Early Clinical and Economic Outcomes of Patients Undergoing Living Donor Nephrectomy in the United States

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Background: Efforts to maximize kidney transplantation are tempered by concern for the live donor’s safety. Case series and center surveys exist, but national aggregate data are lacking. We sought to determine predictors of early clinical and economic outcomes following living donor nephrectomy.

Design: A retrospective cross-sectional analysis using 1999-2005 discharge data from the Healthcare Cost and Utilization Project Nationwide Inpatient Sample was performed. Cases were identified by International Classification of Diseases, Ninth Revision (ICD-9) codes. Clinical and economic outcomes were analyzed with regard to patient and provider characteristics using bivariate and multivariate regression analyses.

Setting: Healthcare Cost and Utilization Project Nationwide Inpatient Sample.

Patients: Patients undergoing living donor nephrectomy, identified by the ICD-9 codes.

Interventions: Clinical and economic outcomes were analyzed with regard to patient and provider characteristics using bivariate and multivariate regression analyses.

Main Outcome Measures: In-hospital complications, mortality, mean length of stay (LOS), and mean total hospital costs.

Results: A total of 6320 cases were identified with 0% mortality and a complication rate of 18.4%. The mean (SD) LOS was 3.3 (0.3) days, and the mean inpatient cost was $10 708 ($505). Independent predictors of donor complications included older age (odds ratio [OR], 1.01), male sex (OR, 1.19), Charlson Comorbidity Index of at least 1 (OR, 1.49), obesity (OR, 1.76), medium-size hospitals (OR, 1.88), and low-volume hospitals (OR, 1.37). Predictors of longer LOS included older age, female sex, Charlson score of at least 1, lower household income, low-volume and urban hospitals, and low-volume surgeons.

Conclusions: Kidney donation is associated with a low mortality rate but an 18% complication rate. Donation by those with advanced age or obesity is associated with higher risks. Informed consent should include discussion of these risks.

Kidney transplantation prolongs survival and improves the quality of life for patients with end-stage renal disease, but it is limited by the severe shortage of organs. Kidneys may be procured and transplanted from either living or deceased donors; consistently superior early outcomes, including graft function, lower rates of rejection, and reduced costs, are obtained with kidneys from living donors. Recipients of live donor kidneys also experience better long-term rates of patient and graft survival. There has been an overall increase in the number of live donor nephrectomies performed in the United States over the past decade. Intense pressure to meet the demand for kidneys also has led some transplant centers to perform elective nephrectomies in donors who are elderly, have hypertension or glucose intolerance, or are obese.

See Invited Critique at end of article

Kidney donation is not without risks to the live donor. Data from single-center institutional reports and surveys suggest that operative mortality is rare, but complications range from urinary tract infections to bowel injury and renal failure. Use of laparoscopy is associated with lower morbidity, decreased postoperative pain, and shorter hospital length of stay (LOS) compared with open techniques. These data may need to be interpreted with caution, however, because a recent study of specific techniques of renal artery stump ligation raised concerns about...
potential severe postoperative bleeding in these patients. Studies
to date have been generally small and possibly underpow-
ered, with the largest study encompassing 3074 kidney do-
nors from 28 institutions.

We report the first study, to our knowledge, to mea-
sure early clinical and economic outcomes of patients un-
dergoing living donor nephrectomy on a population level,
and the association with sociodemographic and clinical pa-
tient and provider (hospital and surgeon) characteristics.

METHODS

This study is a retrospective cross-sectional analysis of 1999-
2005 hospital discharge information obtained from the Health-
care Cost and Utilization Project Nationwide Inpatient Sample
(HCUP-NIS) database, a stratified 20% sample of inpatient ad-
missions to acute care hospitals. The HCUP-NIS is the largest
all-payer inpatient database in the United States and is main-
tained by the Agency for Health Care Research and Quality
(AHRQ). International Classification of Diseases, Ninth Revi-
sion (ICD-9) diagnostic and procedure codes were used to ab-
stract the cases of patients undergoing living kidney donation
from the database. Living kidney donor nephrectomies were
identified as elective cases with both a primary diagnosis of kid-
ney donor (ICD-9 code: V59.4) and primary procedure of
nephroureterectomy (ICD-9 code: 55.51).

