	0.85±0.22	2.73±2.54	0.000
Platelets, cells/mm^3	248328±59377	203186±77133	0.000
AST, IU/mL	20.88±9.38	22.62±16.56	0.632
ALT, IU/ml	20.83±13.97	21.62±17.89	0.576
Liver count	270198±27108	302605±36988	0.000
Liver area, mm^2	16030±2338	17337±2781	0.001
Liver count/area	17.13±2.54	17.86±3.45	0.170
Spleen count	69041±23415	87132±37947	0.004
Spleen area, mm^2	5286±1436	6045±1828	0.008
Spleen count/area	13.23±3.66	14.82±6.73	0.341
S/L ratio	0.79±0.26	0.89±0.53	0.878
Total count	339240±21665	389737±25182	0.000
Background count	24.05±8.13	14.55±6.90	0.000

*Mean±SD; Mann-Whitney U test. RTRs - renal transplant recipients; AST - aspartate aminotransferase; IU - international unit; ALT - alanine aminotransferase; S/L - spleen/liver

Table 2: Significance of the differences in quantitative data analyzed according to qualitative assessment of spleen uptake relative to liver in renal transplant recipientes*.

Variable	Hyposplenism (28)	Hypersplenism (21)	P
Age, y	50.57±10.32	41.43±10.49	0.006
Transplant duration, d	2580±2142	2081±2630	0.233
Creatinine, mg/dL	2.98±2.95	3.31±3.22	0.769
Platelets, cells/mm^3	226852±78848	178952±82340	0.098
AST, IU/L	22.48±7.15	22.65±18.25	0.999
ALT, IU/L	16.90±9.46	19.11±19.36	0.394
Liver count	330675±30076	260547±29380	<0.001
Liver area, mm^2	15814±2165	18030±3870	0.012
Liver count/area	21.17±2.81	14.97±3.19	0.001
Spleen count	57108±26108	124195±38667	<0.001
Spleen area, mm^2	4746±1208	6130±1831	0.005
Spleen count/area	12.32±5.40	21.49±8.94	<0.001
S/L ratio	0.60±0.31	1.50±0.70	<0.001
Total count	387783±22135	384742±36326	0.086
Background count	14.52±8.57	18.19±8.26	0.072

*Mean±SD; Mann-Whitney U test. Y - years; d - days; AST - aspartate aminotransferase; IU - international unit; ALT - alanine aminotransferase; S/L - spleen/liver.

