

# Howell-Jolly bodies and liver-spleen scanning for assessment of splenic filtrative function yields discordant results in renal transplant recipients

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## Abstract

Given discrepancies between methods for diagnosing hyposplenism, the purpose of this study was to evaluate the effect of the spleen size on the correlation between the methods, and to propose a model for improving the interpretation. Patients with renal allografts were included, in whom the spleen was assessed using Doppler ultrasound, scintiscan, and the presence of Howell-Jolly bodies (HJBs) in peripheral smears. In 35 subjects, scintiscan and HJBs were normal (Group 0); 20 had an abnormal result in both methods (Group 1); 34 had discordant results with HJBs present (Group 2); and 14 had discordant results with decreased spleen uptake (Group 3). There was no association between HJBs and scintiscan. The patients of Groups 1 and 2 had smaller spleens. The patients with smaller spleen had more hematological evidence of hyposplenism and exhibit smaller discrepancies between the methods than patients with larger spleen. The spleen can tip the balance from a normal to impaired function provided that the spleen size is below the critical mass required to maintain splenic function. A mild impairment of phagocytic function and slight dyserythropoiesis along with a small spleen would result in decreased take up of radiocolloid or the appearance of HJBs in blood smears.

**Abbreviations:** FH = functional hyposplenism, HJ+ = HJB present, HJ- = HJB absent, HJBs = Howell-Jolly bodies, RES = reticuloendothelial system, RI = resistive indice, SD = standard deviations, SPSS = Statistical Package for the Social Sciences.

**Keywords:** howell-jolly bodies, hyposplenism, methodology disagreement, renal transplant, scintigraphy

## 1. Introduction

Functional hyposplenism (FH) refers to a clinical condition in which a spleen that can be shown by anatomical imaging techniques does not take up radiocolloid properly.<sup>[1,2]</sup>

Besides failure to take up <sup>99m</sup>Tc-sulphur colloid,<sup>[3]</sup> FH can be also demonstrated by increase in pitted red cell counts by interference contrast microscopy,<sup>[4]</sup> or the finding of Howell-Jolly bodies (HJBs) on peripheral stained blood smear.<sup>[5]</sup>

Despite an extensive literature of medical conditions associated with FH, like sickle cell anemia,<sup>[6]</sup> allogenic bone marrow

transplantation,<sup>[7]</sup> inflammatory bowel disease,<sup>[8]</sup> celiac disease,<sup>[9]</sup> systemic lupus erythematosus,<sup>[10]</sup> and amyloidosis<sup>[11]</sup> has been known for decades, to our knowledge a series addressing this issue in renal transplant recipients has been reported only recently.<sup>[12]</sup> FH is now well-recognized as a condition associated with several diseases potentially at risk for developing the disorder and occasionally along with a small spleen.<sup>[5,13,14]</sup>

As a natural consequence of the use of different methods for the detection of hyposplenism, discrepancies between methods may be encountered. Indeed, the correlation between scintigram results and the presence of HJBs in peripheral blood smears has been already assessed in many reports that have yielded conflicting results.<sup>[1,15–21]</sup> Likewise, there is no consensus regarding the relationship between spleen size and function.<sup>[3,13–15,22–26]</sup>

The purpose of this study was a comparative assessment of 2 methods for diagnosing hyposplenism and an evaluation of the effect of the spleen size on the correlation between these 2 methods in a group of renal transplant recipients. Additionally, we propose a model based on mechanisms of decreased spleen uptake of radiocolloid and of appearance of HJBs in peripheral blood smears according to spleen size to improve the interpretation of test results.

## 2. Materials and methods

The study design was a prospective cross-sectional survey based on single samples. One hundred and three patients with renal allografts were included, in whom a standardized investigation of the spleen was performed using color Doppler ultrasound and scintiscan and an examination of a peripheral smear for the

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presence of HJBs. In case of retransplant, only the second renal transplant episode during the study period was included; the results, therefore, reflect one transplant procedure per study patient. Data on the underlying cause of the chronic renal failure were infrequently recorded and are therefore not reported in this article. We included both variables related to the graft and those related to the hyposplenism.