Independent demographic variables included patient age,
sex, race (white, black, Hispanic, and other), which included
but was not limited to Asians, Pacific Islanders, and Native
Americans), patient location (large urban, small urban, subur-
ban, and rural), payer type (private insurance, Medicare, self-
pay, and other), and household income (<$36,000, $36,000-
$44,999, $45,000-$58,999, and ≥$59,000). These variables
were predefined in the HCUP-NIS. Patient comorbidity was
assessed using the Charlson Comorbidity Index and individual
ICD-9 codes for obesity (ICD-9 codes: 27.800, 27.801) and hy-
pertension (ICD-9 code: 40.19). The Charlson Comorbidity In-
dex is based on 17 defined comorbidities. It is a well-validated
measure of illness complexity and has been used extensively
with discharge and claims data to predict short-term out-
comes such as in-hospital mortality, blood transfusion, hospi-
talization charges, and LOS. Patients were identified as either
having comorbidities (Charlson score ≥1) or no comorbid-
ities (Charlson score = 0).

Independent hospital-provider characteristics included hos-
pital size (small, medium, and large), location (urban vs rural),
geographic region (North, East, Midwest, and South), and teach-
ing status (teaching vs nonteaching). Hospital size classification
varied based on geographic region, location, and teaching sta-
tus. For example, large hospitals ranged in size from more than
45 beds in rural hospitals in the West to more than 450 beds in
urban, teaching hospitals in the South. Hospital volume was based
on the mean number of living donor nephrectomies performed
per year. High-volume hospitals were defined as the top 50th per-
centile of hospitals (≥20 cases/y). Surgeons were identified as
the admitting physicians for patients undergoing elective living do-
nor nephrectomy as the principal procedure. They were grouped
according to the total number of living donor nephrectomies they
performed per year during our 7-year study period. High-
volume surgeons were defined as those in the top 25th percent-
tile of surgeons (≥5 cases/y).

OUTCOME VARIABLES

Primary outcome variables were in-hospital patient compli-
cations, mean hospital LOS, total inpatient hospital costs,
adjusted to 2005 dollars, and in-hospital mortality. In-hospital patient complications were identified from the
ICD-9 diagnostic codes present on the discharge records of
the selected cases. Complications were categorized into
bleeding, infection, genitourinary (GU tract), gastrointestinal
(GI tract), respiratory tract, and other. Complications
were treated as a dichotomous variable (none vs ≥1); infor-
mation regarding severity was not available. Total inpatient
costs were calculated using the HCUP-NIS–adjusted,
hospital-specific, cost-to-charge ratios. Costs were adjusted
for inflation, converting all costs to 2005 dollars using rates
from the Bureau of Labor Statistics.

STATISTICAL ANALYSIS

Bivariate analysis of independent variables by outcomes was per-
formed by χ² analysis for categorical variables and analysis of vari-
ance for continuous variables. Multivariate linear regression mod-
els were used to adjust for significant independent variables for
LOS and total inpatient costs. The LOS and total inpatient costs
were log transformed to correct for data skew and kurtosis. Mul-
tivariate logistic regression models were used to adjust for in-
dependent variables for inpatient mortality and complications.
Statistical analyses were performed using SPSS software (ver-
sion 13.0; SPSS Inc, Chicago, Illinois). The HCUP-NIS is pub-
licly available and contains no personal identifying informa-
tion; consequently, this study was deemed to be exempt from
institutional review board approval at our institutions.

RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

There were 6331 patients whose discharge coding indi-
cated that they underwent elective unilateral donor ne-
phrectomy in the HCUP-NIS from 1999 to 2005. Eleven of
these cases were excluded because of the uncertainty of
living kidney donation based on patient age and/or sec-
dondary diagnoses on the discharge record. Of these 11,
7 procedures were not performed at a United Network
for Organ Sharing (UNOS) transplant center and were
deemed unlikely to involve live donors. Two cases in-
cluded total hepatectomies, suggesting erroneous cod-
ing for live donor nephrectomy, in lieu of a deceased do-
nor kidney recovery. One patient had a renal malignant
neoplasm, and this was the case interpreted to have been
misdoded for type of nephrectomy. One patient was un-
dergoing hemodialysis with a preoperative comorbid-
ity of chronic renal failure, but there was no coding for other
complications expected to be associated with such a se-
vere outcome from a live donor nephrectomy. The total
coding error rate for the diagnosis of live donor nephrec-
tomy was 11 of 6331, or 0.2%.

The mean donor age was 40 years; 59% of donors were
female (Table 1). Most of the donors (68%) were white;
blacks represented 12%; Hispanics, 11%; and other mi-
norities, 6%. Most donors were from a large city (60%);
44% had private insurance. Donors with Medicare com-
prised 13% of the population, 13% were self-pay, and 26%
had another form of coverage. There was an increased
number of donors in the higher income quartiles: 29%
earned at least $59,000 compared with 21% who earned
less than $35 999. Overall, donors were healthy; just 5% had a Charlson score of at least 1. Obesity and hypertension were each identified in 2% of donors.

Provider characteristics demonstrated that most living donor nephrectomies were performed at large (86%) urban (99.5%) teaching (95%) hospitals. High-volume hospitals performed 82% of the procedures, and 39% of the procedures were performed by high-volume surgeons. Only 20% of cases were coded as laparoscopic, although the number of laparoscopic cases increased over time (4% in 1999, 29% in 2004).

### UNADJUSTED PATIENT OUTCOMES

No patient-in-hospital deaths were identified. The mean (SD) LOS was 3.3 (0.3) days, and the mean inpatient cost was $10 708 ($505). Over time, the mean LOS revealed a significant and steady decline from 3.9 (0.3) days in 1999 to 2.7 (0.4) days in 2005 (P < .001). The mean inpatient cost significantly increased over time (from $9290 [$331] in 2001 to $10 832 [$644] in 2005; P < .001). The overall complication rate was 18.4%. Gastrointestinal tract complications occurred in 6.1% of cases, respiratory complications in 4.5%, bleeding in 4.3%, infection in 4.2%, and GU tract complications in 1.7%. Conversion from laparoscopic to open technique was reported in 0.8% of cases.

Significant differences in unadjusted clinical and economic outcomes were identified among living kidney donors (Table 2). Patients experiencing a complication were significantly older than patients not experiencing one (41.3 years vs 39.7 years; P < .001). The same trend was observed for patients with bleeding and GI tract complications. Older age also was associated with an increased mean LOS (P = .002).

Male donors had higher total complication rates (20.0%) than their female counterparts (17.8%; P = .03). Men had higher GU tract and respiratory tract complication rates: 2.5% vs 1.3% for women (P < .001) and 5.8% vs 3.7% for women (P < .001), respectively. Costs were higher for male donors ($10 740 vs $10 391 for women; P < .05). However, the mean LOS was shorter for men than for women (3.2 vs 3.4 days, respectively; P < .001).

Patient comorbidity was associated with worse clinical and economic outcomes. Compared with patients without comorbidity (Charlson score = 0), donors with Charlson scores of at least 1 had higher GU tract (3.3% vs 1.7%; P = .03), respiratory tract (6.9% vs 4.4%; P = .04), infectious (6.6% vs 4.0%; P = .03), and total (26% vs 18%; P < .001) complication rates. A Charlson score of at least 1 was associated with longer LOS (3.6 days vs 3.3 days for Charlson score = 0; P < .001). Donor obesity and hypertension were associated with higher complication rates: 28.3% of obese patients had complications compared with 18.2% of nonobese patients (P = .004). Bleeding complications were seen more frequently in obese patients (7.9% vs 4.2%; P < .05) and hypertensive patients (9.9% vs 4.2% for normotensive patients; P = .02). Hypertensive patients also had more GI tract (11.6% vs 6.0%; P = .01) and total (28.9% vs 18.2%; P < .003) complications. They had longer LOS (3.6 days vs 3.3 days; P < .05) and higher inpatient costs ($11 927 vs $10 683; P < .05).

