ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2016 June 24; 6(2): 255-450

Published by Baishideng Publishing Group Inc

WJT World Journal of Transplantation

A peer-reviewed, online, open-access journal of transplantation

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2016-2019

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NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Quarterly

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PUBLICATION DATE
June 24, 2016

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DOI: 10.5500/wjt.v6.i2.255

World J Transplant 2016 June 24; 6(2): 255-271
ISSN 2220-3230 (online)

Exocrine drainage in vascularized pancreas transplantation in the new millennium

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Author contributions: El-Hennawy H and Stratta RJ studier conception, designs, acquisition of data, analysis and interpretation of data; all the authors do drafting of manuscript and critical revision.

Conflict-of-interest statement: The authors acknowledge that the above manuscript represents original work that has not been previously published or submitted for publication. There are no conflicts of interest, grant support, sponsorship, or other financial arrangements to report by any of the authors.

Manuscript source: Invited manuscript

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Received: February 16, 2016
Peer-review started: February 16, 2016
First decision: March 1, 2016
Revised: May 6, 2016
Accepted: May 31, 2016
Article in press: June 2, 2016
Published online: June 24, 2016

Abstract

The history of vascularized pancreas transplantation largely parallels developments in immunosuppression and technical refinements in transplant surgery. From the late-1980s to 1995, most pancreas transplants were whole organ pancreatic grafts with insulin delivery to the iliac vein and diversion of the pancreatic ductal secretions to the urinary bladder (systemic-bladder technique). The advent of bladder drainage revolutionized the safety and improved the success of pancreas transplantation. However, starting in 1995, a seismic change occurred from bladder to bowel exocrine drainage coincident with improvements in immunosuppression, preservation techniques, diagnostic monitoring, general medical care, and the success and frequency of enteric conversion. In the new millennium, pancreas transplants are performed predominantly as pancreatico-duodenal grafts with enteric diversion of the pancreatic ductal secretions coupled with iliac vein provision of insulin (systemic-enteric technique) although the systemic-bladder technique endures as a preferred alternative in selected cases. In the early 1990s, a novel technique of venous drainage into the superior mesenteric vein combined with bowel exocrine diversion (portal-enteric technique) was designed and subsequently refined over the next $\geqslant$ 20 years to re-create the natural physiology of the pancreas with first-pass hepatic processing of insulin. Enteric drainage usually refers to jejunal or ileal diversion of the exocrine secretions either with a primary enteric anastomosis or with an additional Roux limb. The portal-enteric technique has spawned a number of newer and revisited techniques of enteric exocrine drainage including duodenal or gastric diversion. Reports in the literature suggest no differences in pancreas transplant outcomes irrespective of type of either venous or exocrine diversion. The purpose of this review is to examine the

literature on exocrine drainage in the new millennium (the purported "enteric drainage" era) with special attention to technical variations and nuances in vascularized pancreas transplantation that have been proposed and studied in this time period.

Key words: Pancreas transplantation; Portal-enteric drainage; Simultaneous pancreas-kidney transplant; Systemic-bladder drainage; Enteric conversion; Solitary pancreas transplant; Systemic-enteric drainage

Core tip: The history of vascularized pancreas transplantation largely parallels advances in surgical techniques. Prior to 1995, most pancreas transplants were performed with delivery of insulin to the iliac vein and diversion of the pancreatic ductal secretions to the urinary bladder (systemic-bladder technique). Starting in 1995, however, a seismic change occurred from bladder to bowel drainage of the pancreatic secretions that was spurred in part by the success of enteric conversion. In the new millennium, most pancreas transplants are performed as pancreatico-duodenal grafts with either iliac vein and bowel exocrine diversion (systemic-enteric technique) or portal-enteric drainage. With refinements in surgical techniques, exocrine drainage is no longer considered the "Achilles' heel" of pancreas transplantation.

El-Hennawy H, Stratta RJ, Smith F. Exocrine drainage in vascularized pancreas transplantation in the new millennium. *World J Transplant* 2016; 6(2): 255-271 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/255.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.255

INTRODUCTION

Since the inception of the International Pancreas Transplant Registry (IPTR) in 1984, data on > 48000 pancreas transplants has been captured in the ensuing 30 years[1]. There exist 3 major types of vascularized pancreas transplantation; simultaneous pancreas-kidney (SPK), sequential pancreas after kidney (PAK), and pancreas transplantation alone (PTA). Solitary pancreas transplants refer to the PAK and PTA types. They are usually analyzed together because of similar outcomes coupled with the fact that these procedures are performed in the absence of uremia. However, the state of kidney function is quite different; post-uremic in PAK compared to non-uremic in PTA. In the past 3 decades, the results of SPK transplantation have been superior to solitary pancreas transplantation although the disparity in outcomes has decreased over time. In the United States, solitary pancreas transplants (PAK-17%, PTA-9%) represent the minority of activity while 74% are characterized as SPK transplants[1-3].

In uremic patients with type 1 diabetes mellitus, SPK transplantation is a highly regarded treatment alternative because it addresses both kidney failure and diabetes[3]. The number of United States annual pancreas transplants reached a high of 1484 in 2004 and had dropped to < 1000 by 2014[1-3]. The number of annual pancreas transplants reported to the Eurotransplant Network has similarly declined in the past decade whereas annual activity in the United Kingdom has remained relatively stable and activity elsewhere in the world has increased[1-3]. In spite of declining numbers, outcomes have continued to improve and include higher risk groups such as African-Americans, patients with a phenotype suggesting "type 2 diabetes" and solitary pancreas transplant recipients[1-5]. Five year patient survival rates are now nearly 90% across all three transplant types and 10-year patient survival is > 70% in all three groups. Moreover, insulin independence is sustained at 5 years in 73% of SPK, 64% of PAK, and 53% of PTA recipients. The pancreas graft half-life is currently 10-15 years, which is amongst the lengthiest for extra-renal transplants[2].

Evolution in surgical techniques has characterized and paralleled the growth and development of pancreas transplantation. In late 1966 at the University of Minnesota, Kelly *et al*[6] reported the first human pancreas transplant. The initial case was an SPK transplant with a segmental pancreas graft implanted in the iliac fossa with ligation of the pancreatic duct. In the ensuing 13 cases performed between 1966 and 1973, however, Lillehei *et al*[7] transplanted a pancreatico-duodenal graft with either an external ostomy/cutaneous fistula or connection between the recipient bowel and graft duodenum for exocrine drainage. Consequently, optimal management of the pancreatic ductal secretions was identified as a controversy very early in the development of pancreas transplantation. In the late 1970s and early 1908s, partial or segmental pancreatic grafts (based on the body and tail of the pancreas) with pancreatic ductal ligation or occlusion were the preferred methods of controlling the pancreatic secretions[8,9]. During this developmental phase, exocrine drainage techniques were considered to be the "Achilles' heel" of pancreas transplantation. The introduction of bladder diversion of the exocrine secretions into clinical transplantation in the mid-1980s revolutionized the safety and improved the success of pancreas transplantation[10]. From this point in time onward, whole organ pancreaticoduodenal largely replaced segmental pancreas grafts as the preferred method of transplantation. However, segmental pancreas grafts remain the only surgical option in pancreas transplantation from living donors[9,11]. From 1988 to 1995, > 90% of pancreas transplants in the United States were whole organ pancreatic grafts with iliac vein and bladder exocrine diversion (systemic-bladder technique), usually using a trimmed segment of donor duodenum inclusive of the ampulla of Vater as a channel for drainage of the exocrine pancreas[12].

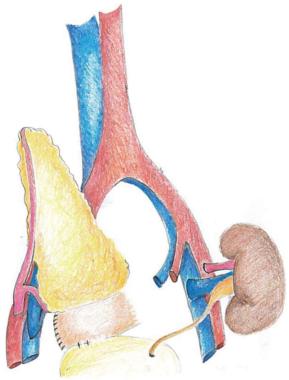

Figure 1 Technique of systemic-bladder drainage with creation of an anastomosis between the allograft duodenal segment and vesical dome of the recipient bladder.

To this day, there remains controversy regarding the optimal method for managing the pancreatic exocrine secretions. By review of data provided by the IPTR, it is evident that the overwhelming majority of pancreas transplants involve whole organ pancreatico-duodenal grafts with either bowel (systemic-enteric) or bladder diversion of the pancreatic ductal secretions coupled with systemic venous delivery of insulin[1,2]. However, starting in 1995, a seismic change from bladder to bowel exocrine diversion transpired coincident with improvements in immunosuppression, preservation techniques, diagnostic monitoring, general medical care, and the success and frequency of enteric conversion[13,14]. Enteric drainage usually refers to jejunal or ileal diversion of the exocrine secretions either as a direct anastomosis or in the presence of a defunctionalized Roux en y limb. By 1998, > 50% of SPK transplants were accomplished with bowel diversion and by 2003 this figure had risen to > 80% of cases in the United States although the systemic-bladder technique was still deployed in 50% of solitary pancreas transplants[13,15]. At present, pancreas transplantation with primary enteric exocrine drainage is performed in 90% of cases in the United States from 2010-2014 although the systemic-bladder technique is a reasonable alternative in selected cases and a preferred option at specific centers[1]. Roux limb diversion is performed in a minority of cases including 21% of SPK and 15% of solitary pancreas transplants[1].

To mimic the natural physiology of the endocrine pancreas, an innovative method of portal vein delivery of insulin (by anastomosing the donor portal vein to the recipient superior mesenteric vein for venous outflow) and bowel diversion of the exocrine secretions (portal-enteric technique) was pioneered in the early 1990s and refined over the past ⩾ 20 years[16,17]. At present, the proportions of enteric-drained cases with portal venous delivery of insulin are 22% in SPK, 11% in PAK, and 13% in PTA cases. Consequently, > 80% of bowel drained pancreas transplants in the United States are performed without a decompressing Roux limb of small bowel and with systemic (iliac or vena cava) venous delivery of insulin[1]. Although the promise of the portal-enteric technique has not been achieved, it has spawned a number of newer and revisited techniques of enteric exocrine drainage including duodenal or gastric diversion[18-32]. Previous reports have not shown any main variances in outcomes for bladder- or enteric-diverted pancreas transplants regardless of method of venous drainage[33-55]. Although one of the three described techniques is deployed in nearly all pancreas transplants at present, the prevailing viewpoint is that the most appropriate procedure to be used is best determined both by recipient and donor anatomy as well as the practicing surgeon's comfort level and experience. A number of previous excellent reviews have emphasized technical aspects of pancreas transplantation but few have been published in the past 6 years[52,56-64]. The purpose of this review is to examine the prevailing literature on exocrine drainage in the past 20 years (the purported "enteric drainage" era) with special attention to surgical techniques that have been introduced over time and with experience in pancreas transplantation.

Bladder drainage of the exocrine secretions (systemic-bladder technique)

Following the groundbreaking studies of Sollinger *et al*[65] and Nghiem *et al*[66] in the 1980s, bladder drainage with a donor duodenal segment became the preferred method of handling the pancreatic ductal secretions in pancreas transplantation until the mid- to late-1990s (Table 1)[67-74]. With this technique, the donor duodenum functions as an exocrine conduit and is anastomosed to the vesical dome either using a 2-layer hand sewn technique or a circular stapled anastomosis[75] (Figure 1). Bladder diversion gained wide acceptance owing to its safety, sterility, convenience, and ease of performance. In addition, bladder drainage enabled direct monitoring of the pancreatic secretions in the urine, permitted a direct approach for trans-cystoscopic biopsy of either the allograft duodenum or pancreatic parenchyma, and provided easy diagnosis and management of anastomotic problems with cystography and urethral catheter drainage[76]. Similar to the use of low pressure cystography to diagnose urine leaks following kidney transplantation, cystography facilitated the detection of anastomotic or duodenal segment leaks following pancreas transplantation with bladder drainage. Prolonged urethral catheter drainage in effect decompressed the anastomosis and enabled control of the exocrine leakage while promoting healing.

Bladder diversion of the pancreatic ductal secretions avoided the inherent bacterial contamination (*e.g.*, peritonitis) that occurred with bowel diversion leaks, contamination that lead to substantial morbidity and even mortality[77]. Consequently, it was associated with a lower risk of intra-abdominal infections and sepsis (because of the sterility of the lower urinary tract) compared to previous techniques of either segmental or whole organ pancreas transplantation with enteric

Table 1 Bladder drainage: Literature review

Center, authors, year, ref., study design, and follow-up	Number and type of transplant	Complications	Enteric conversion	1 yr patient survival	1 yr pancreas graft survival
University of Minnesota, Hakim *et al*[67], Retrospective, mean follow-up 55 mo	*n* = 425 with bladder drainage, SPK - 53%; PAK - 23%; PTA - 24%	Duodenal stump complications - 20%; Duodenal leak - 10%; Recurrent UTI - 9%; Hematuria - 6% (19% required surgery); Bladder stone - 0.5%; CMV duodenitis - 1.5%; Graft loss - 9%	16%	ND	ND
University of Nebraska, Stratta *et al*[68], Retrospective, mean follow-up 44 mo	*n* = 201 with bladder drainage	Duodenal stump complications - 19%; Duodenal leak - 6% (all required surgery); Hematuria - 13% (30% required surgery); CMV duodenitis - 3%	13%	94%	80%
University of Wisconsin, Sollinger *et al*[69], Retrospective	*n* = 500; 338 with bladder drainage, 112 with enteric drainage	Duodenal leak - 15.4%; Graft Thrombosis - 0.7%; Hematuria - 3%; UTI - 52.5%; Graft loss - 13%; Death with a functioning graft - 8%	24%	96.4%	87.5%
The Ohio State University, Henry *et al*[70], Retrospective, mean follow-up 16 mo	*n* = 300 with bladder drainage	CMV - 2%; Intra-abdominal infection - 15%; Wound infection - 8%; Rejection - 55%; Hematuria - 14%; Bladder leak - 10%	4%	92%	82%
University of Maryland, Del Pizzo *et al*[71], Retrospective, mean follow-up 35 mo	*n* = 140; SPK - 68%, PAK - 25%, PTA - 7%	Urological complication - 50%; Bladder stone - 10%; Duodenitis - 11%; Retained foreign bodies - 12%; Bladder tumor - 2%	21%	ND	ND
Mayo Clinic Rochester, Gettman *et al*[72], Retrospective, mean follow-up 44 mo	*n* = 65	UTI - 59%; Hematuria - 26%; Allograft pancreatitis - 19%; Duodenal leaks 17%, (all required surgery); Ureteral lesions - 9%	ND	92%	86%
Hospital Universitario Spain, Medina Polo *et al*[73], Retrospective, mean follow-up 52 mo	*n* = 107, all SPK, bladder drainage in 58, enteric drainage in 49	UTI - 72%; Hematuria - 20%; Bladder stone - 8%; Reflux pancreatitis - 48%	10%	92.7%	78.1%
University of Nebraska, Sudan *et al*[74], Retrospective, mean follow-up 60 mo	*n* = 57, all with bladder drainage	UTI - 15%; Dehydration - 20%; Rejection - 1%	ND	95%	88%

SPK: Simultaneous pancreas-kidney; PAK: Pancreas after kidney; PTA: Pancreas transplantation alone; UTI: Urinary tract infection; CMV: Cytomegalovirus; ND: Not determined/no data.

diversion. In addition, bladder drainage also provided a means to monitor for pancreas allograft rejection by measuring urinary parameters such as amylase, insulin or cytology[78]. However, bladder diversion created an abnormal linkage between the allograft pancreas with intervening donor duodenal conduit and the urinary bladder, which resulted in a number of unique metabolic, urologic, infectious, and miscellaneous complications. Disadvantages and advantages of bladder diversion are specified in Table 2.

With bladder drainage, anastomotic bleeding could be easily diagnosed by the presence of hematuria and usually managed non-operatively with urethral catheter drainage, alkalinization of the urine, administration of blood products, and correction of coagulation parameters. In refractory or persistent cases of hematuria secondary to anastomotic bleeding, however, administration of octreotide, bladder clot removal by cystoscopy with direct fulguration of bleeding sites, or enteric conversion might be indicated. Rates of hematuria are noted in Table 3.

In addition, bladder drainage resulted in obligatory fluid (up to 1-2 L/d of pancreatic exocrine secretions) losses and urinary bicarbonate wasting with consequent changes in the acid-base balance and enzyme-free environment of the lower genitourinary tract. Many patients were prone to dehydration, metabolic acidosis, erythrocytosis, and orthostasis, particularly in the setting of severe autonomic neuropathy secondary to diabetes. For these reasons, the length of donor duodenum transplanted with the pancreas was progressively shortened over time in an attempt to minimize protein

Table 2 Advantages and disadvantages of bladder drainage of the exocrine secretions

Advantages
Safety
Reduced infection rate because of relative sterility of lower urinary tract
Control of anastomosis by urethral catheter decompression
Technical considerations
Relative simplicity because of favorable anatomic location of bladder
Bladder mobilization permits tension-free, multi-layer anastomosis
Bladder vasculature and urothelium promote healing
Direct access to exocrine secretions for monitoring pancreas allograft function
Detection of rejection by urinary parameters (amylase, lipase, insulin, cytology)
Cystoscopic access for either duodenal or pancreatic parenchymal biopsy
Disadvantages
Urologic problems
Hematuria, dysuria, cystitis, urethritis, urethral stricture or disruption, balanitis
Increased risk of lower urinary tract infections, stone formation, and urine leaks (either from bladder or duodenum)
Metabolic and volume problems
Dehydration, orthostasis, constipation, erythrocytosis
Metabolic acidosis
Miscellaneous problems
Reflux-associated hyperamylasemia or pancreatitis
Transitional cell (urothelial) dysplasia
Need for enteric conversion for refractory, persistent, or recurrent problems
Medication burden (massive amounts of bicarbonate supplementation)

and bicarbonate loss from the allograft duodenal mucosa. In some patients, intractable, recurrent, or refractory complications would occur, which were then treated with open conversion from bladder to bowel diversion (enteric conversion) (Figure 2). Paradoxically, the success of "enteric conversion" paved the way for renewed enthusiasm in primary enteric drainage. Enteric conversion frequency ranged from 10% to 40% (Table 3)[79-86]. Several authors reported that enteric conversion resulted in superb long-term graft function coupled with marked symptom improvement even when performed several years following SPK transplant[84,87,88]. Despite urological morbidity and the finite risk of enteric conversion, 5-year actuarial patient and graft survival rates with bladder drainage were excellent and most complications could be managed with conservative (non-operative) therapy.

For diabetic patients with neurogenic bladders, episodes of reflux pancreatitis (managed with urethral catheter drainage) and recurrent urinary tract infections were not uncommon. In the setting of urinary tract infection, the pH of urine would become more acidic, which led to pancreatic enzyme activation and a variety of complications including hematuria, duodenitis, cystitis, urethritis, urethral stricture or disruption, and balanitis. In severe cases, some investigators even reported reduction cystoplasty and bladder re-anastomosis in an attempt to control persistent urologic problems.

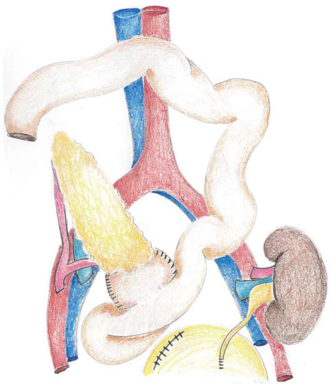

Figure 2 Technique of conversion from bladder to enteric exocrine drainage (enteric conversion) for persistent metabolic, urologic, or other problems.

Most patients required daily oral sodium bicarbonate supplementation and some received chronic suppressive antibiotics to limit the morbidity attributable to the abnormal physiology. Alternative treatments to reduce exocrine drainage side effects included the use of oral pancreatic enzymes or long-acting somatostatin analogues. Other late complications comprised duodenal leaks, stone formation, and the risk of urothelial dysplasia.

At present, bladder drainage remains an important option in selected cases, such as those in which pancreas graft quality in general or viability of the allograft duodenum in particular is suspect. In cases of duodenal ischemia or severe reperfusion injury, the bladder anastomosis can be performed by invaginating the duodenum into the bladder in order to minimize leaks (Figure 1). In addition, if the recipient has severe adhesions from multiple previous intra-abdominal procedures or sclerosing peritonitis, then a bowel anastomosis may be risky. Moreover, until recently, bladder drainage was preferred by many centers in solitary pancreas transplantation (PAK, PTA) because of the increased incidence of acute rejection (early and late) in this setting coupled with the established difficulty in the timely detection of pancreas rejection in the absence of either a urinary marker (with bladder drainage) or serum creatinine monitoring (with an SPK transplant).