The ethics committee approved this study according to local legal requirements, and informed consent was obtained from the patients.

### 2.1. Scintigraphy protocol

Scans were obtained using a gamma camera using frames in  $128 \times 128$  and  $256 \times 256$  matrices. Digital planar images were recorded 20 minutes after the patients received a 5-mCi intravenous injection of  $^{99m}\text{Tc}$ -stannous colloid; particles were in the nanometer range (according to the manufacturer's package insert). In properly exposed images, assessment of the relative uptake of radiocolloid by the spleen versus uptake by the liver in anterior projections was performed; 103 scans were qualitatively interpreted by 2 researchers in single separate sessions. Those patients whose scans demonstrated equal density in the spleen and the liver were designated as normal, whereas patients who had reduced uptake in the spleen compared with the liver were designated as hyposplenic. In the case of disagreements, differences were resolved by joint discussion.

### 2.2. HJBs

All peripheral smears were reviewed for the presence of HJBs by a single observer using light microscopy. A drop of blood was placed onto a slide, and a blood smear was made. The film of blood was incubated with May-Grunwald stain for 3 minutes, after which water was added for 4 minutes. The blood was then Giemsa-stained for 10 minutes. HJBs were counted in the environment of 100 leucocytes. Patients were then further divided into HJB present (HJ<sup>+</sup>) or absent (HJ<sup>-</sup>) groups.

### 2.3. Ultrasonography

All ultrasounds were performed using a Sonoline 40 instrument (Erlangen, Germany) using the 3.5-MHz transducer. The spleen and kidney longitudinal diameters were obtained where the splenic and kidney edges were clearly defined. The resistive indices of 2 intrasplenic and interlobar graft arteries were measured using the built-in software. Spleen longitudinal diameter values were divided into quartiles. The groups (quartiles) were defined by cutoff values reflecting the  $\leq 25$ th,  $>25$ th and  $\leq 50$ th,  $>50$ th and  $\leq 75$ th, and  $>75$ th percentiles of spleen size distribution.

Instead of comparing patients based only on the results of 1 method, we used a composite to compare patients based on both methods. Based on the combined results, we placed the patients into 4 mixed groups, to capitalize on the respective strengths of each approach. The results of the combined examination were considered concordant if results of both methods were positive or negative for diagnosis of hyposplenism and discordant in case of disagreement in either of the 2 tests. Thus, patients with normal results and agreement between the 2 methods were assigned to Group 0, those in whom both results pointed toward hyposplenism were assigned to Group 1, those with HJBs present in peripheral blood smears but normal scintigrams were

assigned to Group 2 and those with absent HJBs but abnormal scintigrams were assigned to Group 3. Additionally, the effect of the spleen size on the agreement between both methods was assessed.

### 2.4. Statistical analysis

Statistical analysis was performed using SPSS statistical package (SPSS for Windows, version 17.0, Chicago, IL). The values were expressed as means  $\pm$  standard deviations. For analysis of the differences between groups, analysis of variance or Student *t* test was used as appropriate.  $\chi^2$ , Spearman rank correlation, and Fisher exact tests were used to discover whether there was a relationship between categorical variables. The results were considered statistically significant at  $P < .05$ .

## 3. Results

We enrolled 103 patients (65 men; 62 cadaveric donors; 8 retransplants). The mean age was  $48.16 \pm 11.67$  years (range 23.0–74.0 years). The mean transplant duration was 2331 days (range 8–8978 days).

Medical treatments included rapamycin and mycophenolate or azathioprine ( $n = 30$ ); calcineurin inhibitors in addition to mycophenolate or azathioprine ( $n=60$ ); or azathioprine or mycophenolate ( $n=13$ ). All treatment regimens were administered in combination with steroids.

Thirty-five subjects had concordant normal results (33.98%) from both methods (normal spleen uptake and no HJBs in peripheral blood smears; Group 0); 20 had a concordant abnormal result (19.42%) in both methods (decreased spleen uptake and HJBs in peripheral blood smears, Group 1); 34 had discordant results (33.01%) with HJBs in peripheral blood smears, Group 2; and 14 had discordant results (13.59%) with decreased spleen uptake, Group 3. There was no evidence of a significant association between the presence of HJBs and spleen scintigraphy for diagnosing hyposplenism (Q-square=0.833;  $P = .362$ ).