High-volume hospitals were associated with significantly lower complication rates, shorter LOS, and lower costs. High-volume surgeons had patients with shorter LOS (3.0 days vs 3.5 days; P < .001) and lower costs ($10 096 vs $11 093; P < .001) than their low-volume counterparts. No significant difference in complication rates was observed between low- and high-volume surgeons (P < .08).

### MULTIVARIATE ANALYSIS

Independent predictors of sustaining a postoperative complication were older age, male sex, Charlson score of at
least 1, obesity, hypertension, and surgery at medium-size and low-volume hospitals (Table 3). Medium-size hospitals, obesity, Charlson comorbidity, and low hospital volume were the strongest predictors of higher complication rates, with odds ratios of 1.88, 1.76, 1.49, and 1.37, respectively.

When examining bleeding complications specifically, older age, female sex, hypertension, and low hospital volume were independent predictors of increased risk with odds ratios of 1.02, 1.31, 1.88, and 1.55, respectively (Figure 1). Factors associated with increased risk of genitourinary complications were male sex and Charlson score of at least 1 with odds ratios of 2.05 and 2.12, respectively (Figure 2).

Independent predictors of longer LOS included older age, female sex, lower household income, Charlson score of at least 1, surgery at low-volume and urban hospitals, and surgery by low-volume surgeons. Predictors of higher costs were older age, male sex, nonwhite race, higher household income, urban center, and surgery at low-volume and nonteaching hospitals (Table 4).

This study of patients undergoing living donor nephrectomy from the HCUP-NIS, 1999-2005, represents 15% (as expected) of the 42,185 living kidney donations reported to UNOS during the same time frame. The HCUP-NIS represents an unbiased random sampling of hospitals in the United States. There was a 0% in-hospital mortality rate, which is consistent with findings from other studies. The survey by Matas et al of US transplant centers based on self-report has been considered the most accurate estimate of donor mortality; it elucidated a rate of 0.02%. Recently, Patel et al reported a 0% mortality rate for donor nephrectomies performed at nonprofit academic centers.

Donor nephrectomy was associated with an overall inhospital complication rate of 18.4%. Independent predictors of outcome included donor-level (older age, male sex, Charlson score ≥1, and obesity) and provider characteristics (medium-size and low-volume hospitals). Previously reported complication rates vary significantly; a review of 44 studies reported complication rates of 3% to 38% for open living donor nephrectomies, and 5% to 26% for laparoscopic living donor nephrectomies.

Increasing demand for organs has translated into less restrictive and controversial exclusion criteria for living kidney donors. Transplant teams are exploring kidney donation from individuals with advanced age, obesity, or medically controlled hypertension. The decision to proceed with a donor nephrectomy must incorporate both the short-term consequences of the surgical procedure and the long-term implications of reduced renal mass and function. There is no consensus with regard to what constitutes a high-risk donor. In a 1997 single-center, retrospective study of patients undergoing open living donor nephrectomy, male sex, pleural entry, and weight greater than 100 kg were associated with perioperative complications. Age, smoking, operative blood loss, operative duration, and body mass index (BMI), calculated as weight in kilograms divided by height in meters squared, were not found to be risk factors. The study by Patel et al of open and laparoscopic nephrectomies determined that smoking, obesity, age older than 50 years, and a center volume of less than 50 cases per year were associated with increased morbidity among 3074 living donors, with a complication rate of 10.6%. Another retrospective study of hand-assisted laparoscopic living kidney donors compared patients with a BMI of less than 25 with those with a BMI greater than 35. Obese patients had longer operative times and more complications, but similar rates of conversion to an open operation, reoperation, and LOS compared with nonobese patients. Our data corroborate the increased risks for obese and relatively old donors. One recent meta-analysis found no difference in perioperative outcomes between donors with and without isolated medical abnormalities, but it was limited by small sample size in the analyzed studies.