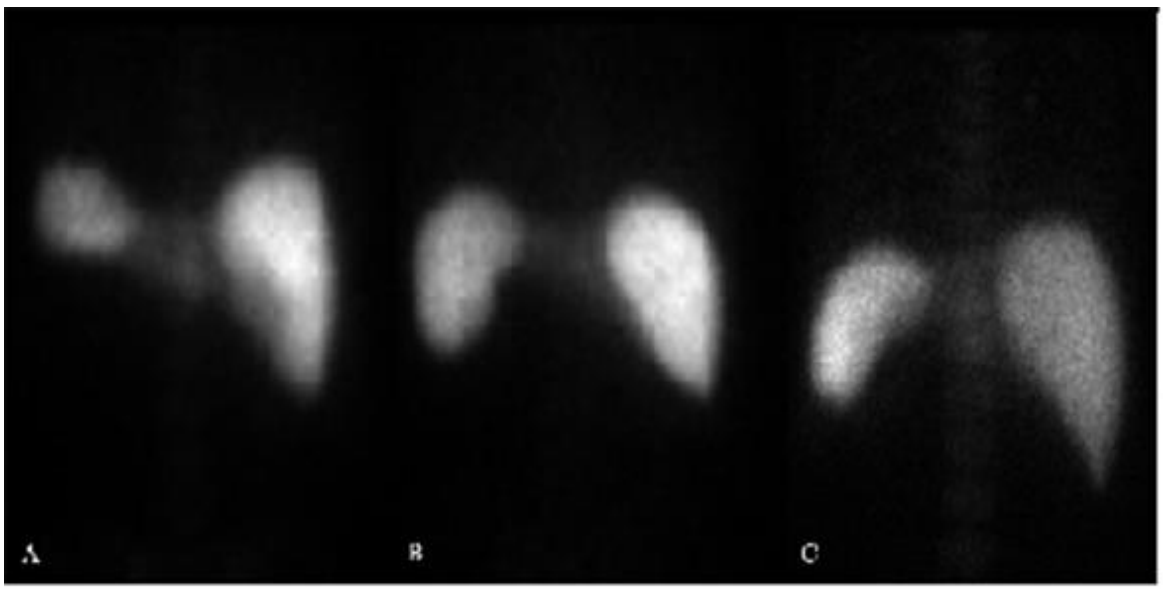

Figure 1: Posterior views of 99mTc-labeled stannous colloid liver-spleen scan show decreased uptake by the spleen (A), equal uptake of colloid by the liver and spleen (B) and uptake by the spleen higher than the liver (C).

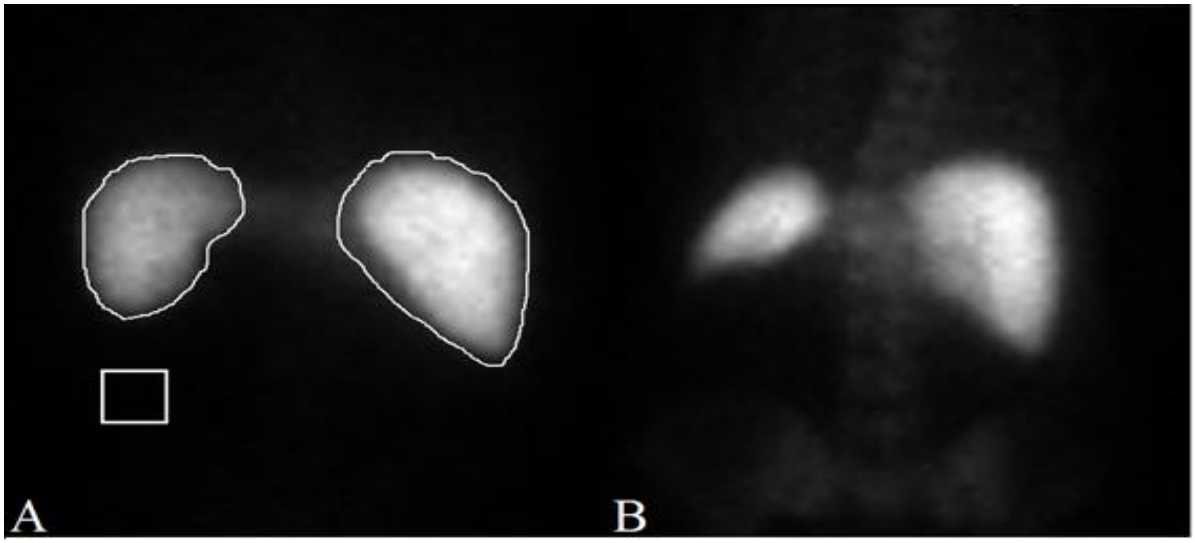

Figure 2: Posterior liver-spleen scan showing both organs encircled, the square area below the spleen and no evidence of colloid uptake by the bone marrow (A), and markedly increased colloid shift to the bone marrow (B).

