ORIGINAL ARTICLE

Changes in Liver and Spleen Volumes After Living Liver Donation: A Report From the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL)

Jean C. Emond,[1] Robert A. Fisher,[2,3] Gregory Everson,[4] Benjamin Samstein,[1] James J. Pomposelli,[5] Binsheng Zhao,[6] Sarah Forney,[7] Kim M. Olthoff,[8] Talia B. Baker,[9] Brenda W. Gillespie,[10] and Robert M. Merion[7,11]

[1]Center for Liver Disease and Transplantation, Columbia University, New York, NY; [2]Department of Surgery, Virginia Commonwealth University, Richmond, VA; [3]Transplant Institute, Harvard Medical School, Boston, MA; [4]Section of Hepatology, University of Colorado Denver, Aurora, CO; [5]Department of Transplantation, Lahey Hospital and Medical Center, Burlington, MA; [6]Department of Radiology, Columbia University Medical Center, New York, NY; [7]Arbor Research Collaborative for Health, Ann Arbor, MI; [8]Department of Surgery, University of Pennsylvania, Philadelphia, PA; [9]Department of Surgery, Northwestern University, Chicago, IL; Departments of [10]Biostatistics, University of Michigan, Ann Arbor, MI; and [11]Surgery, University of Michigan, Ann Arbor, MI

Previous reports have drawn attention to persistently decreased platelet counts among liver donors. We hypothesized an etiologic association between altered platelet counts and postdonation splenomegaly and sought to explore this relationship. This study analyzed de-identified computed tomography/magnetic resonance scans of 388 donors from 9 Adult-to-Adult Living Donor Liver Transplantation Cohort Study centers read at a central computational image analysis laboratory. Resulting liver and spleen volumes were correlated with time-matched clinical laboratory values. Predonation liver volumes varied 2-fold in healthy subjects, even when they were normalized by the body surface area (BSA; range = 522-1887 cc/m^2, n = 346). At month 3 (M3), postdonation liver volumes were, on average, 79% of predonation volumes [interquartile range (IQR) = 73%-86%, n = 165] and approached 88% at year 1 (Y1; IQR = 80%-93%, n = 75). The mean spleen volume before donation was 245 cc (n = 346). Spleen volumes greater than 100% of the predonation volume occurred in 92% of donors at M3 (n = 165) and in 88% at Y1 after donation (n = 75). We sought to develop a standard spleen volume (SSV) model to predict normal spleen volumes in donors before donation and found that decreased platelet counts, a younger age, a higher predonation liver volume, higher hemoglobin levels, and a higher BSA predicted a larger spleen volume (n = 344, $R^2 = 0.52$). When this was applied to postdonation values, some large volumes were underpredicted by the SSV model. Models developed on the basis of the reduced sample of postdonation volumes yielded smaller underpredictions. These findings confirm previous observations of thrombocytopenia being associated with splenomegaly after donation. The results of the SSV model suggest that the biology of this phenomenon is complex. This merits further long-term mechanistic studies of liver donors with an investigation of the role of other factors such as thrombopoietin and exposure to viral infections to better understand the evolution of the spleen volume after liver donation. ***Liver Transpl 21:151-161, 2015.*** © **2014 AASLD.**

Received June 24, 2014; accepted October 26, 2014.

Although living donor liver transplantation (LDLT) has a high success rate[1,2] and increases access to transplantation,[2] concerns about donor safety and the technical complexity of the recipient operation have greatly limited expansion of the procedure in Western countries. Although LDLT is the principal donor source for liver transplantation in Asian countries,[3,4] LDLT has accounted for less than 5% of procedures performed annually in the United States in the past 5 years. Unfortunately, the limited use of LDLT persists despite the demonstration of safety in the donors and efficacy in the recipients. There is a compelling survival benefit for the recipient in choosing LDLT[5] even among

Additional Supporting Information may be found in the online version of this article.

Abbreviations: A2ALL, Adult-to-Adult Living Donor Liver Transplantation Cohort Study; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; BSA, body surface area; CT, computed tomography; INR, international normalized ratio; IQR, interquartile range; LDLT, living donor liver transplantation; M3, month 3; MR, magnetic resonance; SD, standard deviation; SSV, standard spleen volume; Y1, year 1

patients with Model for End-Stage Liver Disease scores as low as 10,[6] and the morbidity of donation in a large multicenter trial of LDLT[7] was acceptable, as previously observed in single-center studies.

Despite the apparent preexisting good health and early clinical recovery of most donors, abnormal laboratory tests were noted in some subjects at least at year 1 (Y1) after donation. We first reported a persistent decline in platelet counts through Y1 after donation in a subset of liver donors from a single center in 2004.[8] This finding was subsequently observed in the much larger Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL).[9] In the 487 subjects in that cohort, platelet counts were significantly decreased at Y1 for the population as a whole and were below the lower limit of normal in 7% of 327 subjects with normal laboratory values before donation who were at Y1 or longer after donation. In a substudy of quantitative liver function of A2ALL donors, we noted that splenomegaly in some subjects corresponded to decreased platelet counts.[10]

In the current study, we sought to determine whether abnormal laboratory tests after donation were associated

TABLE 1. Characteristics of 388 Living Liver Donors

Characteristic	n	Mean (SD) or %	Range
Age (years)	388	37.8 (10.4)	18.2-62.7
Sex			
Male	189	48.7	
Female	199	51.3	
Ethnicity			
Hispanic	53	13.7	
Non-Hispanic	335	86.3	
Race			
White	359	92.5	
African American	11	2.8	
Asian	6	1.6	
Other	12	3.1	
Height (cm)	388	171.8 (10.2)	134.6-195.6
Weight (kg)	388	77.9 (14.8)	43.1-135.0
BMI (kg/m^2)	388	26.3 (3.9)	16.4-42.4
Left lobe donor	25	6.4	

This is study number 25 of the Adult-to-Adult Living Liver Transplant Cohort Study.