A number of centers have reported excellent long-term outcomes in pancreas transplantation with the systemic-bladder technique[9,52,69,70,74,80,89]. For a period of time, the bladder drainage technique was also associated with lower incidences of thrombosis, early technical complications, and graft loss in IPTR reports compared to enteric drainage[12,13,15]. Consequently, many new centers (including those in developing countries) elected to embark on their experience in pancreas transplantation with systemic-bladder drainage owing to its technical simplicity and purported lower technical complication rate. In some instances, centers have adopted a 2-stage approach in which primary bladder diversion is followed by planned enteric conversion in order to avoid the immediate complications of primary enteric diversion

Table 3 Enteric conversion: Literature review

Center, authors, year, ref., and study design	Overall rate (%)	Urologic indications # (%)	Metabolic indications # (%)	Pancreatitis/other indications # (%)	Operative complications # (%)
University of Wisconsin, Van der Werf *et al*[79], Retrospective	95/449 (21%)	90 (95)	1 (1)	4 (4)	21 (22)
Sollinger *et al*[80], Retrospective	160/390 (41%)	93 (58)	1 (0.6)	47 (29)	ND
University of Minnesota, West *et al*[81], Retrospective	79/500 (16%)	43 (54)	26 (33)	15 (19)	12 (15)
University of Nebraska, Sindhi *et al*[82], Retrospective	25/195 (13%)	7 (28)	18 (72)	0	3 (12)
University of Barcelona, Spain, Fernandez-Cruz *et al*[83], Retrospective	16/74 (22%)	0	0	16 (100)	Death 1 (6); Wound infection 2 (12); Anastomotic leak 3 (18)
Leiden University Medical Center, Netherlands, van de Linde *et al*[84], Retrospective	51/ND	39 (76)	23 (45)	Pancreatitis 2 (3); Fistula 1 (1)	UTI 7 (13); Minor bleeding 1 (0.5); Phlebitis 1 (0.5); Paralytic ileus 1 (0.5); Relaparotomy 2 (3)
University of Cincinnati, Kaplan *et al*[85], Retrospective	26 (32%)	13 (50)	13 (50)	0	Death 1 (3); Anastomotic bleeding 1 (3)
Beaumont Hospital, Ireland, Connolly *et al*[86], Retrospective	6/ND	3 (50); 2 hematuria; 1 UTI	3 (50)	ND	Pulmonary edema 1 (16)

UTI: Urinary tract infection; ND: Not determined/no data.

(intra-abdominal infections, early graft loss) and the long-term metabolic and urologic problems related to bladder diversion[84,87]. For example, Marang-van de Mheen *et al*[87] routinely used a two-step approach in SPK transplant; primary bladder diversion followed by planned enteric conversion (Figure 2). They found that this approach resulted in urological complication rates similar to bowel-drained grafts with subsequent excellent survival rates. Conversions were performed by separating the graft duodeno-cystostomy, then re-establishing continuity and diversion by a side-to-side recipient jejunal-graft duodenal-anastomosis either without (most commonly) or with a diverting Roux limb.

The drawback to planned conversion is loss of urinary amylase as an immunological biomarker, especially in PAK and PTA recipients. In SPK transplant recipients, however, the renal allograft and serum creatinine can still be monitored as a biomarker for allograft rejection. Contrary to previous IPTR reports, however, there is no longer a survival, technical complication, or immunological monitoring advantage associated with bladder drainage, so the practice of "intentional" enteric conversion has been largely supplanted by primary bowel diversion[1-3].

Bowel diversion of the pancreatic ductal secretions (systemic-enteric technique)

Initial attempts at bowel exocrine diversion in the 1970-80s were fraught with complications including intra-abdominal sepsis and mortality because of limitations in preservation techniques, immunosuppression, diagnostic monitoring, and general medical care. However, the introduction of University of Wisconsin solution (that was initially developed as a pancreas preservation solution), tacrolimus, mycophenolate mofetil, ganciclovir, newer monoclonal and polyclonal antibody agents, biopsy-directed surveillance, and improvements in general medical and critical care (including higher resolution computerized tomographic scanning, more effective antibiotics, and the development of safe and more sophisticated percutaneous interventions) were pivotal in the re-emergence of primary bowel drainage as an alternative to bladder drainage. During the transitional phase from primary bladder to enteric drainage in the late 1990s to early 2000s, several studies (both prospective and retrospective) reported comparable outcomes with either technique although primary enteric drainage was not associated with the requisite long-term metabolic and urologic complications unique to bladder drainage (Table 4)[90]. In addition, the success of enteric conversion corroborated the safety and feasibility of primary enteric drainage following pancreas transplantation, which in essence eliminated the need for re-operation in 10%-40% patients with urinary bladder diversion. Moreover, bowel diversion of the pancreatic ductal secretions was much more acceptable to the medical community at large because it was more "physiologic" and logical to drain the pancreatico-duodenal secretions into the small bowel. Disadvantages and advantages of primary bowel diversion are noted in Table 5.

Potential risk variables for early bowel leaks include poor characteristics of the allograft duodenum (related to donor hemodynamic instability or trauma), ischemia-reperfusion and preservation injury (related to preservation solution as well as warm and cold ischemia), complications with either the vascular or bowel anastomosis because of adhesions or other technical issues, higher donor or recipient age or body mass index, peritoneal dialysis, and deconditioning in the recipient. In

Table 4 Bladder *vs* enteric drainage: Literature review

Center, authors, year, ref., and study design	Number and type of transplant	Complication/enteric conversion	Acute rejection/ graft loss	Reoperation and readmissions	1 yr patient survival	1 yr pancreas (and kidney) graft survival
University of Maryland, Kuo *et al*[35], Retrospective	23 SPK ED	ED: Fewer UTIs and urologic complications	ND	ND	ED 100%; BD 96%	ED 88%; BD 91%
University of Chicago, Newell *et al*[33], Retrospective	SPK; ED 12; BD 12	Acidosis and dehydration less with ED ($P < 0.005$); Hematuria; BD 25%; ED 0%; No anastomotic leaks in either group; No intra-abdominal infection in either group; Enteric conversion: 33%	ND	BD: 4 patients underwent enteric conversion	BD 100%; ED 83.3%	BD 91.7%; ED 83.3%
University of Wisconsin, Sollinger *et al*[80]; Retrospective	1000 SPK; BD 390; ED 610	Pancreas graft thrombosis; BD 2.3% ED 3.6%; Infection; BD 1.8% ED 0.8%; Pancreatitis; BD 1.3% ED 0.5%; Pancreatic leak BD: 12% ED: 5% ($P = 0.06$)	Kidney rejection; BD 29%; ED 19%; Pancreas rejection; BD 12.1%; ED 5.4%	ND	Similar in both groups	Similar kidney, and pancreas graft survival in both groups
Pirsch *et al*[37], Retrospective	48 BD; 78 ED	Opportunistic infections; ED: 12% BD: 31% ($P = 0.002$); CMV; BD 21% ED 4% ($P = 0.04$); Fungal infection; BD 17% ED 4%; UTI BD 63% ED 20% ($P = 0.0001$)	Kidney rejection; BD 38%; ED 30%; Steroid-resistant rejection; BD 19%; ED 17%			
University of Washington, Friedrich *et al*[90], Retrospective	34; ED 17; BD 17	ED 41%; BD 53%; Enteric conversion: 5%	ED 29%; BD 24%	Readmissions: ED 41%; BD 47%	ND	ND
University of Tennessee-Memphis, Stratta *et al*[41], Prospective	BD 16; ED 16	UTI BD 50% ED 19%; Urologic complications; BD 25% ED 12.5%; Dehydration BD 100% ED 44%	BD 44%; ED 31% P = NS	BD 25%; ED 25%; Readmissions: BD 2.6 ± 1.8; ED 1.75 ± 1.2	BD 88%; ED 94%	Kidney survival; BD 92%; ED 93%; Pancreas survival BD 81%; ED 88%
Albert Einstein Medical Center, Bloom *et al*[34], Retrospective	71 SPK; BD 37; ED 34	Dehydration BD 34% ED 3.4%; Acidosis BD 41% ED 0% Pancreatitis BD 40% ED 3.4% UTI BD 71% ED 27% ($P < 0.005$) Enteric conversion: 19%	BD: 13.5%; ED: 14.7%		Similar between groups	Pancreas allograft survival was similar between groups
Emory University, Pearson *et al*[36], Retrospective	SPK; BD 55; ED 11	BD; UTI 78%; Hematuria 27%; Dehydration 38%; ED no complication				
University of Pittsburgh Corry *et al*[43], Retrospective	BD 44; ED 199	Overall BD 41% ED 26%; Anastomotic bleeding; BD 16% ED 5%; Fistula BD 14% ED 6%		BD 24%; ED 16%		BD 44%; ED 69%
Toronto General Hospital, Cattral *et al*[40], Retrospective	SPK; BD 20; ED 20	UTI: Similar in both groups; CMV infections were significantly less in the ED group	BD 37%; ED 15%; ($P = 0.20$)	BD 1 patient to ligate an arteriovenous fistula in the pancreas graft; ED 4 patients; (bleeding in one, partial wound dehiscence in one, negative laparotomy in two)	BD 95%; ED 100%	Kidney graft survival; BD 95%; ED 100%; Pancreas graft survival; BD 95%; ED 100%

Wake Forest University, Stratta *et al*[46], Retrospective	297 SPK; SE 171 (58%); PE 96 (32%); SB; 30 (10%)	No differences were seen in surgical complications including pancreas thrombosis; Infections: SE 49%; PE 85%; BD 63%	SE 19%; PE 26%; BD 30%	Readmissions: SE 61%; PE 63.5%; BD 63%	SE 97%; PE 99%; BD 97%	Kidney; SE 94%; PE 98%; BD 93%; Pancreas; SE 87%; PE 92%; BD 87%

BD: Bladder drainage; ED: Enteric drainage; SB: Systemic-bladder; SE: Systemic-enteric; PE: Portal-enteric; UTI: Urinary tract infection; CMV: Cytomegalovirus; ND: Not determined/no data.

Table 5 Advantages and disadvantages of enteric drainage of the exocrine secretions

Advantages
Safety
Lower rates of urinary tract infections and urologic complications
More "physiologic"; fewer metabolic and volume problems
Fewer readmissions
Technical considerations
Treats exocrine insufficiency (in patients following total pancreatectomy or in patients with cystic fibrosis
Avoidance of need for enteric conversion; lower relaparotomy rate
Can be used with either systemic or portal venous outflow
Disadvantages
Safety
Higher incidence of leakage of pancreatic enzymes, pancreatitis, peri-pancreatic fluid collections
Higher incidence of intra-abdominal abscess, peritonitis, sepsis
Anastomotic leaks, GI bleeding
Increased risk of wound infections, wound healing problems (contaminated case with GI tract breach)
Technical considerations
Selective need for enterolysis or diverting Roux en y limb
Loss of direct access to anastomosis and allograft for diagnosis and treatment
Miscellaneous problems
Inability to directly monitor exocrine secretions

GI: Gastrointestinal.

addition, late intra-peritoneal infectious complications may occur in bowel-drained transplants[91-93]. In more recent series, however, the incidence of and outcomes associated with surgical complications following enteric diversion are similar to those following bladder drainage and the rates of early graft loss with either technique are comparable[1-3,52,62-64]. The incidence of surgical complications is also similar by type of transplant (SPK compared to solitary pancreas transplantation)[1-3]. Leaks from the allograft duodenum have been reported to occur in 5%-20% of bladder-drained and 5%-8% of bowel-drained pancreas transplants[9,33-52,67-73,80,91-95]. Increasing experience with enteric exocrine drainage is likewise associated with a decreased rate of technical complications[9,38,80,96-103].

Because of lingering concerns regarding the safety of enteric drainage based on historical precedent, the use of diverting Roux limbs was not uncommon in the late 1990s and many centers continued to direct the head and duodenum of the pancreas allograft toward the pelvis just in case "bladder conversion" was required.

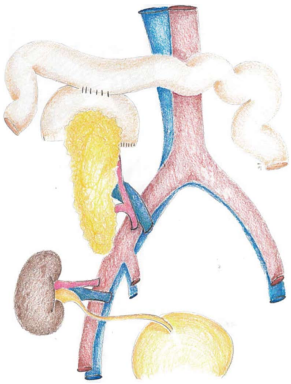

Figure 3 Technique of systemic-enteric drainage with side-to-side anastomosis between allograft duodenum and recipient small bowel.

Techniques that incorporated diverting Roux limbs with temporary external ostomies were also described in an attempt to permit direct endoscopic access and provide decompression of the enteric anastomosis and allograft duodenum[23]. However, with time and experience, most pancreas transplant surgeons evolved to directing the head and duodenum of the pancreas allograft away from the pelvis to simplify the enteric anastomosis, which was typically performed side-to-side between the allograft duodenum and either the recipient proximal jejunum or ileum without a Roux limb (Table 6)[104-108] (Figure 3). Safe techniques of using either the circular or linear stapler were described to simplify the enteric anastomosis[109,110]. If a Meckel's diverticulum was identified, some surgeons would excise the diverticulum and then use this site for the bowel anastomosis[111]. Placement ipsilateral of the kidney and pancreas allografts in SPK transplantation was also introduced to limit the dissection and expedite the procedure[106]. A potential side benefit of enteric drainage was elimination of the need to construct a duodenal segment, which meant less dissection during back bench preparation, less risk of devascularizing the head of the pancreas or duodenum by collateral disruption, and less time spent with the pancreas *ex vivo* and exposed. By transplanting the pancreas as a complete pancreatico-duodenal graft, collateral circulation to the pancreas and duodenum was preserved. Maintaining full duodenal length also facilitated numerous possibilities for performing the bowel anastomosis in the recipient. In addition, the distal donor duodenum could be used as access for stapler

Table 6 Systemic-enteric drainage: Literature review

Center, authors, year, ref., and study design	Number and type of transplant	Complications	Readmission/reoperation/ length of stay	1 yr patient survival	1 yr kidney/ pancreas survival
Medical University of South Carolina, Douzdjian *et al*[105], Retrospective	ED 16; BD 26	Recurrent/persistent urinary complications BD 46% ED 6% (P = 0.01); Dehydration BD 27% ED 6% (P = 0.05); Pancreatitis BD 8% ED 6% (P = NS); Wound infection BD 12% ED 19% (P = 0.5)	Readmissions BD: 1.7 ± 1.5; ED 1.2 ± 1.2 d (P = 0.2) Reoperations BD 23% ED 0 (P = 0.04); Length of stay BD: 12.9 ± 5.6 ED: 20.4 ± 9.6 d, P = 0.007	BD 96%; ED 94%; P = 0.6	Kidney BD 85%; ED 87%; Pancreas BD 90%; ED 85% (P = 0.6)
Institut de Malaties Digestives, Spain, Heredia *et al*[94], Retrospective	205 SPK; ED 97	Duodenal leaks: (n = 11); Acute rejection (n = 6); CMV infection (n = 3); Technical failure (n = 2); Death: (n = 2) as a consequence of sepsis	Reoperation for duodenal leak: Roux-en-Y technique: (n = 3) DJ technique: (n = 2) Transplantectomy: (n = 6)	ND	ND
Toronto General Hospital, Spetzler *et al*[95], Retrospective	Total 284; 191 SPK (67.3%); 93 PAK (32.7%)	Duodenal leak (incidence 6.3%), 12 (67%) occurred within the first 100 d after transplantation	Six grafts (33%) were rescued by duodenal segment resection;	ND	ND
Innsbruck University Hospital, Austria, Steurer *et al*[92], Retrospective	40 ED	Intra-abdominal infection - 11 (27.5%)	Reoperation for intra-abdominal infection Pancreatectomy: 5 Necrosectomy and drainage: 5 Percutaneous drainage: 1	ND	ND
Ruhr-University Bochum, Germany, Ziaja *et al*[104], Retrospective	30 SPK	Perioperative mortality 3.3%	Early relaparotomy was required in 20%; pancreatectomy in 10%	ND	ND
Indiana University, Fridell *et al*[106], Retrospective	49; SPK; All ED	Death: (n = 2) (1 patient died from multi-system organ failure and a second from graft *vs* host disease); Pancreatic graft failures: (2); renal graft failure: (1)	Relaparotomies: (n = 5) bowel obstructions: (2) anastomotic leak: (1) ureteral stricture: (1)	96%	Kidney 94%; Pancreas
University of Pittsburgh, Corry *et al*[107], Retrospective	104 SPK	Graft loss in 6 patients, Death in one patient	Splenic artery hemorrhage: (1) ND	98%	92%; Kidney 95%, Pancreas 83%
University of Maryland, Bartlett *et al*[108], Prospective	27; Solitary pancreas transplants	One graft lost to acute rejection in the tacrolimus group because of patient noncompliance	ND	ND	90% in patients receiving tacrolimus, 53% in patients receiving cyclosporine (P = 0.002)

BD: Bladder drainage; ED: Enteric drainage; CMV: Cytomegalovirus; ND: Not determined/no data; DJ: Duodeno-jejunostomy.

placement to perform the enteric anastomosis[109,110].

Bowel drainage of the pancreatic ductal secretions (portal-enteric technique)

To address the unusual anatomy of pancreas transplantation, Gaber *et al*[16] introduced a new technique in which an anterior intraperitoneal approach to the recipient superior mesenteric vein (SMV) was deployed for venous drainage. This procedure was later modified to a "retroperitoneal" approach to the SMV by Boggi' s group in Pisa. Both of these techniques combined bowel drainage of the pancreatic ductal secretions with portal venous delivery of insulin (portal-enteric technique)[16,17,112,113]. Alternative methods to achieve portal venous delivery of insulin have been reported using either the recipient portal vein directly, the inferior mesenteric vein, or splenic vein. However, in most cases, "portal venous" drainage usually infers that the allograft has a vertical orientation with the body and tail directed towards the pelvis, the head and duodenum directed cephalad, and the recipient SMV as the site for the venous anastomosis[18-22] (Figure 4). The bowel anastomosis is most commonly performed to a bowel loop that is not excluded from the transit of intestinal contents[4,16,17,33,39-42,44-46,49-53,112-121]. Alternatively, the allograft duodenum can be connected directly into the native stomach or duodenum, to a diverting Roux limb without or with a venting jejunostomy, or to an omega loop[23-32,122] (Table 7). Utilizing the native stomach or duodenum affords straightforward access to the allograft duodenum and pancreas for biopsy and surveillance by endoscopic techniques and also expands the possibilities for exocrine drainage sites, particularly in cases of pancreas retransplantation (Table 8)[25-32,123]. However, because up to 5%-10% of transplanted pancreata are at risk for early technical failure that may lead to leaks,

Table 7 Portal-enteric drainage: Literature review

Center, authors, year, ref., study design and follow-up	Number and type of transplant	Complications	Readmissions, reoperation, length of stay	1 yr patient survival	1 yr kidney and pancreas graft survival
University of Tennessee, Stratta *et al*[122], Retrospective, mean follow-up 3 yr	PE 126; 90 SPK; 18 PAK; 18 PTA; Era 1 (10/90-6/95); Era 2 (7/95-5/98); Era 3 (6/98-12/99)	In 3 successive eras, rates of acute rejection were 63%, 33%, and 39%, respectively; rates of major infection were 60%, 43%, and 44%, respectively	In 3 successive eras, rates of relaparotomy were 47%, 31%, and 33%, respectively; rates of thrombosis were 20%, 7%, and 6%, respectively. Mean length of stay: 12.5 d	In 3 successive eras, patient survival was 77%, 93%, and 100%, respectively	In 3 successive eras, kidney graft survival was 77%, 93%, and 94%, respectively; pancreas graft survival was 60%, 83%, and 83%, respectively
Università di Pisa, Italy, Boggi *et al*[17], Retrospective, mean follow-up 21 ± 20 mo	PE 110	10 grafts were lost; 3 acute rejection, 2 chronic rejection, 2 venous thrombosis, 2 deaths, 1 late thrombosis (6 mo). Incidence of pancreas acute rejection was 6%	Relaparotomy rate was 13.6%; Mean length of stay was 26 ± 14 d; One month readmission rate was 13%	98%	Pancreas graft survival was 91%
University of Chicago, Bruce *et al*[116], Retrospective, mean follow-up 16 mo	PE 70	Pancreas graft losses: Thrombosis (3), acute rejection (5), late duodenal perforation (2)	Total 1st year hospitalization: 37 ± 28 d; Relaparotomy in 14 (70%)	88%	Kidney 78%; Pancreas 79%
Louisiana State University, Zibari *et al*[23], Retrospective, mean follow-up 25 mo	PE 21	Postoperative Bleeding in 4, wound infections in 4, acute rejection in 9, pancreas graft loss in 2	Mean length of stay was 16 d	100%	Kidney 90%; Pancreas 90%
Wake Forest Baptist Medical Center, Rogers *et al*[4], Retrospective, mean follow-up 6 ± 3 yr	202; SPK 162, PAK 35, PTA 5; PE 179; SE 23	Thrombosis rate was 8%; acute rejection rate was 28%; major infection rate was 50%	Mean length of stay was 13 d; Relaparotomy rate was 38%	Overall patient survival was 87%; one-year patient survival was 97%	Overall kidney and pancreas graft survival rates are 76% and 65%; death-censored graft survival rates are 84% and 72%, and one year graft survival rates are 94% and 88%, respectively
Monash Medical Centre, Victoria, Australia, Kave *et al*[118], Retrospective, mean follow-up 2 yr	SB 37; PE 27	Pancreas graft thrombosis rates SB 10.8%, PE 7.4% (*P* = NS)		Two-year patient survival was SB 94.3% *vs* PE 96.0%	Two year kidney (SB 89.2% *vs* PE 85.2%); pancreas (SB 77.9% *vs* PE 71.4%)

SB: Systemic-bladder; SE: Systemic-enteric; PE: Portal-enteric.