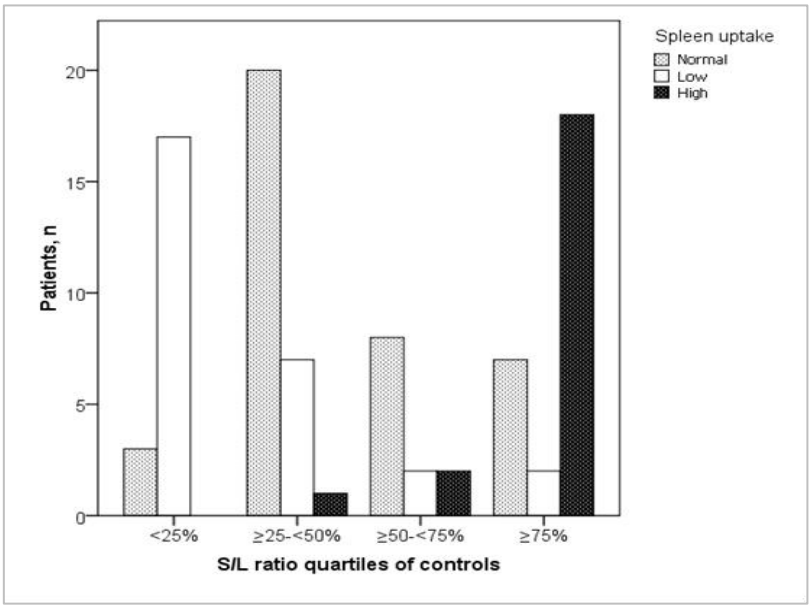

Figure 3: Visually assessed splenic uptake from RTR distributed according to the quartiles of S/L ratio from controls.

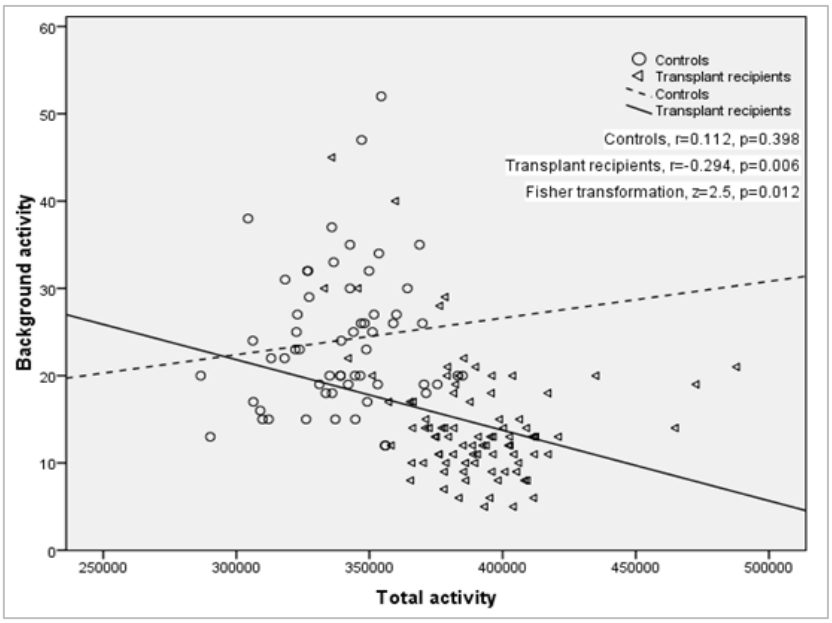

Figure 4: Significance of the difference between correlation coefficient of total and background activity in controls and transplant recipients.

Discussion

There are some relevant findings in this study. The liver and spleen of RTRs were larger and took up more radiocolloid in comparison to the control group. This finding is consistent with activation of the mononuclear phagocyte system, probably by the repeated exposure to infection [12] associated with immunosuppressive therapy. In agreement with overall increased uptake we found a decrease in background activity. Taken together these data suggest that in RTRs the higher the uptake by the mononuclear phagocyte system the lesser the likelihood that radiocolloid remains available to be detected as background activity.