This is publication 26 of the Adult-to-Adult Living Donor Liver Transplantation Cohort Study. The following individuals were instrumental in the planning and conduct of this study at each of the participating institutions: Columbia University Medical Center, New York, NY (DK62483): PI: Jean C. Emond, MD; Co-Is: Robert S. Brown, Jr., MD, MPH, James Guarrera, MD, FACS, Martin R. Prince, MD, PhD, Benjamin Samstein, MD, Elizabeth Verna, MD, MS; Study Coordinators: Taruna Chawla, MD, Scott Heese, MPH, Theresa Lukose, PharmD, Rudina Odeh-Ramadan, PharmD, Jonah Zaretsky, BS. Northwestern University, Chicago, IL (DK62467): PI: Michael M.I. Abecassis, MD, MBA; Co-Is: Talia Baker, MD, Laura M. Kulik, MD, Daniela P. Ladner, MD; Study Coordinator: Patrice Al-Saden, RN, CCRC. University of California Los Angeles, Los Angeles, CA (DK62496): PI: Johnny C. Hong, MD; Co-I: Ronald W. Busuttil, MD, PhD; Study Coordinator: Janet Mooney, RN, BSN. University of California San Francisco, San Francisco, CA (DK62444): PI: Chris E. Freise, MD, FACS; Co-I: Norah A. Terrault, MD, MPH; Study Coordinator: Dulce MacLeod, RN. University of Colorado, Aurora, CO (DK62536): PI: James R. Burton, Jr., MD; Co-Is: Gregory T. Everson, MD, FACP, Igal Kam, MD, James Trotter, MD; Study Coordinators: Carlos Garcia, RN, BS, Anastasia Krajec, RN. University of Michigan Health System, Ann Arbor, MI (DK62498): PI: Robert M. Merion, MD, FACS; DCC Staff: Mary Akagi, MS, CCRP, Douglas R. Armstrong, BSN, MS, Abby Brithinee, BA, Margaret Hill-Callahan, BS, LSW, Lisa Holloway, BS, CCRC, Terese A. Howell, BS, CCRC, Brenda W. Gillespie, PhD, Beth Golden, BScN, Anna S.F. Lok, MD, Monique Lowe, MSI, Akinlolu O. Ojo, MD, PhD, Samia Shaw, AAIT, Abigail Smith, MS, Robert A. Wolfe, PhD. University of North Carolina, Chapel Hill, NC (DK62505): PI: Paul H. Hayashi, MD, MPH; Study Coordinator: Tracy Russell, MA. University of Pennsylvania, Philadelphia, PA (DK62494): PI: Abraham Shaked, MD, PhD; Co-Is: Kim M. Olthoff, MD, FACS, K. Rajender Reddy, MD, Mark A. Rosen, MD, PhD; Study Coordinators: Brian Conboy, PA, MBA, Mary Kaminski, PA-C, Debra McCorriston, RN, Mary Shaw, RN, BBA. University of Virginia, Charlottesville, VA (DK62484): PI: Carl L. Berg, MD; Co-I: Timothy L. Pruett, MD; Study Coordinator: Jaye Davis, RN. Virginia Commonwealth University - Medical College of Virginia, Richmond, VA (DK62531): PI: Robert A. Fisher, MD, FACS; Co-Is: Martha K. Behnke, PhD, Adrian Cotterell, MD, FACS, Ann Fulcher, MD, Pamela M. Kimball, PhD, HCLD, Mary E. Olbrisch, PhD, ABPP, Marc P. Posner, MD, FACS, Mark A. Reimers, PhD, Amit Sharma, MD, R. Todd Stravitz, MD, FACP; Study Coordinators: April Ashworth, RN, BSN, Joanne Davis, RN, Sarah Hubbard, Andrea Lassiter, BS, Luke Wolfe, MS. National Institute of Diabetes and Digestive and Kidney Diseases, Division of Digestive Diseases and Nutrition, Bethesda, MD: Edward Doo, MD, James E. Everhart, MD, MPH, Jay H. Hoofnagle, MD, Stephen James, MD, Patricia R. Robuck, PhD, Leonard B. Seeff, MD, Averell H. Sherker, MD, FRCPC, Rebecca J. Torrance, RN, MS.

Potential conflict of interest: Nothing to report.

For a list of supporting institutions, see the supporting information.

This study was supported by the National Institute of Diabetes and Digestive and Kidney Diseases through cooperative agreements (grants U01-DK62444, U01-DK62467, U01-DK62483, U01-DK62484, U01-DK62494, U01-DK62496, U01-DK62498, U01-DK62505, U01-DK62531, and U01-DK62536). Additional support was provided by the Health Resources and Services Administration and the American Society of Transplant Surgeons.

This study was presented in part at the 63rd Annual Meeting of the American Association for the Study of Liver Diseases; November 9-13, 2012; Boston, MA.

Address reprint requests to Jean C. Emond, M.D., Department of Surgery, Center for Liver Disease and Transplantation, Columbia University, 622 West 168th Street, New York, NY 10032. Telephone: 212-305-9691; FAX: 212-342-5600; E-mail: je111@cumc.columbia.edu

DOI 10.1002/lt.24062
View this article online at wileyonlinelibrary.com.
LIVER TRANSPLANTATION.DOI 10.1002/lt. *Published on behalf of the American Association for the Study of Liver Diseases*

TABLE 2. Donor Laboratory Values and Liver and Spleen Volumes by Time Point: Before Donation and After Donation at M3 and Y1

	Pre-Donation	M3	Y1
Laboratory values at each time point			
Albumin (g/dL)	4.4 (0.4)	4.1 (0.4)*	4.2 (0.4)*
	3.5-5.5	2.8-5.2	2.9-5.2
	387	265	192
Bilirubin (mg/dL)	0.7 (0.3)	0.7 (0.4)	0.8 (0.3)*
	0.1-2.8	0.1-3.6	0.2-2.6
	388	272	197
ALT (IU/L)	24.4 (12.5)	29.5 (16.5)*	25.2 (13.5)
	4.0-110.0	1.5-108.0	6.0-92.0
	388	272	195
AST (IU/L)	23.3 (6.6)	29.9 (13.9)*	26.1 (11.0)*
	11.0-53.0	13.0-130.0	11.0-109.0
	387	272	197
AP (IU/L)	68.4 (24.8)	93.8 (42.9)*	74.1 (26.3)*
	15.0-197.0	30.0-385.0	16.0-186.0
	388	271	196
INR	1.00 (0.08)	1.05 (0.09)*	1.02 (0.09)*
	0.78-1.50	0.89-1.70	0.70-1.50
	382	254	186
Platelet count ($\times 10^3/mm^3$)	264.3 (63.0)	221.6 (67.6)*	214.4 (63.7)*
	126.0-543.0	94.0-660.0	3.6-708.0
	387	270	192
White blood count ($\times 10^3/mm^3$)	6.6 (1.8)	6.6 (1.7)	6.6 (1.7)
	3.1-20.7	0.9-14.0	3.4-16.4
	387	269	194
Hemoglobin (g/dL)	14.6 (1.4)	13.7 (1.8)*	14.4 (1.5)*
	9.1-18.4	5.4 17.3	9.4-18.0
	387	268	192
Volumes at each time point			
Liver volume (cc)	1601.0 (327.3)	1241.2 (257.1)*	1440.5 (274.0)*
	863.8-3250.8	790.0-2024.1	936.3-2122.0
	346	182	90
Spleen volume (cc)	245.6 (107.3)	314.3 (136.4)*	323.6 (181.4)*
	67.1-774.6	77.6-842.2	82.5-1171.7
	346	182	90
Liver/spleen Ratio	7.4 (2.8)	4.6 (1.8)*	5.5 (2.6)*
	2.5-22.8	1.7-11.2	1.2-15.9
	346	182	90

NOTE: For all values, the first row gives the mean (SD), the second row gives the range, and the third row gives the n value.
*Significantly different from the predonation value ($P < 0.05$) according to a paired t test.

with splenomegaly or volumetric alterations in the liver by examining the relationship between liver and spleen volumes and the evolution of laboratory tests up to Y1 after donation. Because spleen size is associated with platelet counts and may be an indicator of portal hypertension, we sought to characterize normal spleen volume before donation and changes after donor partial hepatectomy. In addition, we sought to identify predictors of abnormal laboratory tests and to identify a subset of subjects at risk for marked splenomegaly after donation.