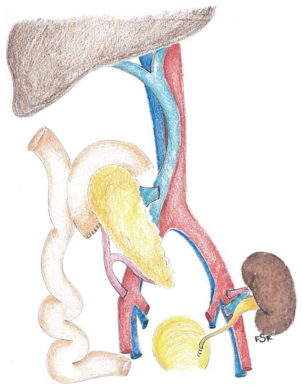

Figure 4 Technique of portal-enteric drainage with side-to-side anastomosis between allograft duodenum and small bowel; this technique is also amenable to using the native duodenum or stomach for exocrine diversion.

many centers are reluctant to perform enteric diversion either to the native stomach or duodenum. Following reperfusion of the transplanted pancreas, if the allograft duodenum does not appear well vascularized, bowel drainage with creation of a diverting Roux limb may be preferred to bypass the enteric stream and promote healing even though this procedure mandates an additional bowel anastomosis.

Although the rate of bleeding at the may be higher, some surgeons prefer to use either a circular or linear stapling device to create the bowel anastomosis[109,110]. However, most commonly, the connection between the allograft duodenum and recipient small bowel is performed using a 2-layer hand sewn technique that comprises a running continuous inner layer of interlocking absorbable suture coupled with an interrupted seromuscular outer layer of simple interrupted non-absorbable sutures to create a "watertight" and hemostatic closure[121]. The bowel anastomosis can be located anywhere between the distal ileum and native stomach although most commonly is performed as a primary side-to-side connection to the proximal jejunum (Figure 4). Other methods of reconstruction may include either an end-to-side or end-to-end anastomosis between the allograft duodenum and recipient gastrointestinal tract. When using portal-enteric drain-

Table 8 Portal-duodenal/gastric drainage: Literature review

Center, authors, year, ref., and study design	Number and type of transplant	Complications	Readmissions and reoperations	1 yr patient survival	1 yr pancreas survival
New York Medical College, Westchester Medical Center, Gunasekaran *et al*[28], Retrospective	DJ: 36; DD: 21; stapled 14, hand-sewn 7	Thrombosis: None in DJ, 2 in DD (P = NS); Enteric leak and small-bowel obstruction: 3 in DJ, 2 in DD (P = NS); Gastrointestinal bleeding: None in DJ, 4 in DD (P = 0.015)	ND	94% with DJ, 95% with DD	89% with DJ, 86% with DD
Louisiana State University, Shokouh-Amiri *et al*[27], Retrospective	Group 1: Allograft jejunum to stomach, n = 30; Group 2: Allograft duodenum to jejunum with Roux-en-Y venting jejunostomy, n = 30	In Group 1: Pancreatectomy in 3, CMV in 7, acute rejection in 4, death in 3; In Group 2: Pancreatectomy in 1, CMV in 2, acute rejection in 6, death in 2 (all P = NS)	Major complications: 4 in group 1, 10 in group 2	94% in group 1, 96% in group 2	85% in group 1, 83% in group 2
Bandeirantes Hospital, Sao Paulo, Brazil, Perosa *et al*[30], Retrospective	43 PAK, 10 PTA with DD	Thrombosis in 5 (9%); 4 additional pancreas graft losses (including 2 deaths with functioning grafts); Acute rejection in 9 (17%); major infection in 24 (45%)	Readmissions: Mean 1.1; Mean length of hospital stay: 11.8 d; Reoperations in 9 (17%)	96%	83%
University Hospital Bochum, Germany, Walter *et al*[31], Retrospective	DD in 125 (64% with portal outflow); DJ in 116 (12% with portal outflow)	GI bleeding in 14 with DD, 4 with DJ; Thrombosis in 5 with DD, 18 with DJ (P = 0.002); Acute rejection in 29% in DD *vs* 31% in DJ	2 anastomotic leaks with DD, 6 with DJ; Pancreatectomy in 14 with DD, 21 with DJ; Early relaparotomy in 42% DD *vs* 48% DJ, all P = NS	96% in both groups	82% with DD, 78% with DJ
Oslo University Hospital, Rikshospitalet, Norway, Horneland *et al*[32], Retrospective	20 SPK, 17 PTA, 3 PAK with DD (n = 40); 30 SPK 7 PTA, 3 APK with DJ (n = 40); In sequential eras	Thrombosis in 13% DD *vs* 5% DJ; Acute rejection in 23% DD *vs* 28% DJ, both P = NS	Reoperations in 40% DD *vs* 30% DJ; Mean length of hospital stay 19 d DD *vs* 16 d DJ, both P = NS	97.5% DD *vs* 92.5% DJ	Overall pancreas survival was 80% with DD, 87.5% with DJ (P = NS)
Scientific-Research Institute of Sklifosovsky, Moscow, Russia, Khubutia *et al*[123], retrospective	Group 1: 15 DJ; Group 2: 17 DD	Acute reject ion in 13% DJ *vs* 12% DD; Major infections in 20% DJ *vs* 6% DD, both P = NS	Surgical complications in 20% DJ *vs* 23.5% DD, P = NS	93% DJ *vs* 94% DD	Pancreas survival 93% DJ *vs* 94% DD; kidney survival 93% DJ *vs* 88% DD

DD: Duodeno-duodenostomy; CMV: Cytomegalovirus; ND: Not determined/no data; DJ: Duodeno-jejunostomy; NS: Not significant.

age, the recipient ileum can be anastomosed to the distal graft duodenum whereas the recipient jejunum can be anastomosed to the proximal graft duodenum. We prefer the former technique with the location of the bowel anastomosis on the posterior aspect of the 3rd or 4th portion of the graft duodenum to promote dependent drainage of the atonic, denervated graft duodenum when the patient is either in the erect or supine position[121]. Anastomotic length can be variable but usually ranges from 3-5 cm.

Unlike bladder drainage, however, anastomotic bleeding with enteric drainage is more occult and harder to diagnose in the absence of gastric, duodenal, or extreme proximal jejunal diversion or in the absence of a diverting jejunostomy. Because most enteric anastomoses are performed in the middle third of the gastrointestinal tract, endoscopic confirmation and treatment are not available. Consequently, the true incidence of anastomotic bleeding with enteric drainage is probably under-reported and the severity may be under-appreciated because of other causes of anemia in the immediate post-operative period. Fortunately, most cases are self-limited and respond to supportive measures such as decompression of the gastrointestinal tract, administration of blood products, and correction of coagulation parameters. In cases of persistent and significant lower (or rarely upper) gastrointestinal bleeding, administration of octreotide may be helpful by inducing vasoconstriction. Rarely, re-operation with revision of the enteric anastomosis (with or without Roux limb diversion) may be indicated for anastomotic bleeding. For severe gastrointestinal bleeding that occurs more than one week post-transplant, however, one must not assume it is secondary to anastomotic bleeding. In this setting, it is imperative to rule out a leaking pseudoaneurysm, which is best diagnosed and treated with angiographic techniques[124].

When using the retroperitoneal approach to the SMV for portal-enteric drainage, in order to perform an anastomosis to the small bowel, one must make a window in the mesentery of the right colon. Bowel drainage can then be accomplished without or with a diverting Roux limb in a standard side-to-side manner[17,113]. If one initially performs a side-to-side bowel

Table 9 Systemic *vs* portal-enteric drainage: Literature review

Center, authors, year, ref., study design and follow up	Number and types of transplant	Complications	Length of stay, readmissions and reoperations	1 yr patient survival	1 yr kidney and pancreas survival
University of Tennessee, Memphis, Stratta *et al*[44], Prospective, mean follow-up 17 mo	SE 27; PE 27	Incidences of acute rejection (33%) and major infection (52%) similar in both groups; Intraabdominal infections were slightly greater in the SE group (26% SE *vs* 11% PE); 2 deaths in SE group compared to one in PE group Pancreas Graft loss: 7 in SE compared to 4 in PE group, all *P* = NS	Readmissions (mean 2.8 SE *vs* 2.2 PE); Mean length of hospital stay: SE: 12.4 d; PE: 12.8 d; Relaparotomy: 8 in SE compared to 7 in PE group, all *P* = NS	SE 96%; PE 93%	Pancreas SE 74%; PE 85%; Kidney SE 96%; PE 93%
University of Maryland, Philosophe *et al*[45], Retrospective	SE: 63 SPK, 42 PAK, 26 PTA	Acute rejection: At 36 mo, the pancreas rejection rates were 21% for PE *vs* 52% for SE (*P* < 0.0001); the kidney rejection rates following SPK were 26% PE *vs* 43% SE (*P* = 0.017)	ND	36-mo patient survival rates were similar in both groups, 89% for PE *vs* 93% for SE	36-mo graft survival rates for all pancreas transplants were 79% with PE *vs* 65% with SE (*P* = 0.008)
Hospital Juan Canalejo, Coruña, Spain, Alonso *et al*[49] and Quintela *et al*[51], Retrospective, mean follow-up 23 mo	PE: 54 SPK, 55 PAK, 40 PTA; SE 18; PE 20	Incidences of intraabdominal infection and acute rejection episodes were not different between groups	Early relaparotomy no difference: SE: 34 d; PE: 20 d	PE: 80% *vs* SE: 86%	Death-censored pancreas (SKP and PAK) graft survival was 73% for PE and 81% for SE (*P* = NS)
Toronto General Hospital, Bazerbachi *et al*[53], Retrospective	SE 147; PE 45	In both groups, a complication occurred in 38% of patients in the first year; Major infections were not different between groups; 3-mo rejection rate was identical (6%) and the 1-yr rejection rate was 12.2% SE *vs* 13.3% PE; Most common reasons for pancreas graft loss in both groups were death with functioning graft (25%), graft thrombosis (13%), rejection (11%) and duodenal leak (9%)	Length of stay - mean 11 d *vs* 10 d in the SE *vs* PE; Most common causes of death in both groups were myocardial infarction (35%), cerebrovascular accident (13%) and cancer (13%); Most common causes of kidney graft loss in both groups were death with functioning graft (61%) and acute rejection (11%)	Patient survival did not differ at 5 yr (94% SE *vs* 89% PE) and 10 yr (85% SE *vs* 84% PE, *P* = NS)	Pancreas survival was similar at 5 yr (82% SE *vs* 76% PE) and 10 years (65% SE *vs* 60% PE); Kidney survival was similar at 5 yr (93% SE *vs* 84% PE) and 10 yr (82% SE *vs* 76% PE)
Medical University Innsbruck, Austria, Ollinger *et al*[120], Retrospective, Mean follow-up 8.3 yr	509 transplants in 4 eras including 34 PE and 146 SE (with DJ) in most recent era (2004-2011)	Thrombosis: 9% PE *vs* 5% SE, *P* = NS		5-yr patient survival 94%	5-yr pancreas survival 77% PE *vs* 74% SE
Hôpital Edouard Herriot, Lyon, France, Petruzzo *et al*[50], Retrospective	SE 36; PE 44; All SPK	No significant differences in long-term outcomes but the SE group had a higher incidence of pancreas graft loss secondary to thrombosis	No difference in total surgical complications	Patient survival rates 92% SE *vs* 95.5% PE	One-, 3-, 5-, and 8-yr pancreas survival rates were 75%, 60.6%, 56.7%, and 44%, respectively, in the SE group compared to 88.6%, 84.1%, 78.4%, and 31.3% in the PE group; One- 3-, 5-, and 8-yr kidney survival rates were 91.7%, 78.1%, 74.1%, and 57.9%, respectively, in the SE group compared to 93.2%, 88.6%, 78.4%, and 38.9% in the PE group

SE: Systemic enteric; PE: Portal enteric; ND: Not determined/no data; DJ: Duodeno-jejunostomy; NS: Not significant.

anastomosis, it is relatively straightforward to convert to a diverting Roux limb for whatever reason by separating the afferent limb with a gastrointestinal stapler just proximal to the anastomosis. The stapled and divided proximal limb can then be placed 40 cm or more distal to the anastomosis on the efferent limb and the second

bowel anastomosis can be constructed either in a side-to-side or end-to-side manner with either sutures or a stapler. A potential advantage of accessing the SMV for venous drainage is that the procedure is no longer pelvic but rather mid-abdominal in location, which is helpful in cases of retransplantation or in patients who have had previous pelvic irradiation or procedures[121].

With any method of enteric drainage, the efferent limb must be placed so as to remove any tension or traction on the bowel anastomosis. By careful positioning, an anastomotic "blow-out" or enteric leak can be averted by preventing bowel angulation just distal to the anastomosis. In addition, it is important close any mesenteric defects and to position the pancreas in such a way that the risk of internal hernia is minimized. Although some surgeons prefer to "wrap" omentum around the bowel anastomosis, we do not advocate this practice because of the concern for liquefaction necrosis that may develop from any fat that comes in direct contact with the pancreas following reperfusion. Fat necrosis may result in peri-pancreatic fluid collections that could subsequently require drainage or become infected.

Alleged gains of pancreas transplantation with portal venous delivery of insulin include immunological, technical, and metabolic, "advantages". However, neither large registry analyses nor prospective cohort studies have been able to corroborate these purported benefits (Table 9)[1,33,39-42,44-46,49-53,112-123]. Conversely, when comparing the three major techniques of pancreas transplantation, there are likewise no well controlled studies to suggest any major drawbacks of portal-enteric *vs* either systemic-bladder or systemic-enteric drainage.

One of most recent and exciting innovations in pancreas transplantation is the advent of laparoscopic pancreas transplantation with robotic support[125-127]. With the da Vinci Robotic system, Boggi *et al*[125] reported the first three whole pancreas transplants performed by using this technology. Their experience constitutes a proof of concept for pancreas transplantation with robotic-assisted laparoscopic surgery. In these cases, enteric drainage of was accomplished using a circular stapler to create an anastomosis between the proximal recipient small bowel and donor duodenum[126]. However, Boggi *et al*[127] have raised concerns regarding the influence of longer warm ischemia duration on viability of the graft because maintaining a cold graft temperature prior to reperfusion is difficult to accomplish laparoscopically. Although several "variations on a theme" exist in the procedural methodology of pancreas transplantation and novel approaches continue to be described, the prevailing viewpoint upholds that the technique with which the individual surgeon feels most confident and comfortable is the best one to be implemented based on donor pancreas quality and recipient anatomic considerations. With improved surgical outcomes over time, exocrine drainage techniques are no longer the "Achilles' heel" of vascularized pancreas transplantation.

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P- Reviewer: Chen ZS, Keller F, Marino IR, Rydzewski A, Salvadori M
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Liu SQ

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DOI: 10.5500/wjt.v6.i2.272

World J Transplant 2016 June 24; 6(2): 272-277
ISSN 2220-3230 (online)

Hepatoduodenal ligament dissection technique during recipient hepatectomy for liver transplantation: How I do it?

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Conflict-of-interest statement: The authors declare no conflict of interest regarding our manuscript.

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Received: October 20, 2015
Peer-review started: October 21, 2015
First decision: December 28, 2015
Revised: April 2, 2016
Accepted: April 14, 2016
Article in press: April 18, 2016
Published online: June 24, 2016

Abstract

Accurate dissection of the hepatoduodenal ligament in the recipient is vital for the success of liver transplantation surgery. High incidence of anatomic variations at the hepatic artery, portal vein and biliary ducts in the hepatoduodenal ligament is well known. Surgical experience is important to be able to foresee the most common anatomic diversities and the possible variations, in order to make a safe and accurate dissection in the hepatic hilum. Before anastomosis, all these hilar structures must be well identified, safely dissected and must also have a sufficient length for the coming implantation process. At the beginning of our program, we were starting the hepatic hilum dissection close to the liver. In time, however, we modified our surgical technique, preferring to start further away from the liver (closer to the duodenum). This length increased progressively over 1500 liver transplantations (80% living donor liver transplantation). During this process, our main purpose was the early control of the hepatic artery (artery first approach). In this paper, our aim is to share our latest version of the hepatoduodenal ligament dissection technique. We also describe alternative approaches used in extraordinary situations.

Key words: Liver transplantation; Living donor liver transplantation; Surgical technique

Core tip: The hepatic artery is one of the main components of the hepatoduodenal ligament and exhibits high anatomic variability, which may change the outcome and success of liver transplantation. In our experience, early control of the hepatic artery (artery first approach) and by the guidance of the hepatic artery, dissection of the rest of the hepatoduodenal ligament components is more practical. In this paper, we share our latest version of the hepatoduodenal ligament dissection technique, developed over the course of 1500 liver transplantations (80% living donor liver transplantation) in our clinic.

dissection technique during recipient hepatectomy for liver transplantation: How I do it? *World J Transplant* 2016; 6(2): 272-277 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/272.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.272

LAPAROTOMY

The Mercedes incision is probably the most widely preferred incision technique for liver transplantation in the world. When we first started performing liver transplantations, we used the Mercedes incision as well. However, we observed a high incidence of incisional hernia with this technique[1]. In time, we reduced the size of the incision and started to use the "reverse L" right upper quadrant incision. Nowadays, we prefer the Mercedes incision only in special occasions (obesity, extensive adhesions due to previous surgery). The extension of "reverse L" incision on the right should extend laterally enough to permit the exposure of the segment Ⅵ of the liver. The tip of the incision on the midline extends up to the xiphoid process, high enough for exposure of the supra-hepatic vena cava. In some selected patients, we performed the liver transplantation only through a supra-umbilical median incision[2]. After laparotomy, the falciform ligament is divided, trimmed and ligated. A sternum lifting mechanical retractor is placed after the suturing of the skin flap to the drape on the right.

MOBILIZATION OF THE LIVER

The left triangular ligament is divided and the gastro-hepatic ligament is examined for an accessory left hepatic artery (HA) arising from the left gastric artery; if there is one, it should first be controlled by a vascular bulldog clamp and then cut close to the liver. We do not prefer to use ligamentum Teres for traction of a cirrhotic liver, which usually tends to bleed from the liver capsule during traction. To achieve better exposure of the hepatoduodenal ligament in a cirrhotic liver, we prefer to first mobilize the right lobe of the liver and place a large piece of gauze behind the liver to move the hepatoduodenal ligament anteriorly. In other words, we position the hepatoduodenal ligament closer to the surgeon. Mobilization of the right liver lobe at the beginning of the procedure provides exposure of the retro-hepatic vena cava at full length. This also allows for total hepatic vascular occlusion when necessary, particularly in emergency conditions. One or two blades of the automatic liver retractors are placed on the visceral surface of the right and/or left lobes of the liver. Then we can easily and clearly expose the hepatoduodenal ligament. A dilated gall bladder may sometimes lay over the hepatoduodenal ligament, preventing good exposure, and its tractions can result in hemorrhage from the liver capsule. In these situations, a partial cholecystectomy may be useful[3].

THE PHILOSOPHY OF THE ARTERY FIRST APPROACH

At the beginning of our liver transplantation program, we began the hepatoduodenal ligament dissection as close as possible to the liver. This was done to avoid injuries to the proximal parts of the components of the hepatoduodenal ligament. However, we experienced some difficulties while working closer to the liver hilum. At first, it was difficult to perform a dissection in such a small area and increased risk of liver capsule bleeding. Secondly, there was difficulty in identifying the arteries from their distal ends, and we observed more intimal injuries during the dissection of these small caliber arteries if there was no proximal vascular control by a vascular bulldog clamp.

HA is one of the three main components of the hepatoduodenal ligament. It has the highest rates of variation, which may change the outcome and success of the liver transplantation. The surgeon performing the recipient hepatectomy is responsible for the protection of all the arteries that may have a potential use during the implantation process. It is obvious that the arteries that must be protected during the dissection are not limited to two (right and left). In every case, five potential arteries (right, left, segment Ⅳ, right HA from the superior mesenteric artery, left HA from the left gastric artery, Figure 1) must be encountered. In our experience, early control of the hepatic artery (artery first approach) and by the guidance of it, dissection of the rest of the hepatoduodenal ligament components is more practical.