The patients in Groups 1 and 2 were characterized by smaller spleen size (Table 1). None of the other analyzed variables were different among groups (Table 1).

The average spleen size was  $104.1 \pm 20.4$  mm and the range was between 57.2 and 168.1 mm. The spleen length was  $<90.0$  mm in 25 subjects (first quartile), between 90.0 and 102.6 mm in 26 subjects (second quartile), between 102.8 and 115.6 mm in 26 subjects (third quartile), and  $>115.6$  mm in 26 subjects (fourth quartile). The patients in the first quartile (smaller spleen) had higher hematocrit and hemoglobin levels and higher lymphocyte, monocyte, and platelets counts than those in the fourth quartile (larger spleen, Table 2).

The correlation between the groups and spleen size quartiles showed that smaller spleens were associated with the appearance of HJBs and decreased colloid uptake by the spleen, as inferred from the relationship between Group 1 and the lower quartile of the spleen size (Spearman rho=0.40;  $P = .003$ ; Fig. 1). Additionally, HJBs (irrespective of scintigram) were more common in the first than in the fourth quartile ( $\chi^2 = 8.63$ ;  $P = .003$ ) (Fig. 1).

Discordance between splenic uptake of Tc-99m colloid and the presence of HJBs has been described in many conditions. To compare our results with those reported in the literature, data concerning agreement between the methods are presented in Table 3. Compared with values reported for a variety of conditions, there was an intermediate level of agreement

**Table 1****Clinical data and measurements of variables of interest from patients studied\*.**

	Group 0=35	Group 1=20	Group 2=34	Group 3=14	P
Age, y	46.46 ± 11.56	53.90 ± 12.51	45.85 ± 10.43	49.79 ± 11.81	.064 <sup>†</sup>
Transplant duration, days	2102 ± 2359	2819 ± 2355	2367 ± 2351	2126 ± 1738	.500 <sup>‡</sup>
Dialysis duration, mo	72.11 ± 55.00	60.10 ± 59.55	53.56 ± 46.65	53.93 ± 40.53	.572 <sup>‡</sup>
Creatinine, mg/dL	2.08 ± 1.97	2.30 ± 2.11	2.26 ± 1.07	2.56 ± 2.57	.094 <sup>‡</sup>
Albumin, g/dL	4.30 ± 0.68	4.43 ± 0.50	4.61 ± 0.40	4.55 ± 0.58	.264 <sup>‡</sup>
AST, IU/L	19.19 ± 9.16	19.53 ± 6.69	22.00 ± 15.25	32.13 ± 23.19	.189 <sup>‡</sup>
ALT, IU/L	22.07 ± 20.38	17.93 ± 8.98	18.62 ± 15.75	29.14 ± 32.93	.583 <sup>‡</sup>
Direct bilirubin, mg/dL	0.17 ± 0.12	0.13 ± 0.09	0.16 ± 0.08	0.21 ± 0.12	.407 <sup>‡</sup>
Indirect bilirubin, mg/dL	0.30 ± 0.31	0.17 ± 0.04	0.27 ± 0.17	0.25 ± 0.09	.097 <sup>‡</sup>
Hematocrit, %	31.35 ± 8.14	36.58 ± 6.76	33.54 ± 8.98	36.20 ± 6.03	.146 <sup>†</sup>
Hemoglobin, g/dL	10.15 ± 2.60	11.82 ± 2.23	10.83 ± 2.89	11.74 ± 1.99	.153 <sup>†</sup>
Leucocytes, cells/mm <sup>3</sup>	7669 ± 2968	7872 ± 5812	8179 ± 2794	7328 ± 3287	.904 <sup>†</sup>
Neutrophils, cells/mm <sup>3</sup>	5738 ± 2721	4241 ± 2832	5824 ± 3072	5004 ± 3119	.258 <sup>†</sup>
Lymphocytes, cells/mm <sup>3</sup>	1284 ± 962	1746 ± 559	1580 ± 861	1696 ± 929	.256 <sup>†</sup>
Monocytes, cells/mm <sup>3</sup>	526 ± 370	583 ± 217	609 ± 296	641 ± 371	.680 <sup>†</sup>
Platelets, cells/mm <sup>3</sup>	195387 ± 83186	228176 ± 63668	213176 ± 69771	197125 ± 62748	.469 <sup>†</sup>
Spleen size, mm	115.21 ± 21.41	93.47 ± 19.06 <sup>§</sup>	99.70 ± 15.04 <sup>  </sup>	102.09 ± 18.90	.000 <sup>†</sup>
Intrasplenic artery RI	0.54 ± 0.07	0.58 ± 0.08	0.54 ± 0.06	0.55 ± 0.11	.278 <sup>‡</sup>
Kidney length, mm	118.07 ± 9.27	116.67 ± 16.64	118.33 ± 12.65	116.64 ± 11.66	.801 <sup>‡</sup>
Intrarenal artery RI	0.66 ± 0.09	0.71 ± 0.09	0.65 ± 0.08	0.68 ± 0.10	.067 <sup>‡</sup>
PRA class I, %	4.25 ± 13.16	7.60 ± 13.66	3.53 ± 13.35	1.53 ± 4.04	.500 <sup>‡</sup>
PRA class II, %	2.26 ± 9.86	9.89 ± 23.13	7.05 ± 22.59	2.83 ± 6.94	.614 <sup>‡</sup>
	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>χ<sup>2</sup></b>
Sex, male	18 (51.4)	14 (70.0)	23 (67.6)	10 (71.4)	0.164
Calcineurin inhibitor	22 (62.9)	10 (50.0)	19 (55.9)	9 (64.3)	0.958
ATG or Anti-IL2	9 (25.7)	3 (15.0)	3 (8.8)	4 (28.6)	0.110
HLA-mismatches, >3	22 (75.9)	13 (81.3)	15 (53.6)	8 (66.7)	0.058