The current study demonstrates that relatively low hospital volume of living donor nephrectomy is associated with
Table 3. Adjusted Odds Ratios (ORs) for All Complications Following Living Donor Nephrectomy, 1999-2005

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.01 (1.01-1.02)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sex</td>
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<td></td>
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<tr>
<td>Male</td>
<td>1 [Reference]</td>
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</tr>
<tr>
<td>Female</td>
<td>0.84 (0.74-0.96)</td>
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<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1 [Reference]</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>1.27 (1.01-1.60)</td>
<td>.04</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.97 (0.75-1.25)</td>
<td>.80</td>
</tr>
<tr>
<td>Other</td>
<td>0.71 (0.49-1.03)</td>
<td>.07</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No comorbidities</td>
<td>1 [Reference]</td>
<td></td>
</tr>
<tr>
<td>Charlson index ≥1</td>
<td>1.49 (1.14-1.96)</td>
<td>.004</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.76 (1.18-2.62)</td>
<td>.006</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.49 (0.99-2.24)</td>
<td>.06</td>
</tr>
<tr>
<td>Hospital bed size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>1 [Reference]</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>1.88 (1.23-2.87)</td>
<td>.004</td>
</tr>
<tr>
<td>Large</td>
<td>1.47 (0.99-2.18)</td>
<td>.006</td>
</tr>
<tr>
<td>Hospital volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1 [Reference]</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>0.73 (0.62-0.83)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Provider (hospital and surgeon) characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-volume hospitals</td>
<td>−0.13</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Teaching hospitals</td>
<td>−0.06</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>High-volume surgeons</td>
<td>−0.04</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NS, not significant.

a High-volume surgeons were defined as those in the top 25th percentile of surgeons (≥5 cases/y).

Figure 1. Odds ratios (ORs) of bleeding complications in patients who underwent living donor nephrectomy, 1999-2005. Error bars represent 95% confidence intervals. *P < .05.

higher donor complication rates, longer LOS, and increased costs. Patel et al.²³ reported similar outcomes associated with low-volume hospitals. The concept that hospital volume has an effect on clinical and economic outcome is not unique to living donor nephrectomy. Pancreatectomy, coronary artery bypass grafting, percutaneous coronary intervention, abdominal aortic aneurysm repair, and esophagectomy have better outcomes and lower costs when performed at high-volume centers.²⁴,²⁵

The potential importance of surgeons’ technical expertise for the safety and economic outcomes of donor nephrectomies recently has been embraced by the UNOS. New regulations have been issued requiring minimal case volumes for certification of each donor surgeon participating in nephrectomies performed at living donor kidney transplant programs.²¹ Our data do not support surgeon volume as an independent predictor of clinical outcomes, although patients of low-volume surgeons had longer LOS. However, this analysis relied on admitting physician coding, whereas living donor kidney transplants are usually conducted under the auspices of a transplant team. There may be discrepancies between the admitting physician on record and the actual surgeon performing the donor nephrectomy.

Several studies have reported decreased operative time, complications, and LOS with increased experience of the surgeon for the initial learning curve of a new technique, such as laparoscopic or hand-assisted nephrectomy. A retrospective study²² of 500 laparoscopic donor
nephrectomies performed by 2 surgeons showed that operative time and complication rates decreased after a surgeon had performed more than 150 procedures and were not associated with patient age, sex, BMI, or side of kidney procurement. Another retrospective study of 381 laparoscopic living donor nephrectomies reported fewer complications, graft loss, and vascular thrombosis with increasing surgeon experience. Surgeon volume has been shown to serve as a proxy for experience in thyroidectomy, coronary artery bypass grafting, aortic aneurysm repair, esophagectomy, and pancreatectomy. There is a paucity of evidence documenting a surgeon-volume outcome association for donor nephrectomy outside of the learning curve observed among surgeons training to use the laparoscopic approach. It is reasonable that increased cumulative experience with open and/or laparoscopic nephrectomy would predict improved patient outcomes.