STARTING NEAR TO THE DUODENUM

The hepatoduodenal dissection is started just above the duodenal margin (Figure 2). We proceed from laterally to medially with the ligation of the peritoneum and the vessels under the peritoneum (Figures 3 and 4). Trimming of the anterior-inferior leaf of the hepatoduodenal ligament makes it possible to identify the common bile duct with the help of 3 and 9 o'clock vessels. The direction of the dissection is towards the common HA, which is the main point of our hilar dissection at this stage. Above the duodenum, while dissecting medially, the right gastric artery that arises from the hilum and travels to the stomach must be ligated and transected. Inexperienced surgeons may worry about transecting this artery for fear of harming the common HA. However, the right gastric artery is more superficial than the HA, and it runs to the stomach and not into the hepatoduodenal ligament.

LYMPH NODES AS LANDMARKS

On the medial side of the hepatoduodenal ligament, just above the duodenum, the largest lymph node of

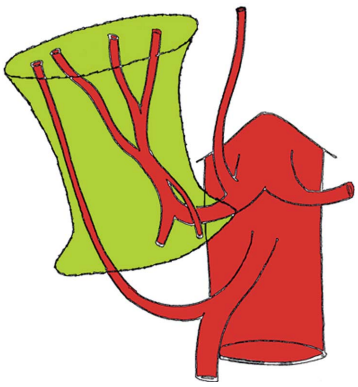

Figure 1 All the potential arterial branches that should be preserved during the recipient hepatectomy.

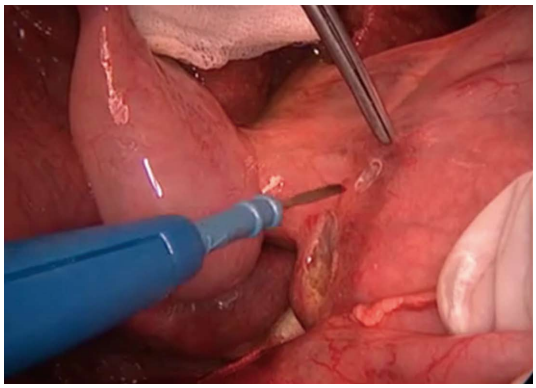

Figure 2 Opening small windows on the peritoneum of the distal hepatoduodenal ligament.

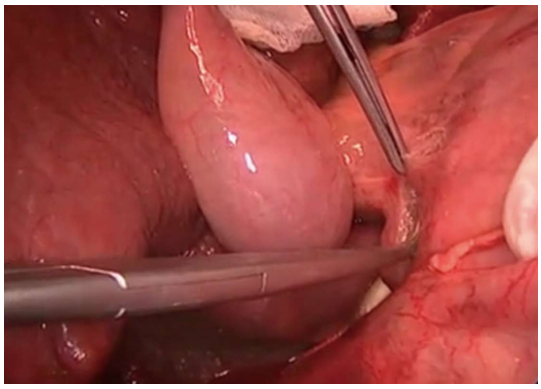

Figure 3 Dissection started just close to the duodenum.

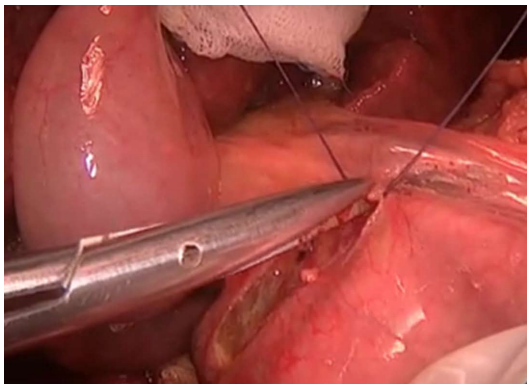

Figure 4 Vessels under the peritoneum were transected after ligation. Avoiding electrocautery at this stage protects the duodenum from thermal injury.

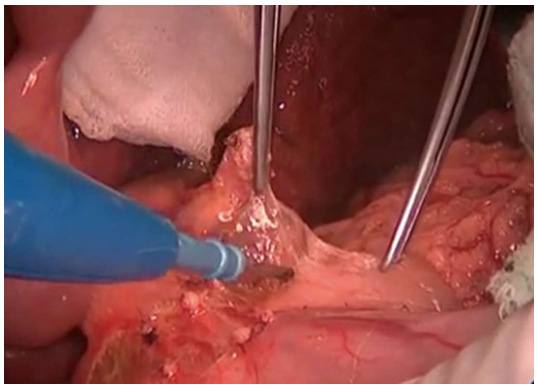

Figure 5 The lymph node located at the infero-medial part of the hepatoduodenal ligament is an important landmark. It locates along the upper border of the pancreas.

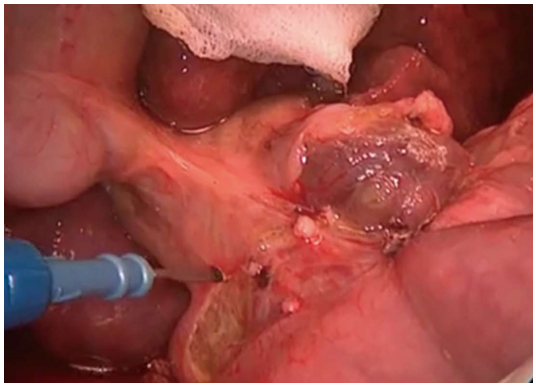

Figure 6 The landmark lymph node is one of the largest lymph nodes of the hepatoduodenal ligament. Its Identification enables to find out the arteria hepatica communis which is just under this lymph node. The dissection is extended to the infero-lateral part of the hepatoduodenal ligament by jumping over the distal common bile duct. Another large counterpart lymph node is exposed near to the distal common bile duct.

the superficial hepatoduodenal lymph nodes can be seen (Figures 5 and 6). This lymph node is located on the trace of the common HA, acting as a landmark for hilar dissection. Once this lymph node is identified, it should be removed carefully after palpation of the common HA under this lymph node. In terms of the newest hemostatic technologies, like Ligasure or Ultrascission[4], we generally prefer to use the suture ligation with low voltage adjusted (25 Watt) monopolar and bipolar electrocautery for hepatoduodenal ligament dissection. Clamps and scissors can also be used for dissection of the common HA to avoid intimal injury due to thermal effects. The common HA is separated from the upper border of the pancreas and completely mobilized. At this stage, the gastroduodenal artery is searched for in the triangle formed by the medial aspect of the distal common bile duct, the trace of the

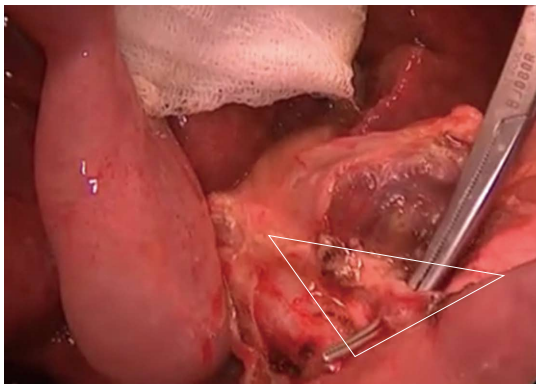

Figure 7 The gastro-duodenal artery is identified in the triangle composed of; distal common bile duct, hepatic artery and upper border of the duodenum.

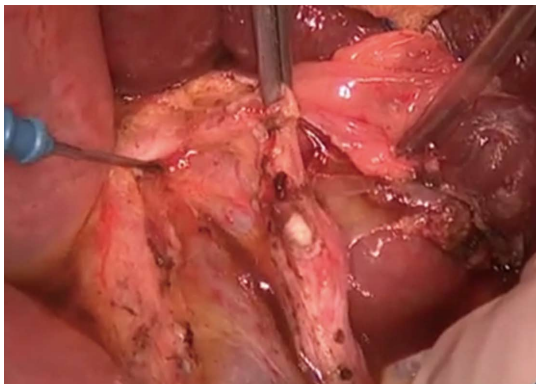

Figure 9 Division of the gastroduodenal artery makes the portal vein visible.

HA, and the upper side of the duodenum (Figure 7). Ligation and transection of the gastroduodenal artery makes the portal vein visible just beneath it (Figures 8 and 9). After this step, the main three components of the hepatoduodenal ligament can be partly identified. We place an atraumatic bulldog clamp to the common HA to decrease the intraluminal pressure in the arterial lumen and to prevent intimal dissection[5] (Figure 10). The gastroduodenal artery is usually divided for several reasons, such as prolonging the HA for a living donor liver transplantation, avoiding steal syndrome through the gastroduodenal artery, and performing an arterial anastomosis to the bifurcation of the gastroduodenal artery and common HA during a deceased liver transplantation.

FOLLOW THE ANTERIOR SIDE OF THE COMMON HA

Generally, there are no main branches arising on the anterior side of the common HA. This knowledge is particularly valuable when dissecting of the hepatoduodenal ligament by the arterial route. Hanging the tissues on the common HA with the help of a right-angle clamp and cutting them *via* electrocautery will

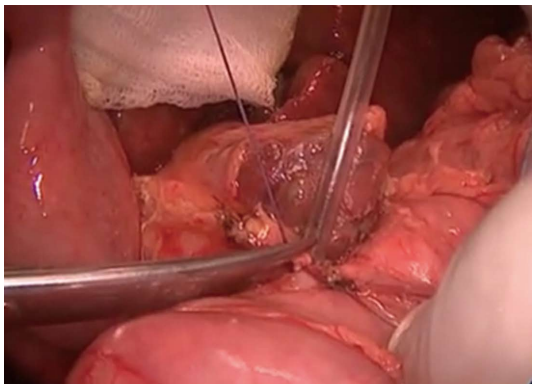

Figure 8 Ligation and transaction of the gastroduodenal artery.

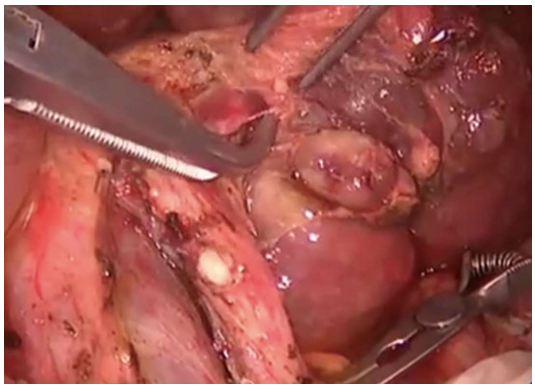

Figure 10 Bulldog clamp applied to the proximal common hepatic artery to prevent the intimal damage in the artery during further dissections.

help in visualizing the distal branches of the proper HA. This dissection meticulously moves toward the bifurcations of the right, left and segment Ⅳ hepatic arteries. There may be some small arterial branches going toward the lymph nodes. However, because the common HA is clamped by a bulldog, these will not cause any major bleedings. Even so, it is advisable to perform careful dissection to prevent hemorrhage. The left HA and segment segment Ⅳ hepatic arteries artery should be followed as far as their entrance into the liver parenchyma. The spatial relationship between the right HA and the bile duct should be evaluated. Generally, the right HA crosses posterior to the common bile duct (Figure 11). However, it sometimes crosses anteriorly and we cut this HA as closely as possible to the liver and continue on to bile duct dissection later. If the right HA is passing posteriorly, then the common bile duct dissection can be started before arterial transections.

BILE DUCTS

The cystic duct is identified, clipped and divided. The common bile duct and ductus choledochus can be identified with the help of the up traction of the cystic duct stump (Figure 12). The lateral side of the common hepatic duct is dissected caudally and cranially.

For better and safer exposure of the extra-hepatic

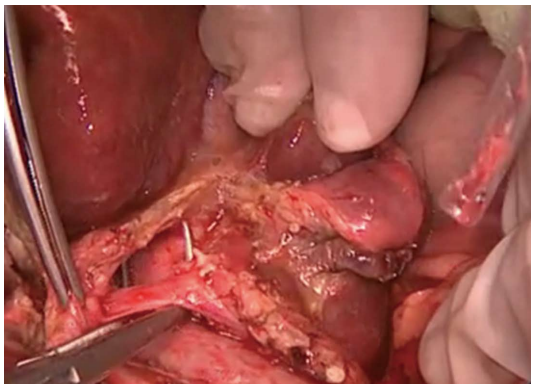

Figure 11 Right hepatic artery is crossing the common bile duct from the posterior.

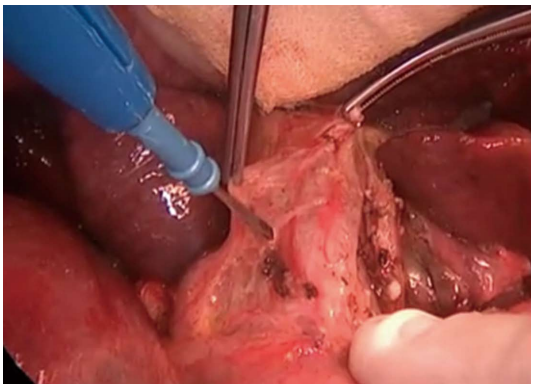

Figure 12 Traction of the cystic duct stump ensures a safer dissection of the lateral border of the common bile duct.

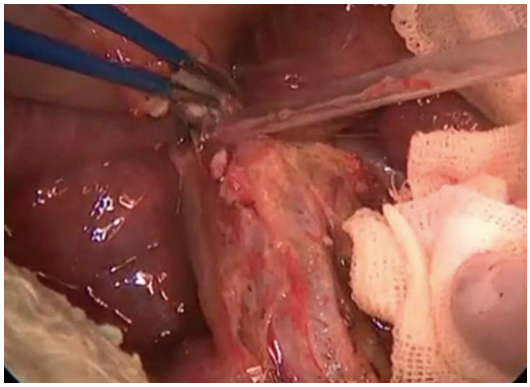

Figure 13 Removing the sheet over the extrahepatic bile ducts provides the identification of the medial and lateral borders of the bile ducts.

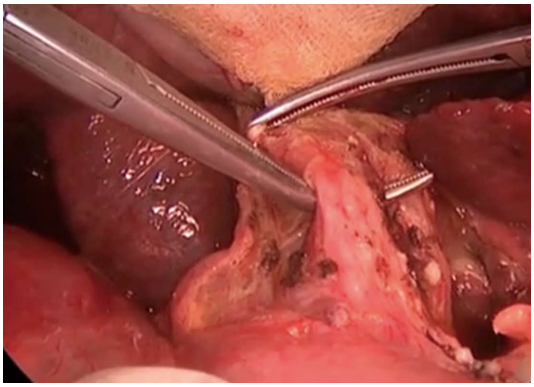

Figure 14 Common bile duct is further liberated and hanged by the right angle clamp.

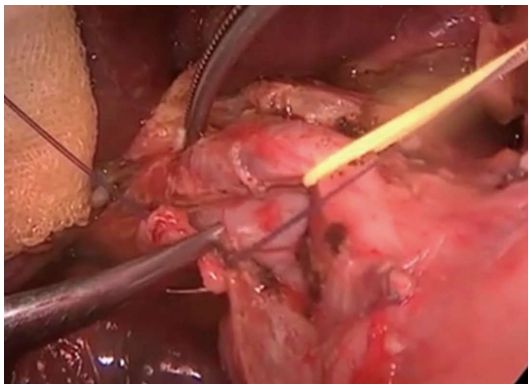

Figure 15 Medial traction applied to the bile duct by a rubber band and the lymphatics are transected. This transaction should be done after being sure that there is no accessory right hepatic artery arising from the superior mesenteric artery.

bile ducts, we peel back the peritoneal sheet that covers the common bile duct, starting from the side of the duodenum and moving to the liver hilum. In our daily surgical practice, we call this "undressing the coat" of the hepatoduodenal ligament. This allows us to safely go underneath the hilar plate that covers the main anatomic contents of the portal hilum. Here, so as not to damage the vascular supply of the choledochus AA and common bile duct, bleeding must be controlled with fine suturing (Figure 13). In this way, the 3 and 9 o'clock arteries become more visible. These are the landmarks for the medial and the lateral margins of the common bile duct. The common bile duct can be lifted using a right-angle clamp (Figure 14). The peri-choledochal plexus supplying the bile ducts from the 3 and 9 o'clock arteries should be preserved. Electrocautery and excessive skeletonization must be avoided here in order to preserve the blood supply of the remnant bile duct, preventing future anastomosis strictures[6]. The remaining tissues along the lateral part of the common bile duct and the portal vein should be divided (Figure 15). However, if there is an accessory right HA arising from the superior mesenteric artery, the surgeon must be careful not to harm it. We prefer to dissect all the lymph nodes around the hepatoduodenal ligament. In this way, the portal vein, bile duct and arteries can be better identified. It also preserves the length of bile duct, arterial branches and the portal vein, which is particularly important for living donor liver transplantations (Figure 16).

ALTERNATIVE APPROACHS

After identification of all hilar contents, we first cut the HA and then the main bile duct as closely as possible to the liver. The portal vein is then cut just before the un-

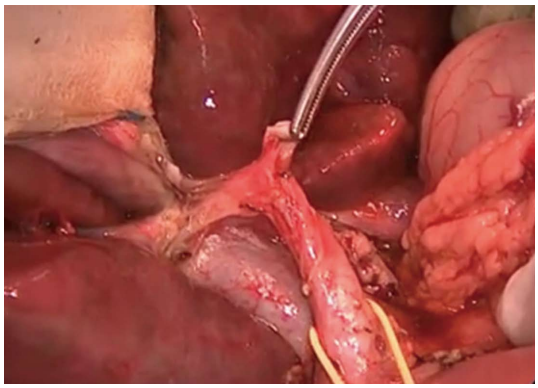

Figure 16 Removing the lymph nodes and the transection of the arteries enables a well visualized and prolonged common bile duct and portal vein.

hepatic phase. In cases in which the hepatoduodenal ligament cannot be dissected easily due to fibrosis or adhesions, we use alternative techniques. In such cases, we perform a double Pringle's maneuver to control the hepatoduodenal ligament, and then resect the ligament together with the contents as closely to the liver as possible. Next, we try to retrospectively identify the contents one by one. Once all of the contents have been identified, the Pringle's maneuvers are released. Also, in cases of portal vein thrombosis, we make available a Foley urinary catheter on the operating table for any unexpected bleeding from the porto-mesenteric veins during endovenectomy[7].

CONCLUSION

Experience and expertise, especially in the surgical field, where many variations can be seen, is extremely important for performing safe and successful dissection. We are still improving our technique for recipient hepatectomy day by day. We hope that these technical details will be helpful to our colleagues dealing with the liver transplantation and hepatobiliary surgery.

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DOI: 10.5500/wjt.v6.i2.278

World J Transplant 2016 June 24; 6(2): 278-290
ISSN 2220-3230 (online)

REVIEW

Liver transplantation and the management of progressive familial intrahepatic cholestasis in children

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Author contributions: All authors contributed to this manuscript.

Conflict-of-interest statement: The authors have indicated they have no potential conflicts of interest to disclose.

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Received: December 14, 2015
Peer-review started: December 18, 2015
First decision: January 18, 2016
Revised: February 24, 2016
Accepted: March 9, 2016
Article in press: March 14 2016
Published online: June 24, 2016

Abstract

Progressive familial intrahepatic cholestasis (PFIC) is a constellation of inherited disorders that result in the impairment of bile flow through the liver that predominantly affects children. The accumulation of bile results in progressive liver damage, and if left untreated leads to end stage liver disease and death. Patients often present with worsening jaundice and pruritis within the first few years of life. Many of these patients will progress to end stage liver disease and require liver transplantation. The role and timing of liver transplantation still remains debated especially in the management of PFIC1. In those patients who are appropriately selected, liver transplantation offers an excellent survival benefit. Appropriate timing and selection of patients for liver transplantation will be discussed, and the short and long term management of patients post liver transplantation will also be described.

Key words: Pediatric liver transplant; Progressive familial intrahepatic cholestasis; Familial intrahepatic cholestasis protein 1; Cholestasis; Multidrug resistance protein 3; Pediatric jaundice; Bile salt excretion protein

Core tip: Progressive familial intrahepatic cholestasis is a rare disorder that predominantly affects young children. If left untreated, children develop debilitating cholestasis and eventually progress to liver failure. Liver transplantation is curative of symptoms related to liver disease but in some cases worsens the extrahepatic symptoms. A multidisciplinary approach is critical to obtaining good long-term outcomes.