The radiocolloid shift to bone marrow seen in some cases might be due to liver impairment [13], however, in the present study no liver dysfunction could be established based on liver enzymes. Moreover, the frequency of bone marrow uptake observed in RTRs was similar to that seen in controls. On the other hand it is well known that bone marrow uptake increases as particle size decreases [14], predominantly at <0.1 µm particles [15]. We are not aware of the exact size of the tin colloid used in the study, however, according to the manufacturer's package insert, particles were in the nanometer range, that is from 10 to 1000 nm (0.01-1 µm) [16]. Therefore, it is possible that the particle size could account for the bone marrow uptake. However, the bone marrow was never seen in the scanning studies carried out in healthy controls, which used both Tc-99 sulfur particles ranged from 0.1 to 1.0 µm and microalbumin particles 0.2 to 3.0 µm in size [17].

Using slightly different methods, the mean value of S/L ratio obtained in our control group, equal to 0.79, was comparable to those from other reports using 99mTc sulfur colloid: 0.77 [18] and 0.84 [19]. In accordance with one of these reports [18], the S/L ratio distribution obtained in our controls did not show a normal distribution. Therefore, we did not use a multiple of standard deviation plus mean from healthy controls to establish the confidence interval of the normal range. Instead, we simply compared the visual assessment of spleen uptake of RTRs with the quartiles of S/L ratio of the controls to infer the overall correlation between the data. Accordingly, there was overall high concordance between the reports provided by the nuclear medicine doctors and the scintillation count in the processed images. Indeed, almost all hyposplenic and hypersplenic patients were in the ranges of the lowest and highest quartiles of controls' S/L ratio, respectively. This finding strongly substantiates visual assessment as a good method for liver-spleen scan analysis. Thereby providing further evidence that the hyposplenism and hypersplenism in RTRs evaluated by visual assessment correspond to a true diagnosis. Moreover, platelet counts were higher in lower uptake spleens patients and lower in higher uptake spleens patients, although there was no statistically significant difference between groups. However, the highest platelet count (419000 cells/mm3) and the two smallest (2575mm2 and 2920mm2) spleens and the lowest platelet count (30000 cells/mm3) and the two largest spleens (9841mm2 and 11623mm2) were found in the lower and higher uptake spleens groups, respectively. These findings corroborate the need for surveillance against hyposplenism and hypersplenism in RTRs. Functional hyposplenism in a series of patients was diagnosed at our institution [6,8] whereas hypersplenism as a complication of renal transplant has received even more attention from different centers [9,20].

The strength of this study was the inclusion of a control group for the purposes of comparison and the use of quantitative methods to support the qualitative assessment. On the other hand, the weakness of the current study is that the correlation between spleen dysfunction and associated relevant clinical events was not included in the study design.

Handicapped by the lack of literature, the most important questions regarding spleen disorders in renal transplant are not yet known. However, results from this study, added new evidence to gradually strengthen the case that previous beliefs about spleen dysfunction in RTRs are well founded. Moreover, in at least six cases reports of infection in RTRs were related to spleen dysfunction, reinforcing the need to bring attention back to this issue [21-23].

Conclusion

We are confident that the current work extends our knowledge regarding spleen dysfunction in renal transplant and future studies focused on the clinical implications of these findings on the overall morbidity of transplants should be carried out. The potential of hyposplenism to contribute to the risk of infection in renal transplants should prompt a consideration of further impairment of immunocompetence in this group of patients.

Author Contributions statement

Nordeval C Araújo - Contributed to the conception and design of study, analysis and interpretation of data, draft of the manuscript, revision of the manuscript critically for important intellectual content. Final manuscript approval for submission and publication.
Margarida M C Orlando - Contributed to the conception and design of study, acquisition, analysis and interpretation of data, revision of the manuscript critically for important intellectual content.
Moises B Neves - Contributed to the acquisition, analysis and interpretation of data.
Raphael Sancho S de Souza - Contributed to the analysis and interpretation of data, revision of the manuscript critically for important intellectual content.
Carlos A Mandarim-de-Lacerda - Contributed to the conception and design of study, revision of the manuscript critically for important intellectual content.

Data availability statement

Data available on request due to privacy/ethical restrictions.

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Declaration of Interest Statement

The authors declare no conflicts of interest.

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