PATIENTS AND METHODS

A2ALL was an observational study at 9 US centers that was conducted to evaluate the efficacy of LDLT in adult recipients and to characterize the impact of donation on healthy subjects. Computed tomography (CT)/magnetic resonance (MR) scans were collected for 388 subjects who underwent donor hepatectomy between July 1998 and May 2010. Demographic and clinical variables were collected both prospectively and retrospectively with follow-up to 11 years after donation. The study was approved by the institutional review boards and privacy boards of the University of Michigan Data Coordinating Center and each of the transplant centers.

Imaging and Analysis

CT/MR scans before donation and at month 3 (M3) and Y1 after donation were collected when they were available from the study sites, de-identified, and transmitted with AG Mednet's system (AG Mednet,

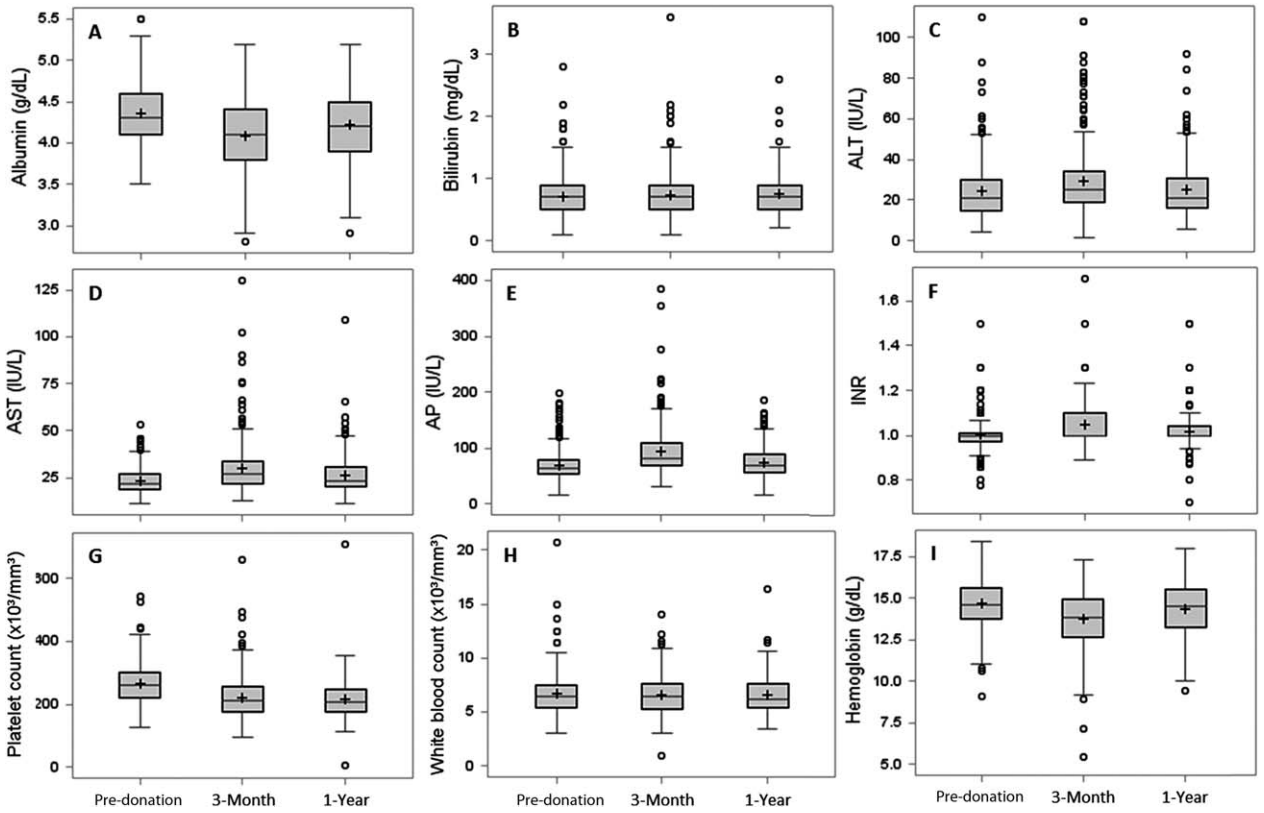

Figure 1. Distributions of laboratory values shown as box plots by time points. Each box spans from the first to the third quartile and shows the median (line crossing the box) and mean (+). Whiskers extend to the farthest data point within 1.5 IQRs from the box ends, with all outlying points shown individually as circles.

Inc., Boston, MA) to the central Computational Image Analysis Laboratory of Columbia University Medical Center.[11,12]

Images were coded to permit merging with clinical information. Liver and spleen volumes were calculated with a proprietary, organ-generic algorithm developed by the Computational Image Analysis Laboratory. Scan-based volumes were correlated with clinical and laboratory features at corresponding times.

Statistical Methods

We used means, standard deviations (SDs), ranges, box plots, and percentages to describe donor characteristics by time point. Correlation coefficients and scatter plots were used to assess relationships between corresponding variables at different time points as well as different variables at the same time point. Spaghetti plots were used to assess within-person trends in variables over time.

Linear regression was used to model the predonation spleen volume as a function of laboratory and patient characteristics. Variables tested in the model included the body surface area (BSA), height, weight, body mass index (BMI), age, sex, predonation liver volume, platelet count, white blood cell count, and hemoglobin. The model fit was assessed with R^2, leverage and influence diagnostics, and residual plots. Variable selection was performed with the method of best subsets. Logistic regression was used to test predictors of predonation spleen volumes greater than 400 cc. All analyses were performed with SAS 9.2 (SAS Institute, Inc., Cary, NC).

RESULTS

Donor characteristics are summarized in Table 1. The sex distribution was nearly equal, and the majority of donors were white (92.5%) and non-Hispanic (86.3%). Heights ranged from 134.6 to 195.6 cm, and weights ranged from 43.1 to 135.0 kg. Although the mean BMI was 26.3 kg/m^2, the range was substantial and included subjects in the overweight and even obese categories [interquartile range (IQR) = 23.3-28.8 kg/m^2, range = 16.4-42.4 kg/m^2]. Only 25 subjects (6.4%) were left lobe donors.

Table 2 summarizes the laboratory and organ volume data before donation and at M3 and Y1. Several laboratory tests of liver function [albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), and international normalized ratio (INR)] were significantly different from predonation values at M3 and/or Y1, although the changes were small and generally remained in the normal range. Similarly, alterations in the hematologic profiles were seen for hemoglobin (although Y1 levels returned to values very close to those before donation) and platelet counts, which declined at M3 and remained significantly lower than

TABLE 3. Linear Regression Models Predicting Spleen Volumes

Model and Variable	Parameter Estimate	*P* Value
Model A: predonation spleen volume (n = 344, $R^2 = 0.52$)		
Predonation liver volume (per 100 cc)	11.14	<0.001
Platelet count at evaluation (×50,000/mm^3)	−19.07	<0.001
BSA (cc)	115.22	<0.001
Hemoglobin at evaluation (g/dL)	11.64	<0.001
Donor age at evaluation (per 10 years)	−13.23	<0.001
Model B: postdonation M3 spleen volume (n = 167, $R^2 = 0.40$)		
Postdonation M3 liver volume (per 100 cc)	16.43	<0.001
Platelet count at evaluation (×50,000/mm^3)	−28.46	<0.001
Sex (reference: male)	77.03	<0.001
Model C: postdonation Y1 spleen volume (n = 75, $R^2 = 0.42$)		
Postdonation Y1 liver volume (per 100 cc)	17.64	<0.001
Platelet count at evaluation (× 50,000/mm^3)	−18.01	0.04
Hemoglobin (g/dL)	32.75	<0.001

predonation values at Y1 [$P < 0.001$]. A subset of patients (8.9%) had platelet counts below the lower limit of normal (150 × 10^3/mm^3) as observed in our previous reports. Graphical representations of the laboratory tests are depicted in Fig. 1. Some of these had skewed distributions.