AST = aspartate aminotransferase, ALT = alanine aminotransferase, RI = resistive index, PRA = panel reactive antibody, ATG = rabbit anti-thymocyte globulin, Anti-IL2 = interleukin-2 receptor antagonists, HLA = human leukocyte antigen.

\* Mean ± standard deviation.

<sup>†</sup> ANOVA test.

<sup>‡</sup> Kruskal-Wallis test.

<sup>§</sup> P < .001 vs. Group 0.

<sup>||</sup> P = .005 vs. Group 0.

between the methods in our renal transplant patients (Table 3).<sup>[1–3,10,11,15–22,24–71]</sup>

We have integrated the examination results and mechanisms associated with the findings of hyposplenism reported in the literature in a theoretical model to help the interpretation of the results (Table 4). Besides the mechanisms, the model includes distributions as required to assign the results of scintigraphy, the presence of HJBs and spleen size according to severity class adjustments. The lowest level of the 3-level patient severity score corresponds to findings in the normal range and is indicative of low-risk patients. The highest level corresponds to patients who have examination findings that indicate severe hyposplenism.

**Table 2****Hematological data according to the spleen size quartile\*.**

Variable	≤25th	>75th	P
Hematocrit, %	35.12 ± 7.68	28.56 ± 9.09	.011
Hemoglobin, g/dL	11.42 ± 2.51	9.39 ± 2.94	.015
Leucocytes, cells/mm <sup>3</sup>	8004 ± 3190	7154 ± 2942	.350
Neutrophils, cells/mm <sup>3</sup>	5192 ± 2990	5706 ± 2792	.559
Lymphocytes, cells/mm <sup>3</sup>	1975 ± 752	878 ± 685	<.001
Monocytes, cells/mm <sup>3</sup>	691 ± 359	448 ± 264	.015
Platelets, cells/mm <sup>3</sup>	246,600 ± 63,543	173,667 ± 65,455	<.001

\* Mean ± SD.

#### 4. Discussion

To date, several useful techniques have been available for the evaluation of spleen function in clinical practice. Among them, excellent procedures such as liver-spleen scintigraphy and the examination of peripheral blood smears for the presence of HJBs are the most common, and are able to provide a specific diagnosis.<sup>[5,72]</sup>

To our knowledge, the present study is the first comparative assessment of these 2 traditional methods for the diagnosis of hyposplenism in a large study enrolling patients with the same condition.