Mehl A, Bohorquez H, Serrano MS, Galliano G, Reichman TW. Liver transplantation and the management of progressive

familial intrahepatic cholestasis in children. *World J Transplant* 2016; 6(2): 278-290 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/278.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.278

INTRODUCTION

Cholestasis in children is caused by many different entities. Progressive familial intrahepatic cholestasis (PFIC), which is also referred to as Byler's disease, Byler's syndrome, or Greenland-Eskimo familial cholestasis, is an autosomal recessive inherited disease that disrupts the genes encoding protein transporters responsible for bile formation[1]. These mutant proteins result in the impairment of bile flow through the liver leading to severe intrahepatic cholestasis and progressive chronic liver disease[2]. Recently, mutations in a gene important for the formation of tight junctions was also reported that leads to progressive intrahepatic cholestasis[3].

Familial conditions of cholestasis were first reported in the 1950s with Ahrens *et al*[4] reporting 4 patients with congenital absence of their intrahepatic bile ducts. These patients had persistent jaundice very early in life, severe growth retardation, malabsorption, pruritus and xanthomatosis with marked hypercholesterolemia. Liver biopsies of these patients revealed complete absence of interlobular bile ducts and bile stasis, despite a normal lobular architecture and extra hepatic biliary system. All four of these children died at an early age[4]. Similarly, in 1966, Gray *et al*[5] reported two sisters with jaundice, marked growth retardation, malabsorption, and pruritus. The course was progressive for both sisters and they died before the age 3[5]. Clayton *et al*[6], Juberg *et al*[7], and Sharp *et al*[8] also reported additional cases of children with progressive cholestasis and liver failure resulting in death. Similarities among these early reported cases were described in an early review on PFIC by Ballow *et al*[9] and included: A familial occurrence, a clinical history of fluctuating jaundice, pruritus, malabsorption, growth retardation early in life and hepatosplenomegaly. Similar biochemical findings included conjugated hyperbilirubinemic obstructive cholestasis with normal blood cholesterol levels[9].

SEARCH STRATEGY

A literature search of English language publications from 1990-2014 was used to identity published data on liver transplantation for PFIC using the Patients Intervention Comparator Outcomes outline (Table 1)[10]. Databases searched were PubMed, Ovid MEDLINE, and Cochrane Reviews. Terms used in the search were "liver transplantation" AND one of the following terms "progressive familial intrahepatic cholestasis", "PFIC", "PFIC1", "PFIC2", "PFIC3", "Byler's Syndrome" or "Byler's Disease".

EPIDEMIOLOGY

The incidence of any of the defective genes involved in the development of PFIC is 1:50000-100000 births and has not shown predominance in any specific geographical area[2,11]. However, there have been communities that have noted cohorts of patients including Faeroe Islands, Inuit (Eskimo) Indians (Greenland and Canada), and the Amish[6,12-15]. PFIC is responsible for 10%-15% of cases of neonatal cholestasis syndrome and is one of the leading indications for pediatric liver transplantation[16,17].

PATHOPHYSIOLOGY

Bile formation at the level of the hepatocytes involves active transport of bile salts, phospholipids, and cholesterol from the portal blood at the basolateral membrane. In PFIC, these transporters function abnormally (Figure 1). Bile then flows from the bile canaliculi lined by adjacent hepatocytes into the canals of Hering that are lined on one side by hepatocytes and one side by cholangiocytes. From there, bile drains into the larger bile ductules.

PFIC1

PFIC1 is an autosomal recessive condition. The mutant gene responsible for the disorder is the *ATP8B1* gene encoding the FIC1 protein[18,19]. The gene locus for ATP8B1 is located on chromosome 18 (18q21-22). FIC1 is a member of the type 4 subfamily of P type adenosine triphosphatase transporters and is involved in phospholipid translocation. The protein is located on the canalicular membrane of hepatocytes and facilitates movement of phosphatidylserine and phosphatidylethanolamine from the outer to inner leaflet of the plasma membrane of the hepatocyte. In addition, it helps to protect the membrane from high bile salt concentration in the canalicular lumen[20].

Mutation of this protein significantly impairs bile salt secretion. The exact mechanism for how deficiency of FIC1 leads to cholestasis is not fully understood[1]. Varying severities of PFIC1 are however noted[11].

PFIC2

PFIC2 is caused by mutation of the ATP binding cassette family B member 11 (*ABCB11*) gene encoding the bile salt excretion protein (BSEP) protein. The gene locus is on chromosome 2 (2q24) and is similarly inherited in an autosomal recessive fashion. BSEP, like FIC1, is a transporter protein that is expressed at the canalicular membrane of hepatocytes, and is the primary exporter of bile acids[21]. BSEP malfunction leads to failure of bile salt secretion from hepatocytes into bile canaliculi and accumulation of bile inside the hepatocytes. This results in severe impaired bile flow and hepatocellular damage[1]. On immunohistochemical staining, BSEP is usually not detectable in PFIC2, and if there is any protein present, it is usually non-functional[22-26].

Table 1 Patients Intervention Comparator Outcomes table for assessment of progressive familial intrahepatic cholestasis

P	I	C	O
Pediatric patients with PFIC	Liver transplantation	Biliary diversion and medical management	Patients survival, graft survival, post operative morbidity

PFIC: Progressive familial intrahepatic cholestasis; P: Patients; I: Intervention; C: Comparator; O: Outcomes.

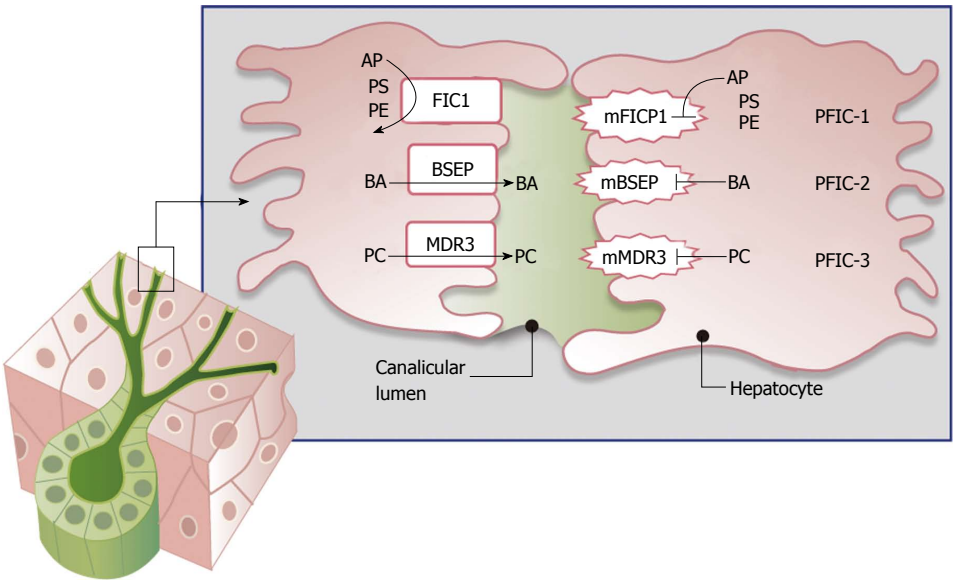

Figure 1 Disruption of bile flow and progressive familial intrahepatic cholestasis. AP: Aminophospholipids; PS: Phosphatidylserine; PE: Phosphatidylethinolamine; BA: Bile acids; PC: Phosphatidylcholine; FIC1: Familial intrahepatic cholestasis protein 1; BSEP: Bile salt exporter pump; MDR3: Multidrug resistance protein 3; mFIC1: Mutant familial intrahepatic cholestasis protein 1; mBSEP: Mutant bile salt exporter pump; mMDR3: Mutant multidrug resistance protein; PFIC: Progressive familial intrahepatic cholestasis.

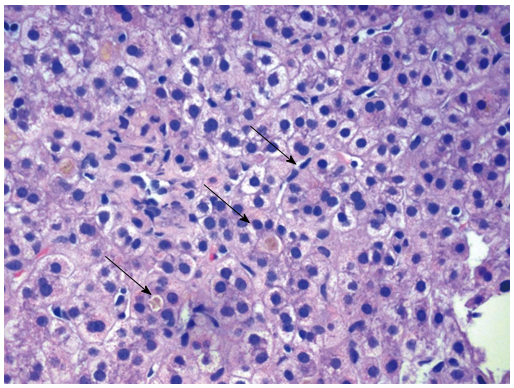

Figure 2 Progressive familial intrahepatic cholestasis type 1 with severe bland lobular cholestasis and lobular disarray. The image shows bile plugging with surrounding pseudorosette formation (arrows). In PFIC1, the canalicular bile is course on electronic microscopy and also referred to as "Byler bile". Thick bile is seen within the pseudorosette here on H and E stain. There is an absence of lobular inflammation and typically no features of neonatal giant cell hepatitis. PFIC: Progressive familial intrahepatic cholestasis.

PFIC3

A mutation in adenosine triphosphate-binding cassette subfamily B member 4 (*ABCB4*) gene encoding the MDR3 protein leads to the development of PFIC3[27,28]. The gene locus is on chromosome 7 (7q21). MDR3 protein is a p-glycoprotein that secretes phospholipids, primarily phosphatidylcholine within bile acid. Dysfunction leads to a decrease in phospholipid excretion[28]. MDR3 defects results in biliary epithelium injury and bile canaliculi injury as well as cholestasis. In addition, there is destabilization of micelles and promotion of cholesterol crystallization that results in increased biliary lithogenicity. This subtype of PFIC is usually present on both alleles and yields complete loss of the MDR3 protein either from a truncated MDR3 from a premature stop codon or missense mutations. All mutations result in severe defective transport of phospholipids and intracellular misprocessing[29].

PFIC4

PFIC4 is a recently described genetic mutation involving the *TJP2* gene that encodes for the tight junction protein 2[3]. TJP2 is a cytosolic protein that interacts with several cytoskeletal proteins and integral membrane proteins and plays an important role in localizing proteins such as Claudins (*e.g.*, CLDN1) to these structures[30]. Patients who presented with PFIC were found to have protein-truncating mutations that resulted in inappropriate localization and disruption of the tight junctions[31].

HISTOLOGIC ALTERATIONS IN PFIC

Even within the different subtypes of PFIC, there are common features and some distinct features. Specific

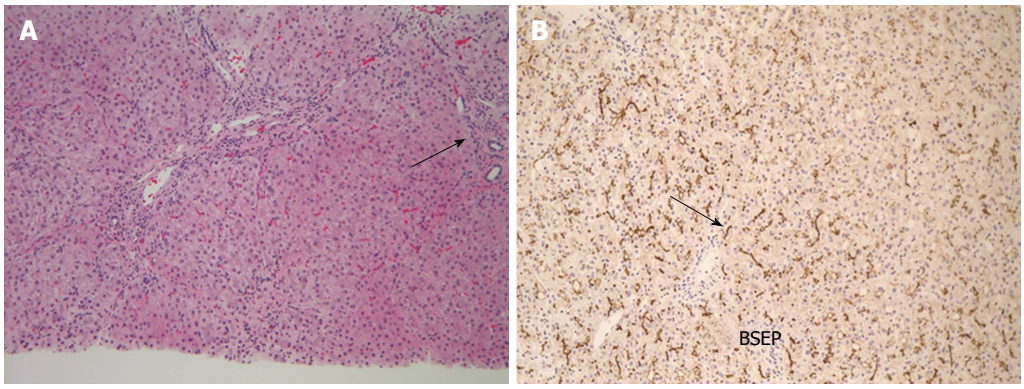

Figure 3 Progressive familial intrahepatic cholestasis type 2 is characterized by mutations in the *ABCB11* gene. A: Patients with progressive familial intrahepatic cholestasis type 2 (PFIC2) can initially present clinically similarly to PFIC1, but with more rapid progression of liver disease. Early on in the disease patients may present with neonatal giant cell hepatitis and lobular inflammation. However, there can be rapid progression with prominent duct reaction and progression to cirrhosis. This figure demonstrates prominent duct reaction in a patient with PFIC2 and advancing fibrosis (arrow). Duct reaction and cholestasis can also occur in patients with extrahepatic biliary obstruction so correlation with clinical findings is required; B: PFIC2 is also called BSEP disease and is characterized by mutations in the *ABCB11* gene. *ABCB11* encodes for the major canalicular bile salt exporter BSEP. Patients with normal BSEP expression show positive immunohistochemistry for BSEP with a canalicular pattern of staining (arrow). In some cases of PFIC2, there is complete lack of staining for BSEP. BSEP: Bile salt exporter pump.

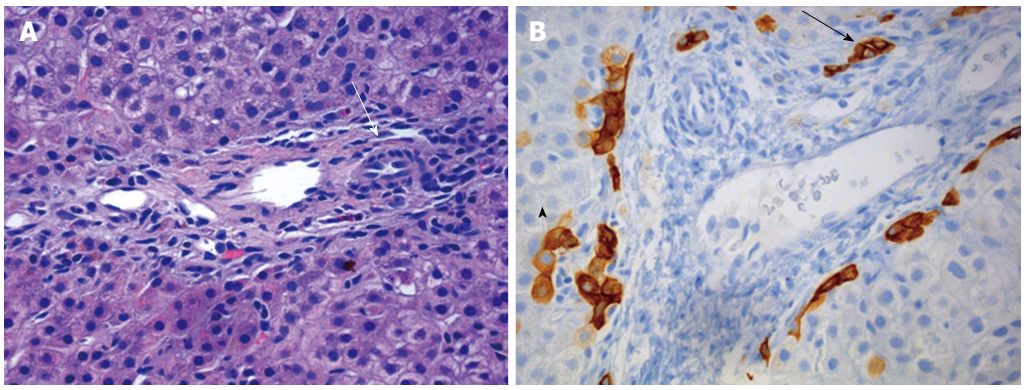

Figure 4 Progressive familial intrahepatic cholestasis type 1 and 2 can also present with duct paucity. A and B: The portal tracts show an absence of bile duct with periportal duct reaction; B: A higher power view of the portal tract with vein on the left artery on the right (arrow) and no appreciable bile duct. Keratin 7 is negative in this portal tract in B and positive in the bile duct reaction (arrow) with some bile duct progenitor cells (paler brown staining arrowhead).

signs on biopsy of PFIC1 (Figure 2) include bland cholestasis, mild lobular fibrosis, and centrilobular canalicular cholestasis with acinar or pseudo rosette formation[1,32]. Early in the disease, the initial biopsy typically demonstrates hypoplastic and threadlike interlobular bile ducts. With progression of the disease, centrilobular hepatocyte loss occurs with resulting pericanilicular and periportal fibrosis. Over time, there is progression to portal-portal and portal-central bridging fibrosis that leads ultimately to micronodular cirrhosis. Interestingly, fibrosis progresses in the absence of significant inflammation and ductular reaction[32].

Findings in the PFIC2 subtype (Figure 3) include cholestasis, giant cell hepatitis, hepatocellular necrosis, portal fibrosis and neonatal giant cell hepatitis with hepatocellular and canalicular cholestasis[32]. The fibrosis begins both in the portal tracts and in centrilobular regions and progresses through a biliary pattern type cirrhosis leading to micro nodular cirrhosis with slight ductular reaction[32]. Both PFIC1 and PFIC2 can show a paucity of bile ducts (Figure 4).

In PFIC3 (Figure 5), there is bile ductular proliferation, inflammatory infiltrate and biliary fibrosis with mild expansion of portal tracts due to a ductular reaction[32]. Canalicular cholestasis is present in centrilobular areas, and biliary/micro nodular cirrhosis supervenes with a biliary halo around cirrhotic nodules. There is also often the presence of ductular reaction and bile plugs[1].

Immunohistochemistry (IHC), electron microscopy (EM) and bile analysis can also provide important information regarding the different subtypes of PFIC. IHC for the different proteins associated with the different PFIC phenotypes is typically performed. However, normal IHC does not necessarily rule out a diagnosis of PFIC since some mutations are solely functional mutations and do not alter protein synthesis or expression[22]. Immunohistochemical stains can be particularly helpful in the identification of the BSEP protein at the canalicular membrane[22]. In PFIC3 patients, canalicular MDR3 immunoreactivity is typically detectable and the diagnosis of PFIC3 requires gene sequencing[24].

EM is also useful in differentiating the different PFIC subtypes. In PFIC1, the EM is coarse and granular in

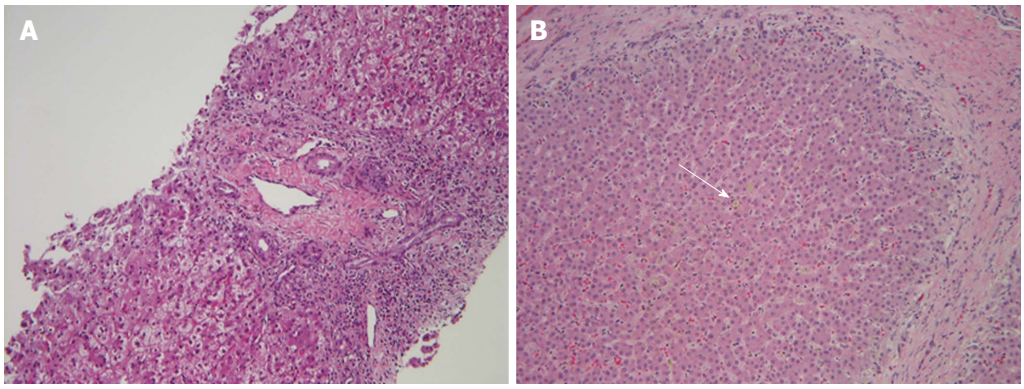

Figure 5 Clinical presentation of progressive familial intrahepatic cholestasis type 3. A: Progressive familial intrahepatic cholestasis type 3 (PFIC3) has a variable clinical presentation and may show nonspecific biliary pattern of injury that can mimic extrahepatic biliary atresia such as bile duct proliferation and cholestasis. In this patient with PFIC3 there is cholestasis, inflammation, and bile duct proliferation; B: Biliary type cirrhosis in a patient with PFIC3 with severe cholestasis (arrow) and micronodular cirrhosis.

appearance that is the characteristic "Byler's bile". In contrast, PFIC2 EM has an amorphous appearance[33,34]. EM findings in PFIC3 patients have not been reported. In PFIC4, EM of the liver tissue of these patients demonstrated elongated tight junctions that lacked the densest part of the zona occludens[3].

CLINICAL PRESENTATION

The hallmark sign and symptom of the disease is jaundice and pruritus. For children and their parents, pruritus is an extremely distressing manifestation of disease and its relief is often the goal of early therapy. Significant pruritus leads to cutaneous mutilation, loss of sleep, irritability, poor attention and impaired school performance. In addition to pruritus, other symptoms include icterus, hepatosplenomegaly, excoriations, hyperpigmentation of the skin, shiny nails, growth retardation, pale stools, and fat malabsorption[1,11]. Most cases of PFIC present in infancy or early childhood with jaundice, and progress rapidly to fibrosis and end-stage liver disease. If left untreated, end stage liver disease will result in death.

There are many similarities and few distinct differences between the different PFIC subtypes[35,36]. Signs specific to PFIC1 include presentation in early infancy as opposed to neonatal period or later in childhood. Foul-smelling, high volume stools and failure to thrive are also hallmarks for PFIC1[35]. Gastrointestinal involvement even after liver transplant with secretory diarrhea can be significant[35,37]. Hemorrhage is also a possible sequelae and is potentiated by vitamin K deficiency and similarly can be the first clinical manifestation[1]. Classic biochemical signs include low or normal gamma-glutamyl transpeptidase (GGT), high alkaline phosphatase and a lower serum albumin as compared to PFIC2. Additionally, there is typically more severe cholestasis and recurrent jaundice, extrahepatic disease and portal hypertension. These sequelae often lead to decompensation in early childhood.

In contrast to PFIC1, PFIC2 tends to present in the neonatal period rather than later in infancy or childhood and tends to progress more rapidly. Biochemically, patients generally have a low or normal GGT, higher serum aminotransferases, higher serum bile acids and higher α-fetoprotein[35]. Patients present with severe cholestasis and persistent jaundice typically within the first month of life. Consistent with the restricted expression of ABCB11 to the liver, there are no extrahepatic manifestations of PFIC2. Progression to end stage liver disease results in portal hypertension and other manifestations of end stage liver disease. PFIC2 tends to progress to end-stage liver disease more rapidly, with cirrhosis, liver failure and death in the first decade of life, most commonly in the first year of life, if a liver transplant is not performed[35].

PFIC3 usually presents in adulthood or late adolescence[38,39]. It is characterized by cholestasis and gastrointestinal bleeds secondary to cirrhosis and portal hypertension. Gastrointestinal bleeding may be the first presenting symptoms in older children or young adults. Biochemically, PFIC3 patients tend to have an elevated GGT. There is also an increased risk of cholesterol and drug induced cholestasis in patients with MDR3 mutations and PFIC3[40,41].