The following results are based on all available data. Because of incomplete follow-up for many donors, we repeated all the following analyses with only right lobe donors with complete volume data (predonation, M3, and Y1 values; n = 48). Only 1 left lobe donor had complete volume data, and that donor was excluded from these analyses. All results were similar to the results presented when the smaller sample size was taken into account. These analyses are available in the supporting information [Supporting Table 1 (corresponding to Table 3) and Supporting Figs. 1-6].

Liver and Spleen Volumes Over Time

The mean predonation total liver volume was 1601 cc (Table 2), and this was on average 2.0% of the donor's body weight (range = 1.4%-5.2%). There were 363 right lobe donors and 25 left lobe donors (6.4%). The mean right lobe volume was 1067 cc, and the mean left lobe volume was 599 cc (67% and 33% of the total liver volume, respectively). The mean predonation spleen volumes were 246 ± 107 cc; these volumes substantially increased at M3 and Y1 (paired *t* tests; M3, n = 165; Y1, n = 75; $P < 0.001$ for both). The ratio of the liver volume to the spleen volume was 7.4 before donation, 4.6 at M3, and 5.5 at Y1; the latter 2 values were significantly lower than the predonation value (paired *t* tests; M3, n = 165; Y1, n = 75; $P < 0.001$ for both).

Figure 2A depicts liver volumes with box plots before donation and at M3 and Y1. Liver volumes were 79% of the predonation volume at M3 (n = 165) and approached 88% of the predonation volume at Y1 (n = 75). Predonation liver volumes were highly variable. Even when they were normalized by BSA, liver volumes varied 2-fold in these healthy subjects; this pattern was observed at all time points (before donation, 522-1887 cc; M3, 428-932 cc; and Y1, 542-980 cc). In Fig. 2B, the spaghetti plot demonstrates the course of liver volumes through resection and regeneration as a percentage of the donor predonation liver volume for individual subjects who had all necessary measurements (n = 46). The comparisons of liver volumes at predonation and postdonation time points are shown individually in Fig. 2C,2D. At both M3 (n = 165; Fig. 2C) and Y1 (n = 75; Fig. 2D), the majority of livers were smaller than they were before donation.

With respect to the spleen volumes (Fig. 3), the mean predonation spleen volume was 246 ± 107 cc, and the volume ranged from 67 to 775 cc (median = 226 cc, IQR = 161-310 cc; Fig. 3A). Even with normalization by BSA, the range was substantial (40-396 cc/m^2), although larger subjects generally had larger spleens (r^2 = 0.52, $P < 0.001$). The spaghetti plot (Fig. 3B) among donors who had volumes at all 3 time points (n = 49) demonstrates the course of the spleen volume over time with a range as high as 234% of the predonation volume at Y1. The majority of the subjects had spleen volumes greater than 100% of the predonation volume at M3 and Y1. Similarly, Fig. 3C,D (n = 165 and n = 75, respectively) shows the increase in spleen volumes over time.

Relationship Between Spleen Size and Platelet Counts

There was a highly significant negative association between the platelet count and the spleen volume at all time points (before donation, Fig. 4A; M3, Fig. 4B; and Y1, Fig. 4C) with a relatively consistent slope but decreasing intercepts over time. The predonation values are superimposed on Fig. 4B,C to compare postdonation and predonation data.

Development of a Model for the Prediction of the Standard Spleen Volume (SSV)

We sought to develop a model for the prediction of the normal spleen volume in healthy donors before donation (model A; Table 3). Up to 14 variables were tested in model selection. The best model included 5 significant variables; a larger spleen size was predicted by a larger predonation liver volume, lower platelet counts, a larger BSA, a higher hemoglobin level, and a