In this prospective observational study, the most important observation was the finding that among 103 patients transplanted, 33 (32.7%) were found to be hyposplenic as measured by <sup>99m</sup>Tc-labeled stannous colloid spleen scans, whereas 54 (53.1%) were hyposplenic on the basis of HJBs.

These results showed that besides the groups in which both methods have a concordant relationship for the diagnosis of hyposplenism (19.42%) or for normal spleen function (33.98%); there are 2 discordant groups: one in which HJBs are present on peripheral blood smears but scintigrams are normal (33.01%) and a second one in which HJBs are absent but scintigrams point toward hyposplenism (13.59%).

Our findings are in accordance with data in previous reports of studies in smaller numbers of subjects in which radionuclide liver-spleen scan and peripheral blood smears did not correlate

First quartile	G0 5	G1 10	G0 6	G1 3	Second quartile
	G2 7	G3 3	G2 13	G3 4	
Third quartile	G0 8	G1 4	G0 16	G1 3	Fourth quartile
	G2 10	G3 4	G2 4	G3 3	

**Figure 1.** Distribution of subsets of concordant and discordant groups (G0–G3) according to the spleen size quartile. While the subgroup of normal concordant examination (Group 0) predominantly had spleen sizes in the upper quartile, the subgroup of hyposplenic concordant results (Group 1) showed the opposite result, with more spleen sizes in the lower quartile (Spearman=0.40; p=0.003) (Numbers highlighted in grey). Group 2 (HJBs present and normal scintiscan) had seven cases of spleen in the lower quartile (Cross-hatched number). HJBs were more common in the first than in the fourth quartile (17/25, 68.0% versus 7/26, 26.9%) (Chi-square=8.63; p=0.003). Spleen length quartiles: A: ≤25th, B: >25th to ≤50th, C: >50th to ≤75th, D: >75th.

well,<sup>[13,17]</sup> although the correlation may vary among disease (Table 3).

There is no consensus concerning the best method for assessing spleen function. Although one study of functional asplenia in adults has suggested that HJBs are a more sensitive indicator of splenic function than sulfur colloid scanning,<sup>[15]</sup> others have found radionuclide sulfur colloid and heat-treated red cell studies to be more sensitive indicators of red cell function.<sup>[6,7,73]</sup>

Based on a compilation of case reports, concordance between the methods is the rule (Table 3). Indeed, in 52 case reports, from 47 articles, in a variety of conditions, the concordance (both normal or both abnormal) was as high as 80.8%, a figure quite a lot higher than that found in the current study (53.4%). However, the figure is substantially lower when the concordance is based on series instead. From 159 cases studied in 13 series (those including at least five cases), also in a variety of conditions, the concordance only reached 54.7% (from 21.8% in miscellaneous conditions to

**Table 4**

**A component-based mechanism involved in the impaired spleen uptake of radiocolloid and appearance of HJBs on peripheral blood smears. Compromised extent.**

Mechanism	Normal	Mild/moderate	Severe
RES impairment			
Liver-spleen scan	Normal	Normal/Decreased	Decreased
HJBs	Absent	Absent/Present	Present
HJBs overload			
Liver-spleen scan	Normal	Normal	Normal
HJBs	Absent	Absent/Present	Present
Spleen critical mass			
Liver-spleen scan	Normal	Normal/Decreased	Decreased
HJBs	Absent	Absent/Present	Present

RES=reticuloendothelial system, HJBs=Howell-Jolly bodies.

100.0% in sickle cell anemia), a percentage very similar to that found in the present study (53.4%).