Low-volume donor nephrectomy centers are associated with poor short-term outcomes. Today, there is frequent collaboration of transplant surgeons and laparoscopists from the fields of general surgery or urology in the perioperative care of donor nephrectomy patients. This team approach may be beneficial for patients and hospitals. Larger centers may have more opportunities to foster such cross-specialty team efforts, which may account for some differences observed in this study between low- and high-volume centers.

Administrative databases have inherent limitations, such as errors in coding and sampling. The HCUP-NIS is a stratified 20% sample of US hospitals; however, it excludes Veteran Administration hospitals. This may account for the apparent undersampling of living kidney donors in our study. We excluded 0.17% of cases because they were consistent with coding errors. The procedure/diagnostic code error rate for the HCUP-NIS is 0.45% to 0.62%. Laparoscopic technique was reported in 20.4% of cases. This is likely an underestimate of the true utilization of minimally invasive approaches, because even today there is no specific ICD-9 code for laparoscopic nephrectomy. The hand-assisted laparoscopic approach is thought to be safer and easier to master than exclusively laparoscopic techniques, and its use has increased. This technique could not be identified in the HCUP-NIS owing to the lack of specific codes. Readmissions are not captured; therefore, complication rates are likely underestimated. Identification of the true payer is complicated by assignment of donor costs to the recipient's insurance; this may be inaccurately coded at hospital discharge and, consequently, explain the large proportion of patients classified as “self-pay.” Our demographic results, however, correlate with recent data from the Organ Procurement and Transplantation Network. The diagnosis of obesity and hypertension among donors in the sample were based on comorbidity codes, not BMI or direct blood pressure measurements; therefore, their rates are likely underestimated.

Patients considering living kidney donation should be presented with an accurate assessment of their risks in order to obtain informed consent. Our data support the successful performance of living donor nephrectomies across numerous transplant centers in the United States, with a reassuringly low mortality rate. Patients considering living donor nephrectomy should be advised that LOS, costs, and perioperative complication rates are generally lower at hospitals with the highest active volumes of living donor nephrectomies.

Living kidney donation is critical to expanding the donor pool given the increasing numbers of Americans with end-stage renal disease. These procedures would not be ethically justifiable if the donor’s risk was known to be excessive. The absence of mortality in this large donor population should be considered supportive evidence for continuation of this practice in low-risk donors. Kidney donations from individuals with increased risk of complications, such as older age, obesity, and hypertension, should be the subject of further study. Long-term outcomes, including renal function and quality of life, should be included in order to present patients with a balanced, informed medical opinion.

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Is It Safe to Donate a Kidney?

Approximately 6000 live donor nephrectomies are performed in the United States annually. Donor safety is of paramount importance. Therefore, there is a need to collect, analyze, and track donor morbidity and mortality to ensure patient safety. Reliable data confirming living kidney donor safety are important to be able to provide educational information for prospective donors and their families.

To my knowledge, Friedman et al are the first to report the use of a population-based approach to obtain this information by analyzing data derived from International Classification of Diseases, Ninth Revision codes in the HCUP-NIS. This is a laudable effort. Of 6320 living donations, there were no donor deaths, an 18.4% complication rate, and a mean (SD) duration of hospitalization of 3.3 (0.3) days. However, there are a number of important limitations to this approach. First, complications are not segregated by severity. Second, there is no consistency in how centers define their complications. Third, the data cannot be wholly verified for accuracy because they are derived from billing information and not confirmed by clinicians. As the name of the database implies, only in-patient data have been examined. The more difficult topic of the long-term consequences of living kidney donation is not addressed.

So, how do we reliably obtain this information regarding the overall safety of living kidney donation? Establishment of donor registries has been proposed. Yet, significant problems exist associated with attempting to follow presumably healthy donors long term in large enough numbers to see statistically significant differences in outcomes. Also, there is a lack of funding for such an enterprise. However, we can rest assured that Friedman et al have reconfirmed the appropriately extremely low mortality of living kidney donation despite a general trend to expand inclusion criteria and the dramatic changes in operative technique that have occurred during the study period.

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