INVESTIGATIONS AND DIFFERENTIAL DIAGNOSIS

Initial investigations of the jaundiced child include a combination of clinical, radiological, and laboratory testing with the goal of ruling out biliary obstruction and extra hepatic causes of jaundice. In addition, infectious or metabolic etiologies should also be ruled out. Important screening and confirmatory laboratory tests include a complete blood count, chemistries including electrolytes, serum glucose, liver enzymes, total and direct bilirubin, GGT, thyroid function studies, C-reactive protein, ferritin, and coagulation studies. In addition to the above labs, serum bile acids, urinary bile acids, lactic acid, alpha-1-antitrypsin phenotype,

Table 2 Review of documented liver transplantation outcomes for progressive familial intrahepatic cholestasis patients (≥ 3 patients)

Ref.	PFIC type	Age at transplant (years old)	Previous management	Graft survival	Patient survival	Notes
Soubrane *et al*[45]	14 "byler disease" PFIC type unspecified	6.5 (0.4-13)	NR	93.3%	92.8%	Consanguineous to the 2nd degree in 8 cases
Emond *et al*[91]	11 PFIC unspecified type	4.6 ± 3.4	2 had previous partial biliary diversion procedures	76.9%	73%	LT performed on those with advanced cirrhosis (6 received diversion procedures only)
Ismail *et al*[80]	8 PFIC of unspecified type	Unknown	1 patient PEBD, all received cholestyramine, phenobarbital, rifampicin, UDCA	100%	85.7%	6 cadaver livers, 2 living donors
Kondo *et al*[63]	4 PFIC of unspecified type	2-7	NR	75%	75%	
Bassas *et al*[56]	5 PFIC3 8 "low GGT PFIC" PFIC1/2	10-40 mo	NR	84.6%	84.6%	Parents of 12 out of 13 were 1st cousins
Cutillo *et al*[57]	6 PFIC1/2 1 PFIC3	4-53 mo	NR	100%	75%	
Englert *et al*[44]	33 patients PFIC2 and 3	Unknown	UDCA 10 of 33 received biliary diversion then LT	100% with prior diversion 89% without prior diversion	100%	
Aydogdu *et al*[52]	10 PFIC1/2 2 PFIC3	43.2 ± 27 mo	UDCA	69.2%	75%	Surviving patients show good quality of life, exacerbation of diarrhea as the exception, mix of LDLT and cadaveric
Hori *et al*[50,51] Miyagawa-Hayashino *et al*[54], Egawa *et al*[53]	11 PFIC1 3 PFIC2	0.6-18.2 years old	Total external biliary diversion performed at time of re-transplantation in one PFIC1 patient	82.4% total graft survival (14/17)	PFIC1 - 90.9% at 5 yr, 72.7% at 10 yr, 54.5% at 15 yr; PFIC2 - 100% at 5 yr	Digestive symptoms in 10 out of 11 PFIC1; 8 out of 11 PFIC1 recipients exhibited steatosis; 9 out of 11 PFIC1 recipients exhibited fibrosis
Kaur *et al*[58]	2 PFIC3 2 PFIC1/2	2, 2.5, 6 and 9 years old males	UDCA, phenobarbital and ondansetron	100%	75%	

LT: Liver transplantation; PEBD: Partial external biliary diversion; UDCA: Ursodeoxycholic acid; PFIC: Progressive familial intrahepatic cholestasis; GGT: Gamma-glutamyl transpeptidase; LDLT: Living donor liver transplantation; NR: Not reported.

alpha-fetoprotein, ammonia, cortisol, viral serologies, carnitine and acyl carnitine profile, and plasma amino acids levels should also be considered[14]. GGT levels not only assist in the differentiation of the type of PFIC, but may also be a helpful prognostic indicator[42]. A serum albumin, which if low, may indicate advanced disease or malnutrition[10]. The presence of coagulopathy may also increase the suspicion of advanced disease[10]. Genetic studies for JAG1 mutations as well as for the described PFIC mutations should also be performed to clarify the etiology of cholestasis. Once a diagnosis of PFIC is made, differentiating between the subtypes, such as PFIC1 and 2 in newborns and young infants, is important since options for optimal treatment may differ between subtypes. Genetic testing is the gold standard for diagnosis using a "gene chip". One chip allows for the analysis of 27 coding regions and their splice junctions from 5 different genes known to be involved in inherited syndromes of intrahepatic cholestasis[43].

In addition to laboratory testing, radiologic investigations are also critical and almost always include an initial abdominal ultrasound. In addition, magnetic resonance cholangiopancreatography can provide additional information especially in older children and help exclude other diagnoses such as primary sclerosing cholangitis that may be high on the differential list particularly in patients with high levels of GGT and cholestasis.

ROLE OF LIVER TRANSPLANTATION

Liver transplantation is currently the only definitive treatment available for PFIC. It corrects the genetic defect and reverses many if not all of the effects of chronic liver disease. Several series have been published examining the outcomes of liver transplantation for PFIC (Table 2). Of the cumulative 131 patients of all subtypes documented, graft survival and patient survival was 76.6% and 85.2% respectively with the

longest reported follow up interval being 19 years post-transplantation.

In the largest series by Englert *et al*[44], 23 patients (PFIC2 or 3) underwent orthotopic liver transplantation as their first line of treatment and 10 received liver transplantation after an initial biliary diversion procedure. The graft survival rate of those who received a liver transplant initially was 89%, whereas graft survival rates of those who first received biliary diversion and subsequent transplantation were 100%. Patient survival between the two groups was 100%[44]. Soubrane *et al*[45] reported similar excellent outcomes. Of the 14 patients transplanted, 13 patients were alive at was an average follow up of 17 mo with normal family life and all children returning to school[45].

Earlier transplantation for PFIC2 appears to be warranted as this subtype appears to progress to cirrhosis faster and also carries an increased risk for the development of primary liver cancers. Hepatoblastoma, hepatocellular carcinoma, and cholangiocarcinoma have all been reported in PFIC2[46-48]. Transplantation in these patients is well tolerated with high graft and patient survival rates as well as great improvements in quality of life. Shimizu *et al*[49] reports two PFIC2 patients that were transplanted prior to the development of end-stage liver disease. Both siblings presented with jaundice and pruritus before 1 year of age. The elder sibling also demonstrated symptoms including acholic stools and failure to thrive. Histopathology revealed the classic findings of PFIC2 but no cirrhotic or malignant changes were identified. Neither sibling experienced major post-surgical complications.

Unlike in PFIC2, early transplantation in PFIC1 is controversial. Although liver transplantation corrects the *FIC1* gene in the liver and theoretically reverses the symptoms related to liver disease, the outcomes post-transplant are mixed. Hori *et al*[50,51] reported one of the largest series for patients that underwent liver transplantation for PFIC1. Eleven PFIC1 patients who received living-donor liver transplants were reported. Post-transplant steatosis was significant (moderate-severe) in 8 of the PFIC1 recipients (72.7%). Four of the 11 recipients eventually showed signs of cirrhosis post-transplant such as esophageal varices and splenomegaly[50]. Two of the 11 PFIC1 patients suffered graft losses, and 10 of 11 patients (90.9%) reported digestive symptoms post liver transplantation. The survival rates of the PFIC1 patients at 5, 10 and 15 years liver transplantation were 90.9%, 72.7% and 54.5% respectively. Additional studies have also highlighted the presence or aggravation of severe digestive symptoms in addition to higher mortality rates following transplantation for PFIC1[52-55]. Therefore, an attempt at medical management of symptoms and/or biliary diversion in PFIC1 patients should be considered prior to transplant. In additions, medical and/or surgical procedures to post liver transplantation should also be considered[52-55].

In addition to considering delaying liver transplantation in PFIC1 patients, the exact mutation specific to the PFIC1 patient may play a role in the development of steatohepatitis in the transplanted liver graft. Three of the 11 patients in this study had distinct mutations in the *FIC1* gene that did not result in persistent post-transplant diarrhea or steatosis[54]. Lykavieris *et al*[37] reported two PFIC1 patients with specific mutations that both resulted in diarrhea exacerbation, appearance of liver steatosis and no catch-up of stature growth at 11 and 7.5 years post-transplant. Nicastro *et al*[55] similarly reported a PFIC1 patient upon whom gene analysis was done and was found to have double heterozygosity for two missense mutations. This mutation was associated with unremitting diarrhea, steatohepatitis and progressive fibrosis.

There is less data reporting on the outcomes of transplantation for patients with PFIC3. In patients that require transplantation, small series have reported excellent graft and patient survival[44,52,56-58]. Like with PFIC2, liver transplantation is curative with resolution of pruritus and other manifestations of chronic liver disease. There are no reported cases of worsening of extrahepatic symptoms. The only post transplantation complications noted specifically for a PFIC3 patient was documented by Kaur *et al*[58] who noted grade 1 acute rejection in 1 post-operative patient. Greater than 80% patient survival rates in the groups that included known PFIC3 transplant recipients have been reported however post-operative quality of life for these patients needs to be further investigated.

In conclusion, liver transplantation is typically viewed as an option when patients have failed medical treatment and/or biliary diversion and have a poor quality of life due to refractory pruritus. Liver transplantation is also considered when patients have end stage liver disease or carcinoma. In regions where wait times potentially are shorter and/or living donation is available, liver transplantation can be considered earlier with excellent long-term survival and quality of life without the need to perform a biliary diversion. However, in cases of PFIC1, liver transplantation can be associated with an increase in extra hepatic manifestations, in particular chronic watery diarrhea and continued growth failure. Transplantation in this setting should be weighed against other options.

LIVING DONOR LIVER TRANSPLANTATION

Living donor liver transplantation (LDLT) has been shown to have outcomes equivalent to deceased donor liver transplantation[59-61]. There is a significant survival advantage to patients transplanted with living donors as compared to those patients on the deceased donor waiting list by preventing death on the waiting list. This can be as high as 20% at some United States centers[62]. In other parts of the world where deceased donation is non-existent, LDLT is the only option for patients with

ESLD. However, given that PFIC is an inherited disease, there was some concern that outcomes post transplant might be compromised when compared with deceased donor grafts from non-related donors. There have been several reports examining outcomes from LDLT that have refuted this notion.

All 13 PFIC patients who received a liver transplantation reported by Bassas *et al*[56] received a living related donor transplant. Eleven of the 13 patients survived and were without complications. The authors commented on the success of the grafts being due to adequate matching and graft size rather than the presence or lack of heterozygosity of gene variants in the donor. Similarly, of the 12 patients reported by Aydogdu *et al*[52], 6 received left lateral segment from living donors. All donors were biological parents. Four of the 6 patients were alive (66.7%) at 1 year follow-up. One patient death was due to hepatic artery thrombosis requiring re-transplantation and subsequent early post-operative death and the other patient developed post-transplant lympho-proliferative disease at 6 mo.

Several other smaller series and case reports have also corroborated these findings[49,57,58,63]. Cutillo *et al*[57] reported 7 PFIC patients who received living related donor transplantation from parental donors. A previous family history of PFIC was found in three families and parental consanguinity in one family. Parental donors had normal liver functions tests and no personal past history of liver disease, gallstones, jaundice or cholestasis of pregnancy. They were alive and well at the time of follow up.

There is a natural concern for living related donor liver transplantation in patients with an inheritable intrahepatic cholestatic disease. However, grafts from related donors do not appear to be at higher risk for failure from PFIC-related causes. Living donation provides an excellent alternative to deceased donation and can provide timely liver transplant to patients.

ADVERSE OUTCOMES FOLLOWING LIVER TRANSPLANTATION

Like liver transplantation for other pediatric disorders, several well known complications have also been recorded such infection and rejection after transplantation for PFIC. These do not appear to occur at increased frequency post-transplant[64]. In addition to the general complications associated with transplantation, there are some that are specifically associated with PFIC.

In patients with PFIC1, an undesired effect of liver transplant is the potential worsening of the extra hepatic manifestations like diarrhea and short stature[52-55]. However, the manifestation and severity of these symptoms is unpredictable[54]. The diarrhea is almost always associated with steatosis on liver histology as well[50]. When these patients are treated with liver transplantation, the impairment of bile salt secretion is corrected, and subsequently, there is a large increase in bile acid secretion relative to what the patient's body is accustomed to. The intestinal manifestations after transplant may reflect an important role for FIC1 in the intestine, where it is highly expressed. This increase in bile acids in the stool causes high volume osmotic diarrhea that has a significant impact on quality of life.

Bile acid resins and partial biliary diversion procedures have been shown to improve these symptoms. Cholestyramine has been reported to be very effective in these patients for managing post-operative diarrhea as well as aiding in overall growth progression[37,50,53,54]. External biliary diversion post-transplantation in patients with PFIC1 who are experiencing an exacerbation of watery diarrhea has also been shown to improve symptoms as well as improve the steatosis on liver histology[55,65].

PFIC2 patients with subtypes that have no immunodetectable BSEP in their native liver also appear to be at risk for the development of recurrent disease[66,67]. Certain patients have developed antibodies against the BSEP protein in the donor liver[66,68,69]. These antibodies cause similar symptoms of cholestasis, steatosis and fibrosis that were present in the original disease process. In some cases, these antibodies have resulted in recurrent graft failure[70]. When allo-antibodies are detected, changes in immunosuppression and implementing plasmapheresis/molecular adsorbent recirculating system therapies have been shown to improve cholestatic episodes post-transplant in some of these PFIC2 patients[70]. The use of rituximab has also been reported and shown to improve symptoms[71].

ALTERNATIVES TO LIVER TRANSPLANTATION: MEDICAL AND SURGICAL THERAPIES

Both medical and surgical therapies play important roles in the management of patients with PFIC both as definitive therapy and as a bridge to transplant. In some cases, they have also been used to manage post-transplant complications.

Medical treatment for portal hypertension includes β-blockers and endoscopic management of esophageal and gastric varices when amenable. Fat-soluble vitamin supplementation and aggressive nutritional support with medium chain triglyceride - rich and high calorie concentrated formulas in infants is also important for the treatment of these patients as well.

Urso-deoxycholic acid (UDCA) increases hepatocyte excretion of bile acids and limits return to the liver by inhibiting their intestinal reabsorption. UDCA has been shown to improve symptoms and liver function tests in some patients with PFIC and is typically viewed as frontline therapy[72-74]. Patients who experience the greatest benefit typically have milder forms of the disease, whereas patients with a total defect in MDR3

tend to be the non-responders to UDCA treatment. Recently, the degree of floppase activity in MDR3 was linked to response to UDCA treatment[39]. In some cases, reversal of fibrosis with long term UDCA therapy has been noted[75]. Combining 4-phenylbutyrate (4-PB) and UDCA treatment together has also been shown to be a promising pre transplant therapy for patients with PFIC2 in an effort to increase BSEP presence at the canalicular membrane[76].

Cholestyramine and rifampicin are also used to provide symptomatic relief. Cholestyramine is a resin that binds bile salts in the intestinal lumen and thus reduces absorption and increases fecal bile salt excretion. Cholestyramine is the first line oral management for pruritus and is effective in up to 80%. Rifampicin aids in the excretion of bile salts and bilirubin in the urine, and aids in the treatment of pruritis.

Recently, Engelmann *et al*[77] documented the use of steroids in PFIC2. These two patients were reported who were incidentally started on steroids for other medical reasons and who subsequently had complete resolution of symptoms and resolution of elevated bile salts.

Biliary diversion procedures

Biliary diversion procedures decrease the enterohepatic circulation of bile reducing its toxic effects. When offered early, biliary diversion is successful in reducing symptoms from pruritus and also slowing the progression of fibrosis[11]. There are both partial external and internal biliary diversions that have been described. Nasobiliary drainage procedures when performed preoperatively can be helpful in the selection of patients that will have the highest success rate from the surgical diversion procedure[17].

Partial external biliary diversion which was first described by Whitington uses a 10-15 cm jejunal conduit between the gallbladder and the abdominal wall creating a permanent biliary stoma[78]. This procedure has been shown to improve growth, normalize liver function, reduce serum bile acids and improve liver histology[79]. In many cases, this procedure is the first line surgical option and should be offered prior to the development of cirrhosis. However, once cirrhosis has been documented, these patients have poorer outcomes and should undergo liver transplantation[80]. Success as documented by not progressing to liver transplantation is reported to be 23%-75%[44,79-83]. This technique is also associated with significant complications including prolapse of the anastomosis, infection, and high volume bile excretion[84]. Additionally, 1/3 of patients experience moderate to severe dehydration and hyponatremia[84]. Modifications of this technique have included the use of a button cholecystostomy and also the use of the appendix in place of the jejunum as a conduit[85,86].

Partial internal biliary diversion has the advantage in that it avoids an external stoma and the complications associated with it. The most common partial internal biliary drainage links the gallbladder drainage to the colon[87-89]. A modification of this procedure involving a laparoscopic cholecystocolostomy has also been described[90]. Initial results from these techniques have been promising, but longer follow-up is needed. Internal diversion to bypass the distal 15% of the small intestine by creating an ileal colonic bypass has also been attempted but outcomes were poor[82].

CONCLUSION

Until more research regarding targeted gene therapies and an increase in the development of the medical management for PFIC, liver transplant remains the most definitive treatment for those with PFIC. However, it is also important to consider current medical therapies and additional surgical interventions like biliary diversion that can potentially create a synergistic outcome. In particular, in patients with PFIC1, often the best clinical outcome and quality of life is an appropriate combination of all three of these therapies. Identification and better understanding of certain mutations in *FIC1* gene might lead to better patient selection. Similarly, in patients with PFIC2, the need for additional medical management can best be determined by pre-operative immunohistochemical studies which can help provide better clinical outcomes. Although the data for liver transplantation for PFIC3 is still lacking, it appears to be the preferred method of treatment with excellent long-term outcomes. There is currently no available clinical data regarding transplantation in the setting of mutations in *TJP2* gene (PFIC4).

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DOI: 10.5500/wjt.v6.i2.291

World J Transplant 2016 June 24; 6(2): 291-305
ISSN 2220-3230 (online)

REVIEW

Massive haemorrhage in liver transplantation: Consequences, prediction and management

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Author contributions: All authors contributed to the conception and design of the review as well as giving final approval of the final version; Cleland S, Corredor C and Ye JJ performed the literature review as well as drafting of initial version and subsequent revisions up to final version; Srinivas C and McCluskey SA provided critical revision and editing of initial and all subsequent versions.

Supported by Department of Anesthesia and Pain Management academic program support.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

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Received: November 10, 2015
Peer-review started: November 12, 2015
First decision: February 2, 2016
Revised: March 22, 2016
Accepted: April 7, 2016
Article in press: April 11, 2016
Published online: June 24, 2016

Abstract

From its inception the success of liver transplantation has been associated with massive blood loss. Massive transfusion is classically defined as > 10 units of red blood cells within 24 h, but describing transfusion rates over a shorter period of time may reduce the potential for survival bias. Both massive haemorrhage and transfusion are associated with increased risk of mortality and morbidity (need for dialysis/surgical site infection) following liver transplantation although causality is difficult to prove due to the observational design of most trials. The blood loss associated with liver transplantation is multifactorial. Portal hypertension secondary to cirrhosis results in extensive collateral circulation, which can bleed during hepatectomy particular if portal pressures are increased. Avoiding volume loading and maintenance of a low central venous pressure together with the use of vasopressors have been shown to reduce blood loss and transfusion during liver transplantation, but may increase the risk of renal impairment post-operatively. Coagulation defects may be present pre-transplant, but haemostasis is often re-balanced due to a deficit in both pro- and anti-coagulation factors. Further derangement of haemostasis may develop in the anhepatic and neohepatic phases due to absent hepatic metabolic function, hyperfibrinolysis and platelet sequestration in the donor liver. Point-of-care tests of coagulation such as the viscoelastic tests rotation thromboelastometry/thromboelastometry allow and more accurate and rapid assessment of these derangements in coagulation and guide the use of factor replacement and antifibrinolytics. Transfusion protocols guided by these tests have been shown to reduce transfusion rates compared with conventional coagulation tests, but have not shown

improvements in mortality or morbidity. Pre-operative factors associated with massive transfusion include previous surgery, re-do transplantation, the aetiology and severity of liver disease. Intra-operatively the use of piggy-back technique and avoiding veno-veno bypass has been shown to reduced blood loss.

Key words: Liver transplantation; Massive transfusion; Coagulopathy

Core tip: The management of bleeding during liver transplantation requires an understanding of the unique coagulopathy of liver failure and the ability to recognize the risk factors for massive transfusion. By avoiding massive haemorrhage and transfusion, patients' outcomes after transplantation are likely to benefit.