It is not entirely clear why concordance in compilations of case reports is different from that in series studies. However, one might hypothesize that part of this inconsistency may derive from fundamental problems associated with the way the patients are enrolled. In case reports, usually only cases with severe infection or fatal outcome are reported, a situation in which hyposplenism is more severe, while in series a wide range of disease severity is included. In accordance with this, it is well known that in mild cases of hyposplenism, although spleen dysfunction can be assessed by using scintiscan, HJBs are regularly absent on peripheral blood smears, that is, the appearance of HJBs in blood smears points towards a more severe form of hyposplenism.<sup>[72,73]</sup> Therefore, based on these assumptions, it is reasonable to believe that our concordant results represent either normal spleen function (HJBs absent and normal spleen scintigraphy) or severe hyposplenism (HJBs present and impaired spleen uptake of colloid) and in cases of disagreement where there is an absence of HJBs, we are dealing with a less severe dysfunction of the spleen. The more problematic discrepant results are the 34 patients in which the HJB tests are positive but the scintigrams are negative. This discrepancy may be caused by conditions other than hyposplenism.

This disagreement (HJBs present along with normal spleen scan) has been attributed to a bone marrow abnormality (dyserythropoiesis), in which an overload of HJBs delivered to the circulation can overwhelm the ability of a normal spleen to clear them from circulation.<sup>[58]</sup> In accordance with this, HJBs

**Table 3**

**Comparison of the results obtained in this study with the summation of several case reports and series found in the literature.**

	Concordant			Discordant		
	Total	Abnormal	Normal	Total	HJBs absent	HJBs present
Case reports <sup>[2,10,11,21,22,24–66]</sup>	78.6% (44/56)	88.6% (39/44)	11.4% (5/44)	21.4% (12/56)	58.3% (7/12)	41.7% (5/12)
Series <sup>[1,3,15–20,67–71]</sup>	54.7% (87/159)	77.0% (67/87)	23.0% (20/87)	45.3% (72/159)	67.6% (48/72)	33.3% (24/72)
Current	53.4% (55/103)	36.4% (20/55)	63.6% (35/55)	46.6% (48/103)	29.2% (14/48)	70.8% (34/48)
Distribution of series cases according to specific diseases						
Amyloidosis <sup>[15,16,18]</sup>	46.6% (14/30)	92.9% (13/14)	7.1% (1/14)	53.3% (16/30)	68.7% (11/16)	31.3% (5/16)
Sickle cell anemia <sup>[1,67,68]</sup>	87.0% (20/23)	95.0% (19/20)	5.0% (1/20)	13.0% (3/23)	66.7% (2/3)	33.3% (1/3)
Asplenia/ Splenectomy <sup>[19,20,69]</sup>	67.3% (33/49)	54.5% (18/33)	45.5% (15/33)	32.7% (16/49)	12.5% (2/16)	87.5% (14/16)
SLE, Sjögren syndrome <sup>[3,70]</sup>	63.7% (7/11)	85.7% (6/7)	14.3% (1/7)	36.4% (4/11)	0.0% (0/4)	100.0% (4/4)
Heterotaxy <sup>[71]</sup>	75.0% (9/12)	77.8% (7/9)	22.2% (2/9)	25.5% (3/12)	100.0% (3/3)	0.0% (0/3)
Miscellaneous <sup>[17]</sup>	11.8% (4/34)	100.0% (4/4)	0.0% (0/4)	88.2% (30/34)	100.0% (30/30)	0.0% (0/30)

HJBs=Howell-Jolly bodies, SLE=systemic lupus erythematosus.

have also been reported in some hematological abnormalities, such as iron deficiency anemia, hemolytic anemias, and megaloblastosis.<sup>[74,75]</sup> Thus, it is reasonable to speculate that the finding of HJBs in peripheral blood smears along with a normal spleen scan might represent the hematological disorders mentioned in renal transplant recipients. However, as no comprehensive study to detect hematological disorders was performed in the present study, it is not possible to confirm this hypothesis.

Whether the disassociation of radiocolloid uptake from the appearance of HJBs is a qualitative or quantitative phenomenon is unknown. However, there are several pieces of evidence that support the influence of spleen size on the blood film features of hyposplenism.

We performed some further analyses to examine whether incorporating a spleen size parameter in interpreting the traditional methods for diagnosing hyposplenism would improve the concordance between the methods. In our study, the findings on peripheral blood smears correlated better with spleen size than with the findings on radionuclide liver-spleen scans. Indeed, the appearance of HJBs increases from 26.9% to 70.0% as spleen size reduces from the upper to the lower percentile, respectively. Accordingly, HJBs was found in all patients submitted to total splenectomy,<sup>[69]</sup> whereas no case of HJBs could be detected in patients undergoing subtotal splenectomy.<sup>[20]</sup> To a lesser extent, a similar relationship was also found between spleen size and spleen colloid uptake.