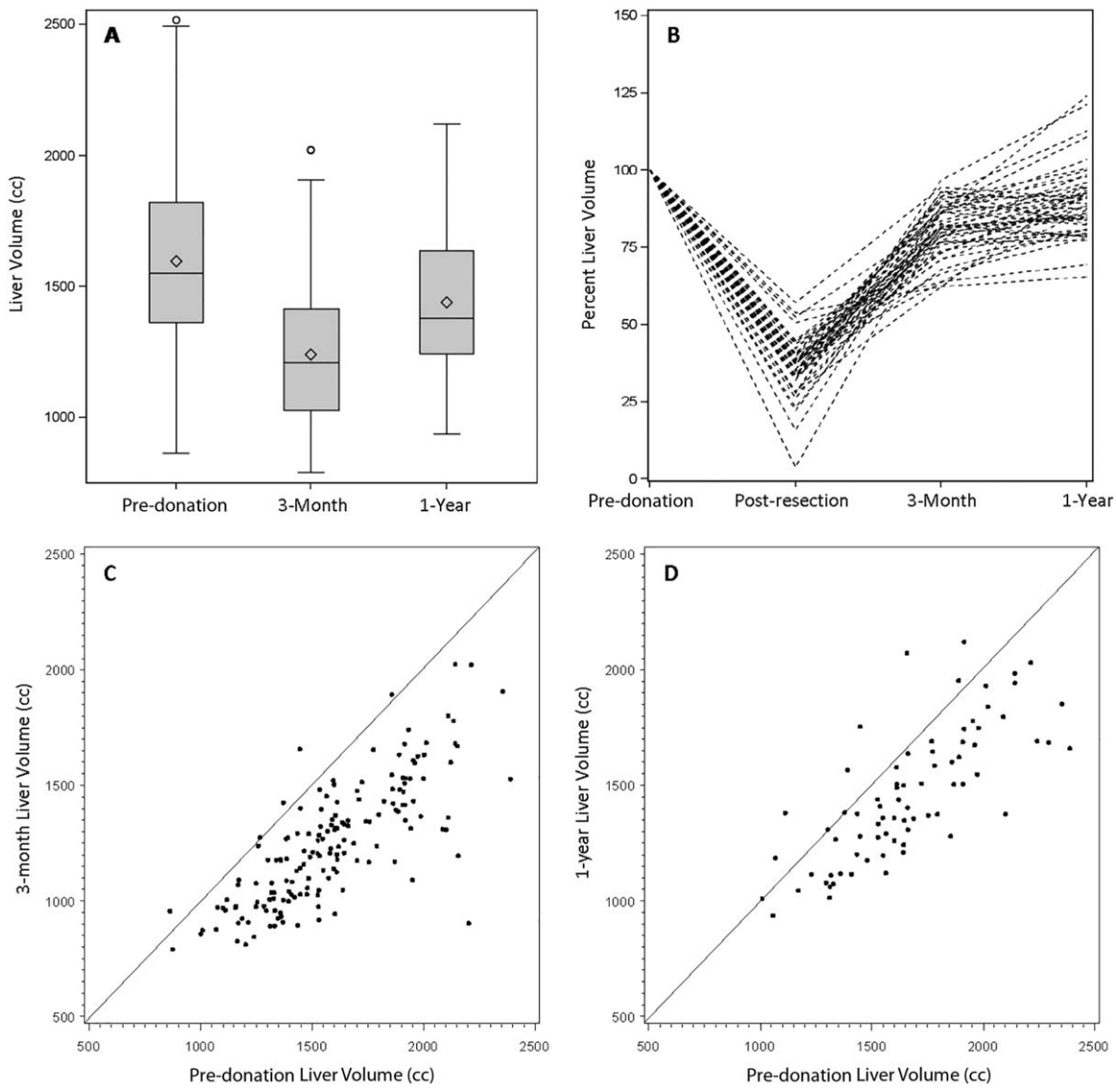

Figure 2. Liver volumes before donation, after resection (Fig. 2B only), and after donation at M3 and Y1. Volumes are shown as (A) a box plot by time point (before donation, n = 346; M3, n = 182; and Y1, n = 90), (B) a spaghetti plot showing volumes over time as percentages of predonation volumes for donors with complete data at all 4 time points (n = 46), and as scatter plots of (C) the M3 volume (n = 165) and (D) the Y1 volume (n = 75) plotted against predonation volumes with a diagonal identity line.

younger donor age. With an R^2 value of 0.52, the model performed well for most observed spleen volumes, but the fit suffered in a small group of observed large spleens for which the model consistently underpredicted the volumes. A logistic model was fit to predict the probability of predonation spleen volumes greater than 400 cc to further investigate the outliers in the linear regression model, but the significant predictors were BSA and platelet counts, which were already seen as predictors of larger spleen volumes in the SSV model.

Models for Spleen Volume Over Time

First, we assessed the applicability of the SSV model to postdonation spleen volumes. Using predictions from the predonation model, Fig. 5 presents predicted and observed spleen volumes. At M3, we found that the model consistently underestimated the spleen volumes across all observed volumes. At Y1, underprediction continued, but only in the larger observed spleen volumes. To investigate whether other variables might be important for predicting spleen volumes after liver hepatectomy that were not seen before donation, we developed models to predict postdonation spleen volumes at both M3 and Y1 [models B (n = 167) and C (n = 75), respectively; Table 3]. Although sample sizes in both postdonation models were smaller than those in the predonation model, lower platelets and larger liver volumes were still seen as significant predictors of larger spleen volumes. Lower platelets predicted even larger spleens at M3 than those seen in the models at other time points (based on parameter estimates in the 3 models);

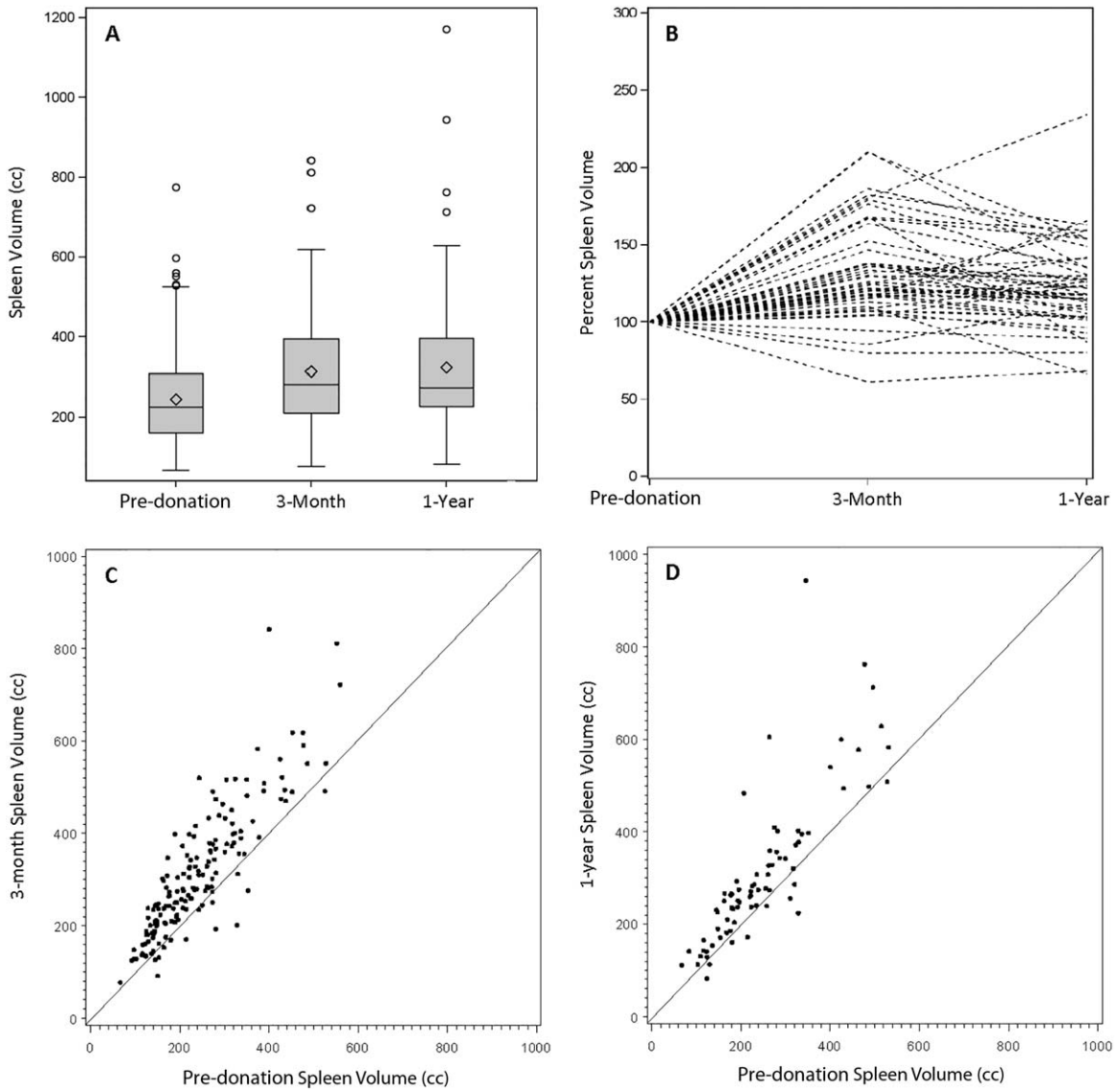

Figure 3. Spleen volumes before and after donation at M3 and Y1. Volumes are shown as (A) box plots by time point (before donation, n = 346; M3, n = 182; Y1, n = 90), (B) a spaghetti plot showing volumes over time as a percent of predonation volume for donors with complete data at all 3 time points (n = 49), and as scatter plots of (C) M3 volume (n = 165) and (D) Y1 volume (n = 75) plotted against predonation volumes, with a diagonal identity line.

similarly, larger liver volumes predicted slightly larger spleen volumes in both postdonation models versus the predonation model. The Y1 model also showed that higher hemoglobin levels predicted larger spleen volumes, with nearly 3 times the magnitude seen in the predonation model. At M3, males were predicted to have larger spleen volumes. Neither age at donation nor BSA were significant predictors in either postdonation model. Using predictions from the postdonation models, Fig. 5 also presents predicted and observed spleen volumes. As seen in the application of the predonation model to postdonation volumes, the postdonation models showed underprediction of the observed large spleen volumes. This suggests that persistent splenomegaly after donation occurs in a variety of donors and that changes in such spleen volumes cannot be completely explained through the application of linear models using patient demographics and laboratory values alone. Portal vein thrombosis, although of interest, could not be tested because only 1 case was reported for the donors in this analysis.