Cleland S, Corredor C, Ye JJ, Srinivas C, McCluskey SA. Massive haemorrhage in liver transplantation: Consequences, prediction and management. *World J Transplant* 2016; 6(2): 291-305 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/291.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.291

INTRODUCTION

The first human solid organ transplantation was performed in 1954 when Dr. Joseph Murray led a team in successfully transplanting a kidney between identical twin brothers[1]. Liver transplantation proved far more difficult as patient decompensation was inevitable and the challenges of operating with massive and uncontrollable haemorrhage were substantial[2]. In 1963, Starzl *et al*[3] published the first case series of 3 patients, two of whom died shortly after the procedure and one bleeding to death on the operating table. Throughout the remainder of the 1960's liver transplantation was an experimental procedure with the first survival beyond a year not coming till 1967[4]. Improvements in surgical outcomes became possible with the dramatic improvement in the graft quality due to the acceptance of the concept of brain death[5], and with the introduction an effective immunosuppressive agent, cyclosporine[4,6]. Survival after liver transplantation has steadily improved[7,8], and orthotopic liver transplantation (OLT) is now an accepted treatment of advanced liver failure.

With the expansion of OLT programs in the early 1980's, there was an increasing demand on blood transfusion services. Butler *et al*[9] reported red blood cell (RBC) transfusion rates in the range of 6-254 units per person in the first few years of their programme. With experience the same group was able to reduce their mean RBC, fresh frozen plasma (FFP) and platelet transfusion rates from 40 to 20 units per patient[10], which was comparable with other groups at the time[11]. The reduction in transfusion rates was attributed to improved surgical technique and faster laboratory processing times to allow more rapid diagnosis and treatment of developing coagulopathies[11]. Despite these advances, liver transplant recipients accounted for up to 25% of all the blood transfused in a hospital[10] and had by far the greatest requirement for blood products of any solid organ transplants[12].

Outcomes following liver transplantation have dramatically improved with 5-year graft survival rates in the United States of at least 70% despite transplants being performed on patients with a worse clinical condition due to the Model for End-Stage Liver Disease (MELD) score based organ allocation system[13]. MELD was adapted by the United Network for Organ Sharing from a survival model used for patients undergoing transjugular intrahepatic portosystemic shunts[14,15], and objectively predicts 3-mo mortality and therefore the need for transplantation[16]. There has been an equally impressive decline in blood product use over the same period[17,18] with case series describing OLT without the use of any blood products[19-23]. Yet despite the notable improvements made in the management of blood loss and transfusion there remains a large variability in transfusion practices[24]. This variability in transfusion practice of a precious resource is an important consideration as there may be implications for transplant morbidity and mortality[25-29]. The impact of blood transfusion on surgical outcomes is an area of active debate, but the impact of massive transfusion is more convincing.

Recent reviews have discussed prediction of blood loss during liver transplantation[30,31], and summarised strategies to reduce blood loss[32,33]. This review will focus on massive haemorrhage in liver transplantation including consequence, prediction, and management as well as considering some of the lessons learned from other surgical specialties such as trauma and obstetrics.

DEFINITION

The classical definition of massive haemorrhage is the loss of one blood volume within a 24-h period[34]. Correspondingly massive transfusion in an adult has commonly been defined as 10 or more units of packed red cells in a 24-h period, which approximates to replacement of one blood volume based on the approximate blood volume of a 70-kg male[35].

These definitions are retrospective and often used as the basis for risk prediction models for massive blood loss and the implementation of resuscitative transfusion strategies and protocols. Their use has been questioned particularly in the setting of trauma as it excludes information regarding the patient's condition, institutional transfusion practices and the risk of survival bias as patients who die from exsanguination before receiving 10 units will not be included in the massive transfusion group[36]. Haemorrhage is the main cause

of death following major injury in patients surviving to hospital admission with the highest incidence 1 to 3 h following admission[37]. To address this researchers in trauma suggested more dynamic definitions of massive transfusion including the use of 4 red cell concentrates within one hour with likely on-going need[38], 5-plus units within first four hours of admission[39] or 10 units within 6 h[35]. The PROMPTT trial investigators suggested two different approaches. Rahbar *et al*[35] demonstrated that resuscitation with four or more units (with 1 L crystalloid classed as 1 unit) of fluid within the first 30 min of admission for trauma was significantly associated with 6-h mortality and was a surrogate for sickness in severely bleeding patients. Alternatively Rahbar *et al*[36] using baseline admission characteristics (systolic blood pressure < 90 mmHg, HR > 120 bpm, pH < 7.25 and haemoglobin < 9) were able to develop a latent class model for those at risk of severe haemorrhage and in need a massive transfusion protocol (MTP). The British Committee for Standards in Haematology have suggested a similar dynamic definition as "bleeding which leads to a heart rate of more than 110 beats/min and/or systolic blood pressure less than 90 mmHg"[40] in their most recent guidelines.

In obstetrics massive haemorrhage remains an important cause of morbidity and mortality with 13 death per 100000 maternities in the United Kingdom reported in the most recent confidential enquiry into maternal deaths. Post-partum haemorrhage (PPH) is defined as more than 500 mL from the genital tract within 24 h of birth and subdivided into minor (500-1000 mL), moderate (1000-2000 mL) and severe (> 2000 mL)[41]. These definitions form the basis for activating protocols of resuscitation measures. The Royal College of Obstetrics and Gynaecology state that moderate PPH (1000 mL plus) with on-going bleeding or signs of shock should trigger such measures[41].

Liver transplantation surgery in contrast to trauma and obstetrics is largely an elective or semi-elective procedure where blood loss can be anticipated and a strategized around. Death from exsanguination, common in the early days of transplantation is now a rare event and therefore the traditional definitions of massive haemorrhage/transfusion are less at risk of survivor bias. Defining massive transfusion as 6 unit or more in 24 h has been used in a number risk prediction studies for massive transfusion[42-44].

EPIDEMIOLOGY OF HAEMORRHAGE DURING LIVER TRANSPLANTATION

Liver transplantation requires operating on patients with the pathophysiological changes associated with advanced cirrhotic liver disease. The presence of portal hypertension and the haemostatic changes that occur both as a consequence of hepatocyte death and during the stages of liver transplantation itself are important causes of bleeding that are unique to this procedure.

Portal hypertension

As chronic liver disease progresses hepatocyte death leads to inflammation and the subsequent generation of fibrosis that marks the onset of cirrhosis[45]. Increased intrahepatic vascular resistance (HVR) with maintained portal blood flow requires increased portal pressures. Approximately, 70% of the portal hypertension is attributed to structural factors (fibrosis, vascular re-modelling, vascular occlusion, nodule formation) whilst the remaining 30% is thought to be due to dynamic functional abnormalities in the liver microvasculature[46]. A reduction in intrahepatic vasodilators (of which nitric oxide may be the most important) combined with an increased activity and sensitivity to endogenous vaso-constrictors contribute to the dysfunctional nature of sinusoidal endothelial cells with vasoconstriction of microvasculature and increased HVR[46]. As portal hypertension develops portosystemic collateral vess-els form and blood from the splanchnic circulation is diverted into these collateral vessels[46]. In addition to increased portal blood flow, thinning of arterial walls in these circulatory beds increase the susceptibility for blood loss.

Coagulopathy of liver disease

The liver synthesises most of the circulating proteins of coagulation needed in haemostasis, therefore there is a decreased level of many of these proteins in liver failure[47]. Conventional tests of coagulation are often deranged in advanced liver disease reflecting the deficiency in procoagulant factors. The prothrombin time (PT) and international normalised ratio (INR) are useful markers of hepatic synthetic function. The INR is also used in combination with recipient age, bilirubin and creatinine to calculate the MELD score.

Conventional coagulation tests are, however, poor predicators of peri-procedural bleeding in end-stage liver disease with no increase in bleeding seen in patients undergoing invasive procedures such as cardiac catheterisation[48] or dental extraction[49]. The main source of bleeding seen in liver disease pre-transplant is secondary to variceal haemorrhage, with portal hypertension and splanchnic haemodynamics the proposed mechanism for bleeding rather than coagulopathy.

The haemostasis in liver failure is neither shifted towards bleeding nor thrombosis, but has been referred to as a balanced coagulopathy[50]. Thrombocytopenia and reduced platelet function is offset by elevated levels of von Willebrand factor (vWF) and decreased levels of ADAMTS 13 (a metalloprotease which cleaves vWF)[51]. All pro-coagulant proteins are reduced in hepatic insufficiency with the exception of factor Ⅷ, but so too are the levels of anti-coagulants antithrombin and protein C and S[50]. It has been suggested that the relative excess of plasma coagulation factors in health provides a "margin of safety" to account for physiological or pathological stresses to the system[50]. Without this excess of coagulation factors the balanced coagulopathy of liver failure can be thought of as more susceptible

to the perturbations associated with the perioperative period.

This revised understanding of the coagulopathy of liver failure challenges the ubiquitous use of plasma to correct abnormal blood tests and should focus the use of blood products to manage overt microangiopathic bleeding[2]. In fact, the aggressive correction of derangements in INR without supportive evidence of impaired clotting may not only be unnecessary, but harmful in and of itself. In portal hypertensive rats subjected to a period of haemorrhage, replacing the exact volume lost with blood results in an increase in portal pressures by 20%[52], higher rates of haemorrhage and worse outcome[53]. This has subsequently been demonstrated in patients with severe acute upper GI bleeds. Those treated with a restrictive transfusion strategy had lower portal pressures, lower rates of further bleeding and higher rates of survival compared to those treated with a liberal stratergy[54].

Phases of transplantation

During the pre-anhepatic phase of transplantation the surgeon has to perform a hepatectomy whilst contending with the numerous porto-systemic collaterals and the hyperdynamic, dilated, thin walled splanchnic circulation. Adhesions from previous surgery can be another source of blood loss[2]. During the anhepatic phase, hepatic synthesis and clearance is absent, and hyperfibrinolysis can increase rapidly with the accumulation of tissue plasminogen (t-PA)[55]. Plasma t-PA increases the conversion of plasminogen to plasmin. The end result is that during the anhepatic phase fibrinogen production is stopped and the consumption of fibrin is promoted leading to a rapid consumption of the primary building block of clot formation and increased blood loss[56]. In the neohepatic phase, fibrinolysis is further stimulated by the release of t-PA from the ischaemically injured endothelium of the donor liver[57]. Platelet counts commonly decrease due to sequestration into the sinusoids, extravasation of platelets into disse spaces and phagocytosis by Kupffer cells[55].

CONSEQUENCES OF MASSIVE BLOOD LOSS AND MASSIVE TRANSFUSION

Transfusion of RBCs and blood products has been linked to adverse outcomes in OLT patients[28,58]. Even modest transfusion requirements have been linked to prolonged lengths of hospital stay, with the use of of more than 6 units of red cells having the greater impact in decreased survival rates[44]. de Boer *et al*[59] demonstrated a dose related effect in one year survival rates, with a HR of 1.37 per unit of platelets and 1.07 per unit of packed red blood cells, in their multivariate analysis of a cohort of 433 adult OLT patients.

Both short and long-term survival appears to be affected by intraoperative massive blood transfusion (MBT). Rana *et al*[28] found that an intraoperative blood transfusion of > 28 units was as significant risk factor for decreased 3 mo survival in a study of 233 consecutive liver transplant recipients performed by the same experienced surgeon. Intraoperative blood transfusion greater than 5 units was independently associated with reduced 3 and 5 years survival in a study of 102 living donor liver transplant patients[60].

Observational studies have demonstrated a link between blood loss and transfusion requirements and increased morbidity in OLT patients. Transfusion requirements of > 17.5 packed red cell units and > 3.5 platelet units in a study including 291 consecutive OLT patients were found to accurately predict the requirement for post-transplant renal replacement therapy[29]. Transfusion of > 2 units of packed red cells was identified as a risk factor for development of surgical site infections in liver transplant recipients[61]. Intraoperative blood loss was also found to be the main determinant of early surgical re-intervention after OLT[62].

It is important to highlight that studies investigating outcomes following liver transplantation are limited by their observational nature in that they demonstrate association and not causality between blood loss, transfusion requirements and morbidity and mortality outcomes.

PREDICTION OF MASSIVE TRANSFUSION IN LIVER TRANSPLANTATION

A number of studies have identified factors associated with massive blood loss and transfusion requirements in liver transplant patient populations (Table 1)[42-44,60,63-79]. Risk factors can be classified based on the perioperative period and surgical factors.

Preoperative risk factors

Patient, donor organ or other factors that increase the duration or technical difficulty of the surgical procedure such as previous abdominal surgery[25,60,73,80] or redo transplantation[42] are independently associated with higher blood loss and transfusion requirements. Observational studies suggest that haemostasis, coagulopathy and risk of bleeding differ according to the cause of liver failure. For instance, patients with primary biliary cirrhosis exhibit a preserved capacity for thrombin generation and less fibrinolytic activation during the anhepatic phase compared with other cirrhotic states[81]. Case series of patient with portal vein thrombosis undergoing liver transplantation report greater operation times and consumption of blood products[80,82]. Increasing age of the recipient has been reported as predictor of MBT in a number of studies[42,72,79]. McCluskey *et al*[42] found age to be a weak predictor and the authors remarked that age is likely to be a surrogate for other unidentified risk factors.

Severity indexes of liver disease have been investigated as predictors of blood loss during liver transplant surgery. The Child-Turcotte-Pugh (CTP) score uses

Table 1 Studies evaluating red blood cell transfusion requirements and prediction variables in adult liver transplantation

Ref.	No. of patients	Population	Data methodology	Outcomes	Final model prediction variables	Performance of model
Motschman *et al*[63]	83	OLT	Retrospective univariate and multivariate	RBC transfusion requirement	History of previous GI bleed, Previous RUQ surgery	
Deakin *et al*[64]	300	OLT	Retrospective univariate and stepwise multivariate	⩾ 7 units RBC	Urea levels and platelet count	Specificity 62% Sensitivity 68%
Findlay *et al*[65]	583	OLT	Retrospective univariate and multiple linear regression analysis	RBC transfusion requirement	Age, creatinine and bilirubin	R = 0.22
Steib *et al*[66]	410	OLT	Retrospective univarite and stepwise multivariate analysis	High blood loss ⩾ 12 units RBC	Preoperative Hb, previous abdominal surgery, preoperative FDP	Sensitivity 18% Specificity 98%
Pirat *et al*[67]	40	OLT	Bivariate and multiple linear regression	RBC transfusion requirement	Preoperative albumin	R = 0.48
Ramos *et al*[44]	122	OLT	Univariate and multivariate regression	> 6 units RBC	UNOS class and placement of caval shunt	
Massicotte *et al*[68]	206	OLT	Retrospective univariate and multivariate logistic regression	> 4 units RBC	Starting INR, platelet count and duration of surgery	
Yuasa *et al*[69]	635	LDLT (adult and pediatric)	Univariate	Arbitrary high blood loss quartile (344 ± 272 mL/kg)	Univariate = age < 1 yr, Hct < 30%, T-Bil > 20 mg/dL, BUN > 30 mg/dL. Dx Pre-op atresia, Re transplantation	
McCluskey *et al*[42]	460	OLT	Multivariate regression. Risk index internally validated	> 6 units RBC in 24 h	Age > 40, Hb < 10 g/dL, NR 1.21-1.99 and > 2, platelet < 70, creatinine > 110 mmol/L female and > 120 mmol/L males, albumin < 28 h/L and redo transplant	C statistic model = 0.79
Mangus *et al*[70]	526	OLT "piggy back"	Univariate and multivariate regression	RBC transfusion requirements	Pre-op Hb MELD score, Initial CVP	
Massicotte *et al*[71]	505	OLT	Nomogram risk model based on multivariate regression analysis		FFP transfusion. High starting Hb and phlebotomy protective for blood loss	Bootstrapped AUC prediction model = 89.8%
Araújo *et al*[72]	758	OLT	Retrospective univariate and multivariate regression	RBC requirements	PT, Hb, age, liver malignancy	R = 0.30
Bang *et al*[73]	555	LDLT	Multivariate regression	Intraoperative blood loss > 1000 mL	MELD, albumin, ascites and previous abdominal surgery	
Roullet *et al*[74]	148	OLT	Univariate and multivariate regression	> 8 units RBC and loss of > 1 blood volume	Preoperative Hb and Child-Pugh A protective for blood loss > 1 blood volume	
Esmat Gamil *et al*[43]	286	OLT	Univariate and multivariate logistic regression	> 6 units RBC	INR > 1.6, Ascites	
Li *et al*[60]	181	LDLT	Univariate and multivariate regression	> 6 units RBC	Platelet count < 70 × 10^9/L, Hb < 100 g/L, fibrinogen < 1.5 g/L and previous abdominal surgery	
Wu *et al*[75]	522	LDLT	Univariate and multivariate regression	Re-exploration for hemostasis	> 10 mL/kg FFP transfusion	
Varotti *et al*[76]	219	OLT	Univariate and multivariate regression	RBC transfusion requirements	MELD	
[77]	291	OLT (no malignancy or re-transplant)	Multivariate logistic regression	RBC transfusion requirements	Baseline Hb and Fibrinogen	
De Santis *et al*[78]	166	OLT "piggy back"	Univariate and multivariate regression	Blood product requirements	Child-Pugh, preoperative Hb and INR, graft ischemia time	
Cywinski *et al*[79]	804	OLT	Multivariate regression Bootstrapping for prediction model	RBC and cell saver requirement, > 20 and > 30 RBC units usage	MELD and preoperative platelet count	RBC + CS > 20 units c = 0.70 (RBC + CS > 30 units c = 0.67

OLT: Orthotopic liver transplantation; LDLT: Living donor liver transplantation; RBC: Red blood cells; GI: Gastrointestinal; RUQ: Right upper quadrant; UNOS: United Network for Organ Sharing; INR: International normalized ratio for prothrombin activity; FFP: Fresh frozen plasma; Hct: Hematocrit; Hb: Hemoglobin; PT: Prothrombin time; MELD: Model for End-Stage Liver Disease; CVP: Central venous pressure; AUC: Area under the curves.

levels of serum bilirubin, albumin, PT and the presence of ascites and encephalopathy to quantify of disease severity. Multiple studies have included the CTP score in multivariate analyses of factors associated with increased blood loss during liver transplantation with diverging results[44,60,68,78]. De Santis *et al*[78] found in a population of 166 "piggy-back" OLT that the CTP score together with haemoglobin and graft ischaemia time to be associated with blood and blood products transfusion requirements. A CTP class A was found to be a protective risk factor for bleeding more than one blood volume in a study including 148 OLT patients[74].

Multivariate analysis found an association between pre-operative MELD scores and blood products usage or massive blood loss in different liver transplant patient populations such as hepatitis B related cirrhosis[83], living donor[73], piggyback[70] and mixed OLT populations[76,79]. MELD was significantly associated with patients requiring the use of blood products, but failed to predict those requiring massive blood transfusions[79]. MELD was also a poor predictor of blood loss or blood transfusion requirements in a series of 350 patients with mean MELD scores of 20 ± 10[71]. It is important to note to mention that the reported mean transfusion requirement was only 0.5 ± 1.3 unit which is lower than the reported by other studies in similar populations[76].

Preoperative haemoglobin is an important predictor of blood transfusion in a number of multivariate models[42,60,66,70,72,77,83]. Preoperative haemoglobin of more than 12.6 g/dL was found to be a protective factor for blood loss of one blood volume or more in a series of 148 patients receiving OLT[74]. Thrombocytopenia pre-transplant is also associated with massive blood transfusion requirements[60,80].

Coagulation variables such as the INR and fibrinogen are predictors of blood loss and transfusion requirements. A cut-off INR of $\geqslant$ 1.6 was found to be predictor of > 6 units blood transfusion requirement in an study of 286 patients receiving OLT[43]. Preoperative INR values were also found to be independent predictors of risk for MBT in a study of 460 liver transplant recipients[42]. Fibrinogen levels below 1.5 g/dL were associated with increased risk for transfusion of > 6 units of RBC in living donor related transplant patients[60].

The presence of ascites was found to be predictive of a transfusion requirement of > 6 units RBC[43] and of high intraoperative blood loss (> 1000 mL)[73]. The development of ascites may serve as a marker of portal hypertension with an associated increase in collateral circulation and dilated blood vessels that may be transected during surgical dissection.

Models to improve prediction of blood loss and MBT requirements have been developed from preoperative risk predictor variables that are readily accessible to the clinician during the preoperative assessment. The McCluskey risk index for MBT includes seven preoperative variables: Age > 40 years, haemoglobin concentration ($\leqslant$ 10.0 g/dL), INR 1.2-1.99 and > 2), platelet count $\leqslant$ 70 × 10^9/L), creatinine (> 110 μmol/L for female subjects and > 120 μmol/L for male subjects,) and repeat transplantation. The model was internally validated achieving a high c statistic (0.79)[42]. External validation of the McCluskey index attained reasonable sensitivity (80%) and specificity (84.21%)[84]. However, more recently, Cywinski *et al*[79] also attempted to create a prediction model for intraoperative blood product requirements based on preoperative variables. The authors used several advanced statistical techniques to analyse data from 804 primary OLTs performed during a 9-year period. Although, they found a strong relationship between transfusion and postoperative mortality, the model proved to be an unreliable predictor of transfusion requirements[79].