There is also further supporting evidence from the literature. Indeed, based on data from the literature, 55 of 90 (61.1%) patients with a presence of HJBs had an absent or small spleen,<sup>[1–3,10,11,13,16,18,19,21,22,24–27,29,32,33,35,36,41–45,49,50,52,54,55,57–59,61,62,66,68,69,71]</sup>, whereas only 1 of 13 (7.7%) patients with absence of HJBs had a small spleen.<sup>[16,26,28,37,40,53,64,65]</sup> Moreover, of 35 cases of HJBs present with normal or enlarged spleen, 9 were sickle cell anemia cases<sup>[11]</sup> and in 5, microscopic sections showed extensive splenic parenchymal replacement by tissue infiltration of the underlying disease.<sup>[18,24–26,50]</sup>

Moreover, agreement between the methods increases depending on the spleen size. The lower the spleen size, the better the agreement between methods for diagnosing hyposplenism: 50% versus 15% agreement in the lower quartile and upper quartile, respectively. However, agreement on negative results for hyposplenism (normal results using both methods) presented the opposite trend (14.3% in the lower vs. 45.7% in the upper quartile).

If one assumes that the lower quartile is below the critical size of spleen required to clear HJBs, this assumption supports the appearance of HJBs in the peripheral blood smears of further 7 patients from the discordant group with HJBs, which in turn, further reduces the percentage of unexplained cases of HJBs from 33.0% to 26.2% (Fig. 1).

From a diagnostic perspective, it is comforting when the results of both methods agree, that is, both methods point toward hyposplenism or normality. However, when combinations of methods often vary, it seems to be a good idea to add other parameters to improve diagnosis. Clinicians need to be aware of the limitations of both methods when making management decisions. Adding information from the measurement of the spleen to the results of HJBs and scintiscanning might help to refine the diagnosis of hyposplenism. The addition of spleen size measurement increases the agreement between HJBs and scintiscanning, and it would be reasonable to include it to

further improve prediction in subgroups of patients with borderline degrees of hyposplenism.

Accordingly, a theoretical model is proposed (Table 4) to explain the discordance between the scintigraphy and HJBs methods based on the interaction of 3 mechanisms underlying medical tests for spleen function. This developed model is expected to maximize the strengths and minimize the weaknesses of each type of data. Like other models, this contains approximations and assumptions that limit the range of validity and predictive power, but may help us to gain insights into the disagreements between different methods for diagnosing hyposplenism. This process was performed for 3 different grades of severity for each variable.

The model assumes that as the impact of each mechanism (or the summation of more than one mechanism) impairment on the spleen function (as assessed by the method) are expected to vary substantially according to the degree of severity, then a stratified composite mechanism can help in interpreting the examination results and its consistency across these subgroups.

One might hypothesize that if all components are normal, the spleen uptake of radiocolloid should be normal and HJBs absent. However, if all components are abnormal—as in a severe disease extent—the spleen uptake of radiocolloid should be decreased and HJBs present, the classical features of hyposplenism. However, a composite score of abnormal components at different extents could yield a spectrum of spleen impairment severity that could explain the disagreements in results between liver-spleen scintiscanning and peripheral blood smears in hyposplenism, as reported in different clinical conditions.

Overall, the hypothesized model fits well. Both normal (Group 0) and abnormal concordant (Group 1) results can be associated with normal and at least 1 severely abnormal mechanisms, respectively (Table 4). If the spleen shows a decreased uptake by liver-spleen scan, without HJBs in peripheral blood (Group 3), the reason may be mildly or moderately compromised reticuloendothelial function of the spleen, without HJB outpouring and a spleen larger than a critical size. However, if HJBs are present and a liver-spleen scintiscan is normal (Group 2), reticuloendothelial function should be normal (or only mildly compromised) and either HJB outpouring or small spleen size or both should be taken into account.