Effect of the Donated Liver Lobe on the Spleen Volume

Because right hepatectomy involves resection of more than half of the liver and results in a smaller remnant liver, we hypothesized that the impact of donation on spleen size might be different between right lobe and left lobe donors. We present the changes in the spleen volume from the predonation period to the

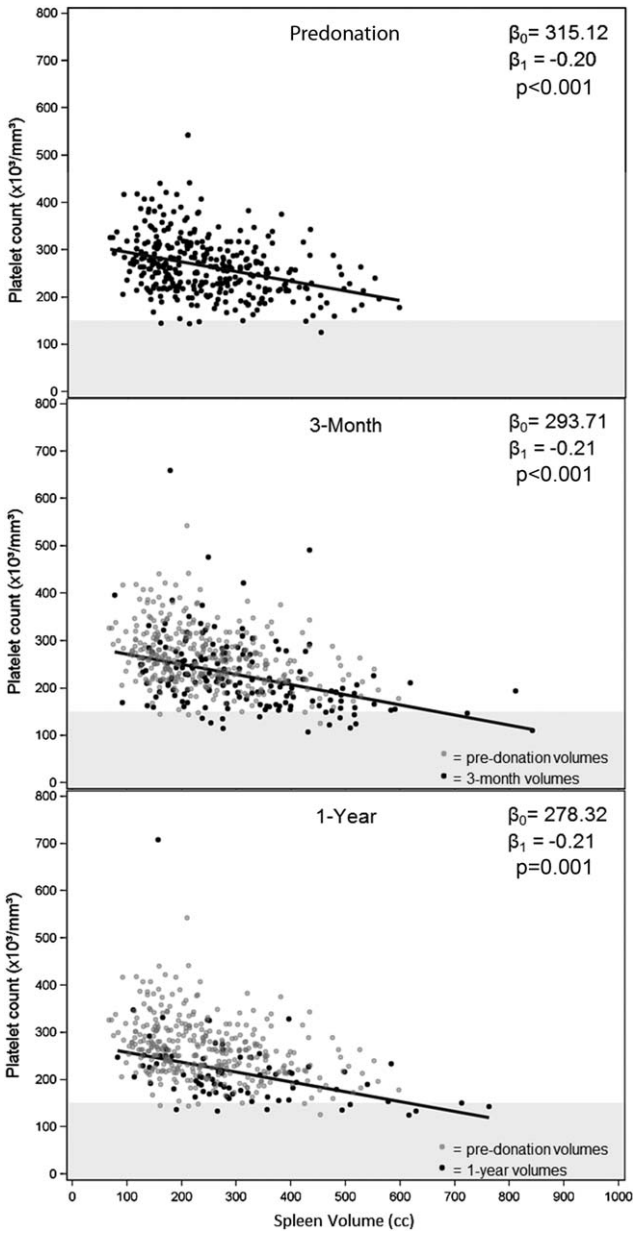

Figure 4. Scatter plots of platelet counts versus spleen volumes at 3 time points. On the M3 and Y1 plots, the predonation values are plotted as gray dots (n = 345) behind the postdonation values (black dots; M3, n = 167; Y1, n = 76). Regression lines are shown, and the intercepts (β_0), slopes (β_1), and *P* values are given. Values below the lower limit of normal for platelet counts ($150 \times 10^3/mm^3$) are shown in the gray horizontal bands.

postdonation period for right and left lobe donors in Fig. 6. At M3, the average change in spleen volume (δ = M3 value − predonation value) was significantly different between right and left lobe donors (right lobe mean δ = 72 cc, left lobe mean δ = 41 cc, $P = 0.01$, n = 165). At Y1 (δ = Y1 value − predonation value), the left lobe effect was even more pronounced (right lobe mean δ = 66 cc, left lobe mean δ = −22 cc, $P = 0.2$, n = 75), although power was limited with only 2 left lobe donors. Although the samples were small, these observations are consistent with the possibility that the extent of hepatic resection is a contributor to the risk of splenomegaly after donation.

Long-Term Laboratory Abnormalities

We examined laboratory abnormalities beyond Y1 and found that among the 11 patients with abnormal platelet counts at either M3 or Y1 who had at least 1 measurement between year 2 and year 4, 8 had abnormal platelet counts at least 1 time from year 2 to year 4, and this indicated the persistence of this condition in a subset of patients. The incidence of complications recorded for donors, however, was not different for the 12 donors with spleen volumes greater than 500 cc at Y1 ($P = 0.81$).

DISCUSSION

This study demonstrates that thrombocytopenia observed after living donor hepatectomy is highly correlated with persistent changes in spleen size. An increase in spleen size was observed in nearly all subjects after hepatectomy, with a subset in the abnormal range. Our efforts to develop a predictive model for spleen size were complicated by the presence of a subset of subjects skewed toward larger spleen sizes both before and after liver donation, and this indicates that the biology of this is not subject to simplistic interpretation. Our SSV modeling identified a number of highly significant predictive variables for SSV. A population of outliers emerged when this proposed SSV equation was applied to postdonation measures. Furthermore, separate models developed in the postdonation setting with a smaller sample of donors were not able to identify any predictors to sufficiently model these large spleens either. The clinical significance of these findings will require long-term study of donors with particular attention to these subjects.

Understanding the significance of persistent splenomegaly after donation is complicated by the paucity of studies of spleen size in the literature. Pozo et al.[13] characterized the spectrum of conditions associated with splenomegaly, which range from infections to malignancy. Perhaps more relevant to the population of potential donors, clinically apparent splenomegaly was identified in 2.5% of healthy college freshmen,[14] none of whom developed clinical disease in over a decade of follow-up. Among the pathological conditions associated with splenomegaly, infections and hematologic disorders figure prominently. We observed in the donor population that the spleen size was critically related to hematologic parameters even in healthy subjects. However, most of the published data on spleen size are from the hematology literature, and these studies generally have not taken advantage of 3-dimensional volumetrics based on a more advanced interpretation of integrated sequences from cross-sectional imaging.

Our efforts to develop a predictive model for spleen size included liver size as well as a variety of

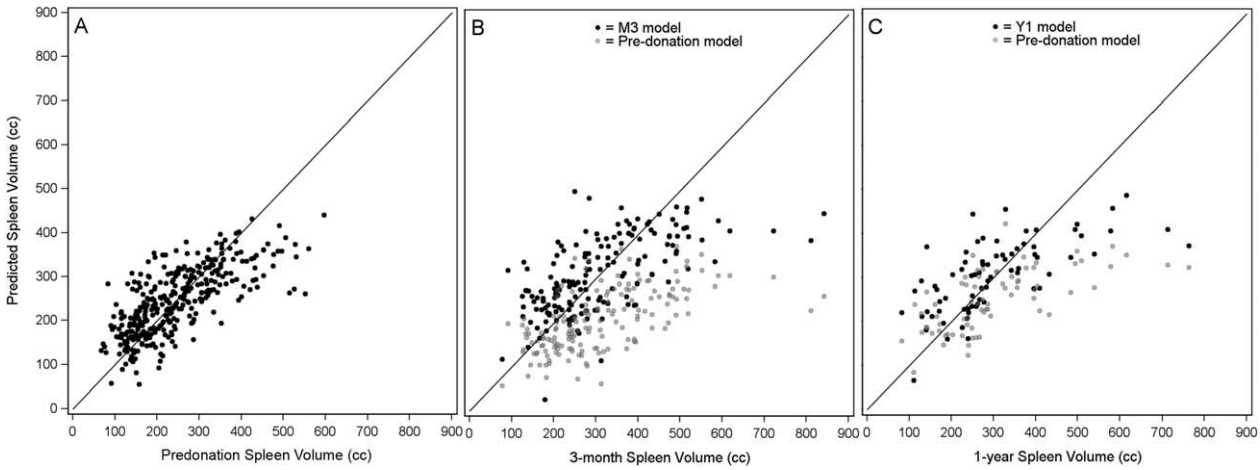

Figure 5. Predicted spleen volumes versus observed spleen volumes by time point. (A) Predonation predicted values calculated with the predonation spleen volume model (n = 345). (B,C) The black dots show postdonation predicted values with the M3 (n = 167) and Y1 spleen volume models (n = 75), respectively, and the light gray dots show predicted values calculated with the predonation spleen volume model.

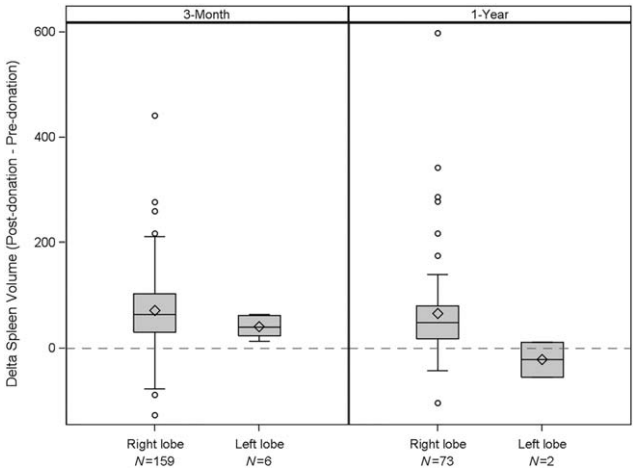

Figure 6. Spleen volume changes from before donation to after donation: a comparison of right and left lobe donors at M3 ($P = 0.01$) and Y1 ($P = 0.20$). Note that the small numbers of left lobe donors limit the statistical power. Each box spans from the first quartile to the third quartile and shows the median (line crossing the box) and the mean (+). Whiskers extend to the farthest data point within 1.5 IQRs from the box ends, with all outlying points shown individually as circles.

hematologic parameters. The importance of liver size in our model can be attributed to the intrinsic relationship of blood flow between the liver and the spleen. In a recent cohort of patients with congenital hepatic fibrosis, a highly significant correlation between spleen size and platelet counts was observed.[15] Interestingly, however, the authors found no correlation between hepatic function and platelets or spleen size, and this is similar to the findings of the current study, although perhaps albumin is too superficial a marker of hepatic function to fully explore this relationship. In addition to its role as an immune regulator and hematologic modulator, the spleen has been implicated as a contributor to capacitance in the cardiovascular system,[16] and the splanchnic circulation is a well-known regulator of intravascular volume. Clearly, the complexities of these functions are well beyond the capacity of our study to investigate. Nonetheless, alterations of both liver volumes and spleen volumes over time are worth investigating on a more mechanistic level.

The relationship between the regenerating liver and the spleen is the core issue in the current study because the subjects selected for donation are clinically healthy and are screened for a broad spectrum of health conditions. In addition to the simple parameters available in the ordinary donor workup, there is likely to be a relationship between spleen size and previous exposure to common viruses such as Epstein-Barr virus, although the variability of such screening in the donor population did not permit us to address this issue. Surprisingly little has been reported on the issue of spleen size, although the question has been addressed in ultrasonographic studies of athletes with mononucleosis.[17] Our data reaffirm that liver volume is not restored to the predonation size in the majority of subjects at Y1 despite normalization of laboratory tests of liver function, and this is consistent with Pomfret's early report and a recent publication from a Korean group with a large cohort of right lobe donations.[9,18,19]

Although portal hypertension may influence spleen size in extreme instances, the relationship between the not fully regenerated liver and the spleen may also affect platelet counts because of decreased levels of thrombopoietin.[20] This hormone has been implicated in platelet development and is decreased in the presence of liver disease.[20-22] In LDLT, Nagasako et al.[23] demonstrated that low platelet counts were correlated with decreased levels of thrombopoietin on day 7 after liver donation. Thrombopoietin in liver donors has not been studied in the long term, and in light of our findings, further mechanistic studies are warranted. The

overall examination of platelet counts in our patients indicates that although counts were significantly lower over time in all subjects, very few fell outside the normal range. This is similar to the issue of liver regeneration; although the livers at Y1 were, on average, 88% of predonation values, none of the subjects had clinically evident functional impairments. The Everson study cited earlier[10] included a corresponding quantitative liver function assessment that might yield better information if repeated with a larger number of patients.

Our data are dependent on the accuracy of cross-sectional imaging, which has been widely used in surgical planning in LDLT.[24,25] To overcome variability in volumetrics associated with center practices and techniques, we collected all available scans and re-analyzed them with a central computational laboratory.[9-14] In addition, liver and spleen volumes are not routinely assessed in clinical evaluations of patients with liver disease, although this is clearly a useful adjunct in characterizing the course of recovery after liver surgery. Our data demonstrate the wide variability of liver and spleen sizes in healthy subjects. Although this is peripheral to the main aims of this article, we caution against the rigid use of equations of standard liver volumes in surgical decision making in hepatectomy. Although it is sometimes necessary to have an objective norm, the clinician should be aware of variations among individuals.

The most important practical question raised by this study is the clinical significance of persistent abnormalities of liver and spleen volumes and platelet counts after donation. Although we have volumetric data for only a quarter of our subjects at Y1, we have essentially complete data for the subjects' laboratory values, and the correlation of platelet counts with volume data are consistent over time. Clinical follow-up of right lobe donors is now well over a decade at many centers, and few subjects with chronic disease have been identified. However, we feel strongly that the small subset of donors who are outliers with respect to organ volumes and laboratory results merit close long-term follow-up.