It seems plausible to speculate that according to its size, the spleen can tip the balance from normal to impaired function as assessed by the tests, provided that the spleen is below a critical size that is required to maintain splenic function. Accordingly, an association of mild impairment of phagocytic function and slight dyserythropoiesis along with a small spleen would result in a clear decreased take up of radiocolloid or the appearance of HJBs in peripheral blood smears.

There are separate lines of evidence that support the hypothesis that spleen size may help in the understanding of disagreements between liver-spleen scintiscanning and peripheral blood smears. Some studies have shown an association of reduction in spleen size with a heterogeneous group of diseases that include hematological disorders,<sup>[14,23]</sup> chronic intestinal inflammation,<sup>[76]</sup> and in systemic erythematosus lupus.<sup>[3]</sup> However, there is significant uncertainty and divergence of opinion regarding the correlation between spleen size and function.<sup>[1,57]</sup> Moreover, the occurrence of hyposplenism in patients with splenomegaly or palpable spleen<sup>[1,58,70,77]</sup> seems an apparent contradiction. Nonetheless, in cases in which there was no clear evidence of hematologic stress and no outpouring of reticulocytes, a small

spleen, below a critical necessary size, cannot adequately clear all HJBs from red cells.<sup>[22]</sup>

Data from experimental<sup>[78]</sup> and clinical studies<sup>[79]</sup> showed that a greater amount of implanted spleen tissue reduces the likelihood of appearance of HJBs in peripheral blood smears. Based on the summation of the results from the reports mentioned above<sup>[78,79]</sup> and those of the present study, we are confident that small spleen size in renal transplant patients plays an important role in the finding of HJBs without impaired uptake in spleen scans. It is noteworthy that small spleen has also been associated with the use of rapamycin, a common immunosuppressant used in renal transplant.<sup>[80]</sup>

Nonetheless, our results are in line with the concept that a small spleen below a crucial size is an important cause HJB presence in peripheral blood.<sup>[78]</sup> Interestingly, spleen size but not HJBs and spleen scintiscanning correlated with peripheral blood cell counting, suggesting that a small spleen size is a more specific variable for the identification of some features of hyposplenism than spleen reticuloendothelial system function alone. Accordingly, small spleen correlated better with higher hemoglobin levels and lymphocyte and platelet counts.

As the blood filtrative function of the spleen relies on the amount of clarified senescent cells that travel through venous sinusoids and the elevation of spleen blood flow may play an important role in the development of hypersplenism,<sup>[81]</sup> it is reasonable to assume that the clearance of HJBs from peripheral blood might be compromised if the spleen blood flow decreases. On the one hand, an increase in the cardiac output ratio to the spleen in splenomegaly has been reported;<sup>[81]</sup> on the other hand, it seems plausible to infer that a decrease in cardiac output ratio to a small spleen could produce features of hyposplenism. Indeed, in patients with chronic graft-versus-host disease after bone marrow transplantation, HJBs were associated with smaller spleen and lower splenic flow.<sup>[82]</sup>

A weakness of this study was the fact that we were not able to answer why a large number of renal transplant recipients present HJBs in peripheral smears without signs of impaired radiocolloid uptake by the spleen. The assumption that the presence of HJBs is a feature of severe splenic dysfunction is in disagreement with normal spleen colloid scintigraphy, at least at first sight. However, if different mechanisms, such as RES dysfunction, HJB overload, and spleen critical mass, take place to different extents, an intricate summation effect could be expected that might explain the discordant findings in different clinical situations.

According to the proposed model, it is possible that even an otherwise normal small spleen overwhelmed by an outpouring of HJBs cannot adequately clear all corpuscles from red cells, although clearance of colloidal particulate remains intact. This assumption would be valuable for interpreting the finding of HJBs in peripheral blood smears in patients with normal liver-spleen scans and could be used as an alternative to the present system.

The results of this study extend our knowledge concerning discrepancies between tests used for diagnosing hyposplenism by examining the underlying mechanisms responsible for the abnormal results. However, further exploration with longitudinal studies is necessary before any generalizations can be drawn regarding the significance of this model for refining the next steps in identifying limiting, worst-case, or special scenarios for diagnosing hyposplenism.

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