The strengths of the current study include the large number of subjects and the multicenter collection of experience, which best reflects the diversity of practice and experience. Thus, this report is a valuable complement to the single-center reports on this issue. Weaknesses include the lack of complete follow-up with an increasing number of missing scans with time after donation. Furthermore, an analysis of correlative mechanistic data will be essential to a better understanding of the physiology of splenomegaly after donation. Finally, the small number of left lobe donors with complete follow-up has provided some confirmation but prevents us from fully addressing the argument made by Makuuchi et al.[3] that left lobe donors might fare better than right lobe donors because of the larger residual liver volume and less portal hypertension.[26]

In conclusion, we have confirmed that the persistent thrombocytopenia observed in living liver donors is associated with splenomegaly that is persistent up to 1 year after donation. However, abnormalities outside the normal range are limited to a small number of subjects. The variability of liver and spleen volumes before donation is reiterated and reminds us that the biological complexity bedevils the creation of rigid formulas for surgical planning. Nonetheless, the extreme outliers clearly need close follow-up to ascertain whether these findings are harbingers of subclinical disease that may evolve. The physiologic role of the spleen after hepatectomy needs further clarification in two respects, first its role as a regulator of cardiovascular tone mediated by hormones with circulatory effects, and secondly, whether alterations in thrombopoeitin levels alter platelet counts in this setting. Finally, there is an unquestioned need to extend follow-up for subjects to ascertain the effects of donation on long-term health.

REFERENCES

1. Olthoff KM, Merion RM, Ghobrial RM, Abecassis MM, Fair JH, Fisher RA, et al.; for A2ALL Study Group. Outcomes of 385 adult-to-adult living donor liver transplant recipients: a report from the A2ALL consortium. Ann Surg 2005;242:314-325.
2. Shiffman ML, Brown RS Jr, Olthoff KM, Everson G, Miller C, Siegler M, Hoofnagle JH. Living donor liver transplantation: summary of a conference at the National Institutes of Health. Liver Transpl 2002;8:174-188.
3. Makuuchi M, Miller CM, Olthoff K, Schwartz M. Adult-adult living donor liver transplantation. J Gastrointest Surg 2004;8:303-312.
4. Moon DB, Lee SG, Hwang S, Kim KH, Ahn CS, Ha TY, et al. Toward more than 400 liver transplantations a year at a single center. Transplant Proc 2013;45:1937-1941.
5. Berg CL, Gillespie BW, Merion RM, Brown RS Jr, Abecassis MM, Trotter JF, et al.; for A2ALL Study Group. Improvement in survival associated with adult-to-adult living donor liver transplantation. Gastroenterology 2007; 133:1806-1813.
6. Berg CL, Merion RM, Shearon TH, Olthoff KM, Brown RS Jr, Baker TB, et al. Liver transplant recipient survival benefit with living donation in the Model for Endstage Liver Disease allocation era. Hepatology 2011;54:1313-1321.
7. Abecassis MM, Fisher RA, Olthoff KM, Freise CE, Rodrigo DR, Samstein B, et al.; for A2ALL Study Group. Complications of living donor hepatic lobectomy—a comprehensive report. Am J Transplant 2012;12:1208-1217.
8. Rudow DL, Brown RS Jr, Emond JC, Marratta D, Bellemare S, Kinkhabwala M. One-year morbidity after donor right hepatectomy. Liver Transpl 2004;10:1428-1431.
9. Trotter JF, Gillespie BW, Terrault NA, Abecassis MM, Merion RM, Brown RS Jr, et al.; for A2ALL Study Group. Laboratory test results after living liver donation in the Adult-to-Adult Living Donor Liver Transplantation Cohort Study. Liver Transpl 2011;17:409-417.
10. Everson GT, Hoefs JC, Niemann CU, Olthoff KM, Dupuis R, Lauriski S, et al. Functional elements associated with hepatic regeneration in living donors after right hepatic lobectomy. Liver Transpl 2013;19:292-304.

11. Guo X, Zhao B, Schwartz L. Computer-Aided Tumor Segmentation Using Local Region-Based Active Contours. Columbia University Invention Report IR #290. Available at: http://innovation.columbia.edu/technologies/2906_cumc-radiology-imaging-lab-available-technologies. Accessed 12/29/2014
12. Guo X, Zhao B, Schwartz L. Techniques for segmentation of organs and tumors and objects. 2013. Google Patents. Available at: http://www.google.com/patents/WO2013155300A1?cl=en Accessed 12/29/2014.
13. Pozo AL, Godfrey EM, Bowles KM. Splenomegaly: investigation, diagnosis and management. Blood Rev 2009;23:105-111.
14. Ebaugh FG Jr, McIntyre OR. Palpable spleens: ten-year follow-up. Ann Intern Med 1979;90:130-131.
15. Gunay-Aygun M, Font-Montgomery E, Lukose L, Tuchman Gerstein M, Piwnica-Worms K, Choyke P, et al. Characteristics of congenital hepatic fibrosis in a large cohort of patients with autosomal recessive polycystic kidney disease. Gastroenterology 2013;144:112-121.
16. Hamza SM, Kaufman S. Role of spleen in integrated control of splanchnic vascular tone: physiology and pathophysiology. Can J Physiol Pharmacol 2009;87:1-7.
17. Hosey RG, Kriss V, Uhl TL, DiFiori J, Hecht S, Wen DY. Ultrasonographic evaluation of splenic enlargement in athletes with acute infectious mononucleosis. Br J Sports Med 2008;42:974-977.
18. Kim SJ, Na GH, Choi HJ, You Y, Kim DG. Effect of donor right hepatectomy on splenic volume and platelet count for living donor liver transplantation. J Gastrointest Surg 2013;17:1576-1583.
19. Pomfret EA, Pomposelli JJ, Gordon FD, Erbay N, Lyn Price L, Lewis WD, Jenkins RL. Liver regeneration and surgical outcome in donors of right-lobe liver grafts. Transplantation 2003;76:5-10.
20. Ikura Y, Ohsawa M, Okada M, Iwai Y, Wakasa K. The significance of platelet consumption in the development of thrombocytopenia in patients with cirrhosis. Am J Med Sci 2013;346:199-203.
21. Peck-Radosavljevic M, Zacherl J, Meng YG, Pidlich J, Lipinski E, Längle F, et al. Is inadequate thrombopoietin production a major cause of thrombocytopenia in cirrhosis of the liver? J Hepatol 1997;27:127-131.
22. Jelkmann W. The role of the liver in the production of thrombopoietin compared with erythropoietin. Eur J Gastroenterol Hepatol 2001;13:791-801.
23. Nagasako Y, Jin MB, Miyazaki H, Nakayama M, Shimamura T, Furukawa H, et al. Thrombopoietin in postoperative thrombocytopenia following living donor hepatectomy. Liver Transpl 2006;12:435-439.
24. Krupski G, Rogiers X, Nicolas V, Maas R, Malagó M, Broelsch CE, Bücheler E. Computed tomography versus magnetic resonance imaging–aided volumetry of the left lateral segment before living related liver donation: a case report. Liver Transpl Surg 1996;2:388-390.
25. Higashiyama H, Yamaguchi T, Mori K, Nakano Y, Yokoyama T, Takeuchi T, et al. Graft size assessment by preoperative computed tomography in living related partial liver transplantation. Br J Surg 1993;80:489-492.
26. Ishizawa T, Sugawara Y, Hasegawa K, Ikeda M, Tamura S, Makuuchi M. Extent of hepatectomy on splenic hypertrophy and platelet count in live liver donors. Clin Transplant 2006;20:234-238.