Surgical factors

Advances in surgical techniques and experience have been crucial for the reduction in blood loss. The piggyback technique involves a single anastomosis of the donor vena cava to the recipient inferior vena cava and a shortened warm ischemic time[85]. Additionally, the preservation of the recipient's vena cava reduces the requirement for extensive resection of the retroperitoneum. Large case series of patients undergoing OLT using the piggyback technique report a reduction in transfusion requirements[86-88] compared with the classic technique or use of veno-venous bypass. Veno-venous bypass has been found to be an independent predictor for increased blood loss and transfusion requirements[44,89]. It is thought that the contact with the bypass circuits triggers fibrinolysis, haemolysis and platelet activation, thus impairing or worsening haemostasis. Despite the encouraging data from case series, a Cochrane review that included two trials with high risk of bias comparing the piggyback with the conventional method of liver transplantation did not find enough evidence to recommend or refute the use of the piggyback method[85].

MANAGEMENT OF MASSIVE BLOOD LOSS

Lessons from the Battlefield

Many of the developments in the management of the exsanguinating patient have come from the trauma literature and the experience gained by treating military casualties in the Iraq and Afghanistan wars. Haemorrhage is the leading cause of death in the first hour following traumatic injury and causes 40% of all trauma deaths[90]. Treatment of massive haemorrhage was historically concerned with restoration of the circulating volume using crystalloids until a transfusion trigger was met (commonly 6 g/L) after which packed red cells were to be given. Both British and American guidelines advised only giving FFP after the loss of approximately one blood volume and aiming for an INR < 1.5[34,91]. Coagulation abnormalities with trauma patients were thought to be as a result of closed head injury or iatro-

genic due to massive blood transfusion or excessive fluid resuscitation. Two papers from 2003 challenged this concept and demonstrated that patients presenting with major trauma commonly had a significant coagulopathy that was present before resuscitation had commenced and was an independent predictor of mortality[92,93]. This coagulopathy was termed acute coagulopathy of trauma.

Acute coagulopathy of trauma is characterised by ooze-type bleeding from mucosal regions, serosal surfaces and vascular access sites distinct from simple massive bleeding[94]. It consists of endogenous primary pathologies - disseminated intravascular coagulation (DIC) and acute coagulopathy trauma shock (ACOTS), and exogenous secondary pathologies that mimic DIC and ACOTS - hypothermia, acidosis, anaemia and dilutional coagulopathies[95]. Similarities between the pathophysiological changes that occur in liver transplantation have been discussed in a recent review on haemostasis in liver transplantation[96]. Derangements in thrombin-thrombomodulin-protein C system lead to anticoagulation in both trauma and liver transplantation patients[96]. Catecholamine release during traumatic injury is thought to directly damage the endothelium resulting in progressive de-endothelialisation. High levels of syndecan-1, a marker of endothelial degradation is association with inflammation, coagulopathy and increased mortality in trauma patients[97], and patients with end-stage liver disease have recently been demonstrated to have significantly higher levels than controls[98]. These levels are further elevated following graft reperfusion during liver transplantation.

MTPs with fixed ratios of red cells to plasma more closely approximating whole blood transfusions came to the fore following a retrospective analysis of United States army combat patients requiring massive transfusion. Those that were treated with a high plasma to RBC ratio had a significantly improved survival to hospital discharge compared with those treated with low ratio transfusion, primarily through decreasing death from haemorrhage[99]. These results led to a proliferation of studies reporting beneficial outcomes from high plasma:RBC ratio MTPs in trauma[100,101] as well as obstetrics[102,103]. Part of the benefit must be attributed to the decreased delay in obtaining blood products and improved communication between the laboratory and the team treating the patient. One criticism of the studies investigating MTPs is that they are largely retrospective before and after studies that are subject to survivor bias. Given the lack of high quality trials the Canadian National Advisory Committee on Blood and Blood products took the decision in 2011 that fixed ratio formula based care could not be recommended as a standard of care[104]. In an attempt to address these concerns two large concurrent prospective multicentre trials have been conducted in severely injured adult civilian trauma patients.

The observational trial PROMMTT, demonstrated reduced 30-d mortality in patients treated with a higher FFP/Platelet to red cell ratio early in resuscitation and went on to inform the design of the randomised control trial PROPPRR[105,106]. Here, while 30-d mortality was not improved in patients treated with a 1:1:1 ratio *vs* 1:1:2 (plasma:platelets:red cells), fewer patients died from exsanguination in the first 24 h[107]. Criticism of the use of fixed ration protocols cite the potential waste of blood products and the one-size fits all approach to massive haemorrhage. MTPs promote the early use of plasma and platelets, which might otherwise be delayed if waiting for conventional laboratory coagulation test results to guide treatment. The increasing availability of point of care (POC) haemostatic tests such as the viscoelastic assays, rotational thromboelastometry (ROTEM™) and thromboelastometry (TEG™), provide an alternative. Tapia *et al*[108] demonstrated that TEG™ guided resuscitation was superior to standardized MTP resuscitation of penetrating trauma patient and Karkouti *et al*[109] were able to demonstrate a significant reduction in transfusion rates for all blood products for patients undergoing cardiac surgery through a ROTEM™ based algorithm. Recent state of the art papers on the management of traumatic haemorrhage have viscoelastic tests integrated into MTPs[38,110-112]. In the presence of uncontrolled haemorrhage, fixed ratio transfusion packages are instigated converting to viscoelastic test-guided goal-driven resuscitation once bleeding slows[110]. While trials comparing fixed ratio-guided resuscitation with viscoelastic test-guided in liver transplantation are lacking it is usually a well-controlled procedure and most centres have access to POC coagulation monitors to guide transfusion, the fixed ration MTP's are possibly only required in the most uncontrolled of settings.

Fluid management

Another strategy to reduce blood loss is fluid restriction similar to liver resection surgery. However, excessive fluid restriction may have deleterious consequences including hemodynamic instability and postoperative renal impairment. Schroeder *et al*[113] conducted a retrospective record review comparing two liver transplant centres using "low" central venous pressure (CVP) (< 5 mmHg) and "normal" CVP (7-10 mmHg) targets during liver transplant. Even though transfusion rates were reduced, increased rates of postoperative renal failure and 30 d mortality were observed in the "low" CVP group.

Reduction of blood loss through maintenance of a low CVP must be balanced against adequate tissue perfusion. Static pressure measurements such as CVP are unreliable indicators of volume status and adequacy of organ perfusion[114]. Dynamic (pulse and stroke volume variation) and thermodynamic (Intrathoracic Blood Volume Index) have demonstrated superior performance compared to static pressure measurements in terms of volume status assessment and preload dependence prediction in critical care and perioperative settings[115]. Studies looking at the performance of dynamic parameters during liver transplant surgery have

produced mixed results[116,117] and their impact on liver transplantation outcomes requires further research.

Vasopressors

A variety of pharmacological agents can produce selective vasoconstriction of the splanchnic vascular bed and reduce portal blood flow. Vasopressin, octreotide and phenylephrine are examples of agents that have been studied as potential interventions for blood loss reduction during OLT. Use of low dose vasopressin (0.04 U/min) infusion during the dissection phase was associated with reduce blood loss compared with control group in a retrospective non randomised study of 110 OLT patients[118].

The effect of an octreotide infusion was studied in a randomised controlled trial of 79 patients undergoing OLT. The study found that an octreotide infusion was associated with an increased urine output during the operation compared to control, but it failed to show any significant difference in terms of blood loss or blood transfusion requirements[119].

Phenylephrine administration was found to be associated with decreased blood loss and lower lactate levels compared to patients receiving inotropes (dobutamine or dopamine) for cardiovascular support during liver transplant[120]. Phenylephrine was also found to be useful in restoring systemic arterial pressure following phlebotomy aimed at reduced portal venous pressure and thus blood loss during the dissection phase of OLT[121].

Transfusion thresholds and coagulation monitoring

There is significant variability among liver transplantation centres in methods of coagulation monitoring, transfusion triggers and transfusion protocols[24]. There is no evidence supporting specific haemoglobin or haematocrit triggers for packed RBC transfusion in OLT. However, data from other surgical and critical care populations indicates that transfusion strategies targeting lower perioperative haemoglobin levels are safe and can lead to a reduction in RBC transfusion. A transfusion threshold of 70 g/L for hemodynamically stable critically ill is suggested by data from the Transfusion Requirements in Critical Care trial[122]. The Transfusion Reduction Threshold Reduction Trial (TITRe2) compared the outcomes of a large population of cardiac surgical patients finding no evidence of harm with the use of a restrictive threshold of 75 g/L compared with a "liberal" threshold of 90 g/L[123]. Similarly, results from a randomized surgical trials of hip surgery patients with pre-existing cardiovascular disease indicate that a restrictive RBC transfusion strategy is not associated with harm[124]. Some guidance can also be extrapolated from a randomize study performed in the setting of severe acute gastrointestinal bleeding excluding massive exsanguinating bleeding, concurrent acute coronary syndrome, stroke or peripheral vascular disease. All patients received endoscopic and treatment for bleeding within 6 h if required. Patients were randomized to a "liberal" RBC transfusion threshold of 90 g/L or "restrictive" of 70 g/L. Thirty-one percent of patients in both groups had cirrhosis and bleeding was due to oesophageal varices in 21% of the patients. The authors observed improved mortality rates, reduced risk of further bleeding, and less complications such as pulmonary oedema, in patients randomised to the restrictive strategy.

There is some evidence that erythrocytes stimulate thrombin generation and play a concentration dependant role in accelerating the initial coagulation reaction[125]. Therefore, higher haemoglobin concentrations may be desirable during acute bleeding associated with hemodynamic instability.

Blood loss during liver transplant surgery can occur in a slow and protracted manner or can be rapid and cause severe hemodynamic instability limiting the applicability of haemoglobin thresholds. During exsanguinating blood loss transfusion should be guided by the rate of bleeding and the likelihood of surgical control: Guided by transfusion indicators and POC testing where possible and guided by fixed ratio transfusion of RBC, plasma and platelets when bleeding is acute and time does not permit real time assessment of the coagulation status.

Viscoelastic tests of coagulation (TEG™, ROTEM™) provide a dynamic picture of the interaction of the whole blood coagulation and fibrinolytic systems. Viscoelastic methods have faster turnaround times compared to traditional tests and are POC or bedside tests, performed in close proximity to the patient in the operating room or critical care areas.

The use of POC viscoelastic methods of coagulation monitoring and their inclusion in blood and blood products transfusion algorithms has been found to be associated with reduced blood and blood products requirements in cardiac surgery[126]. A Cochrane review including 9 RCTs concluded that the use of ROTEM™ or TEG™ to guide transfusion strategies in patients with massive bleeding appears to reduce the amount of bleeding and requirement for blood and blood products, but found no evidence of benefit in terms of morbidity and mortality[127].

Another Cochrane review studying interventions to reduce blood loss in liver transplantation analysed two randomised studies using thromboelastography in liver transplant populations[128]. The studies were both single centre and included a population of adults undergoing OLT[129,130]. The authors concluded that thromboelastography-guided transfusion was associated with a reduction in FFP transfusion requirements but had no impact on 3-year survival rates, RBC or platelet transfusion requirements. The trials were however deemed to have a high risk of bias by the Cochrane reviewers.

Viscoelastic tests can detect the presence and degree of fibrinolysis at different stages of the transplant procedure and can be used effectively to guide the need

for and response to anti-fibrinolytic therapy[131].

Antifibrinolytics

There are 2 major classifications of antifibrinolytic agents, the lysine analogues [aminocaproic acid, Amicar and cyclokapron, tranexamic acid (TXA)], and the trypsin inhibitor (aprotinin, Trasylol). Hyperfibrinolysis may lead to significant blood loss due to diffuse microvascular bleeding, however, much of the fibrinolysis is self-limiting which might help to explain why our ability to predict massive transfusion is difficult and it calls into question the routine prophylactic use of antifibrinolytic therapy. In most circumstances the risk of thromboembolic complications with an antifibrinolytic is low providing an excellent therapeutic index, but in liver failure our inability to identify thromboembolic risk is also limited[132] and therefore the judicious use of these agents is recommended. Patients with a prothrombotic state, such as primary biliary cirrhosis, primary sclerosing cholangitis, hepatocellular carcinoma, portal vein thrombosis and Budd-Chiari syndrome, may be at particularly increased risk of thromboembolic complications.

In 1987 Royston demonstrated a dramatic reduction in blood loss with aprotinin in patients under undergoing repeat open heart surgery and its use in cardiac surgery was approved by the Food and Drug Administration in 1993. Concerns regarding an increased risk of renal dysfunction were raised in several observational trials[133,134]. The publication of the Blood Conservation Using Antifibrinolytics in a Randomized Trial (BART) trial raised additional concerns where patients undergoing high risk cardiac surgery were shown to have a significantly higher 30-d mortality when given aprotinin *vs* tranexamic acid or aminocaproic acid[135] led to its licence being withdrawn in a number of countries. A number of concerns regarding the methodology of the BART trial have subsequently been raised and a review by Health Canada found that the trial was too small to reliably assess mortality and concluded that the benefits of aprotinin outweighed it's risks[136]. Studies investigating the aprotinin ban on blood loss in liver transplantation give mixed results with both an increase in blood transfusion rates following its withdrawal[137] and no change[138] being reported.

Several systematic reviews have investigated the use of antifibrinolytics in liver surgery. A recent Cochrane review focused on methods to decrease blood loss and transfusion requirements in liver resection surgery including 33 trials involving 1913 patients with interventions comparing aprotinin *vs* control, TXA *vs* control and TXA *vs* aprotinin[139]. There was no significant difference in 60-d mortality or thromboembolic episodes and while aprotinin was associated with a significantly lower allogenic blood transfusion requirements, it did not confer any outcome benefit. Importantly, the reviewers deemed all the trials to have high risk of bias thus further weakening the strength of the conclusions[139].

In liver transplantation recipients a systematic review and meta-analysis of 23 studies including 1407 patients analysed the effect of either TXA or aprotinin on blood loss, transfusion requirements and incidence of thromboembolic[132]. Blood loss and transfusion requirements were lower with TXA compared to controls, but the thromboembolic risk was unchanged in groups of patient receiving anti-fibrinolytic therapy[132].

In OLT, thromboembolic events are relatively rare and as such trials studying TXA lack statistical power to detect clinically significant important increases on thromboembolic risk[140]. However, it would be prudent to treat with TXA only in presence of fibrinolysis, observed clinically as microvascular bleeding or evidenced by POC test such as TEG™ or ROTEM™. Routine used is no longer recommended in international guidelines[141] and should be carefully considered in patients at risk of thromboembolic complications.

Cell salvage

Intraoperative cell salvage has been adopted in a variety of surgical settings in an effort to reduce allogeneic blood transfusion rates and thus potential complications and cost associated with the transfusion of allogeneic blood[142]. Controversy exists surrounding the use of cell salvage in liver transplantation. The washed RBCs are devoid of clotting factors and platelets and there is potential for accumulation of fibrinolytic factors released by the processed RBC or the transplanted liver. Older studies appeared to substantiate these concerns suggesting that transfusion of salvaged blood was associated with increase blood loss and requirement for blood products[143]. The cost effectiveness of cell salvage has also being questioned[144]. More recent studies have demonstrated the efficacy of cell salvage in reducing the need for allogeneic blood transfusion for both OLT[145] and living donor liver transplantation[146]. The cost effectiveness of cell salvage was also established in a large prospective study including 660 liver transplant patients where a total cost saving of $188618 United States dollars was achieved over the study period[147].

Malignant disease is a relative contraindication for cell salvage due to the risk of metastasis arising from cancerous cells that are not eliminated by the cell salvage process. Intraoperative cell salvage has however been used in the setting of hepatocellular carcinoma with no apparent increase in recurrence rates[148]. Leucocyte depletion filters incorporated into cell salvage circuits have shown to effectively remove malignant cells when used during liver transplantation of patients with non-ruptured hepatocellular tumours[149].

Bacteria can contaminate salvaged red cells when suctioned blood is mixed with biliary, bowel secretions or is in contact with the skin. A study analysing bacterial contamination of salvaged blood during liver transplant found that even though micro-organisms can be observed in to up to 70% of the processed and reinfused units, none of the postoperative blood cultures revealed growth of the same micro-organisms[150]. It is however, advisable to avoid aspiration of blood after initiation

of the biliary anastomosis stage of the liver transplant procedure.

CONCLUSION

The management of bleeding associated with liver transplantation remains an important area of investigation and no one change in clinical practice will have a dramatic impact. What is required is a concerted effort including the identification of patients at risk for massive blood loss, POC evaluation of medically manageable bleeding, and cost effective blood conservation strategies designed specifically for each patient. The beneficiaries of our efforts will be the transplant recipients in prolonged disease free survival and our health care systems in reduce cost per patient by both reducing blood product utilization and hospital length of stay.

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DOI: 10.5500/wjt.v6.i2.306

World J Transplant 2016 June 24; 6(2): 306-313
ISSN 2220-3230 (online)

Loco-regional therapies for patients with hepatocellular carcinoma awaiting liver transplantation: Selecting an optimal therapy

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Author contributions: Byrne TJ and Rakela J contributed equally to the work; Byrne TJ and Rakela J conceptualized the review; Byrne TJ drafted the original manuscript; both authors reviewed and approved the final manuscript as submitted.

Conflict-of-interest statement: The authors declare no conflicts of interest regarding this manuscript.

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Received: October 19, 2015
Peer-review started: October 21, 2015
First decision: December 28, 2015
Revised: February 2, 2016
Accepted: March 22, 2016
Article in press: March 23, 2016
Published online: June 24, 2016

Abstract

Hepatocellular carcinoma (HCC) is a common, increasingly prevalent malignancy. For all but the smallest lesions, surgical removal of cancer *via* resection or liver transplantation (LT) is considered the most feasible pathway to cure. Resection - even with favorable survival - is associated with a fairly high rate of recurrence, perhaps since most HCCs occur in the setting of cirrhosis. LT offers the advantage of removing not only the cancer but the diseased liver from which the cancer has arisen, and LT outperforms resection for survival with selected patients. Since time waiting for LT is time during which HCC can progress, loco-regional therapy (LRT) is widely employed by transplant centers. The purpose of LRT is either to bridge patients to LT by preventing progression and waitlist dropout, or to downstage patients who slightly exceed standard eligibility criteria initially but can fall within it after treatment. Transarterial chemoembolization and radiofrequency ablation have been the most widely utilized LRTs to date, with favorable efficacy and safety as a bridge to LT (and for the former, as a downstaging modality). The list of potentially effective LRTs has expanded in recent years, and includes transarterial chemoembolization with drug-eluting beads, radioembolization and novel forms of extracorporal therapy. Herein we appraise the various LRT modalities for HCC, and their potential roles in specific clinical scenarios in patients awaiting LT.

Key words: Liver transplantation; Loco-regional therapy; Transarterial chemoembolization; Radioembolization; Hepatocellular carcinoma

Core tip: Hepatocellular carcinoma has increased in incidence in recent decades. Liver transplantation is an excellent therapy for carefully selected patients. Due to the risk of tumor progression while awaiting liver

transplantation, loco-regional therapy is frequently used in this setting. An expanding array of treatment options exist and are herein characterized, including descriptions of which modality may be ideal in various settings.

Byrne TJ, Rakela J. Loco-regional therapies for patients with hepatocellular carcinoma awaiting liver transplantation: Selecting an optimal therapy. *World J Transplant* 2016; 6(2): 306-313 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/306.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.306

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common human malignancy and the third leading cause of cancer-related death[1,2]. Driven largely by the hepatitis C virus (HCV) epidemic, the age-adjusted incidence of HCC in developed nations has approximately tripled since the early 1970's[3]. Cirrhosis is the major risk factor in HCC formation and is present in the vast majority of cases.

Therapy for HCC has evolved during recent decades. While some small HCCs may be fully eradicated with percutaneous ablation[4], surgery with resection or liver transplantation (LT) is considered the only curative option in most situations. That cirrhosis is present in the majority of patients diagnosed with HCC may explain this, since localized ablation would not address the diseased non-cancerous liver which still harbors the potential for hepatocarcinogenesis.

Resection and LT both achieve favorable survival in selected patients with early-stage and/or unifocal HCC[5,6]. However, a review of a large North American cohort (> 20000) of liver cancer patients using the Surveillance, Epidemiology and End Results 1973-2003 database showed a dramatically superior actuarial survival for LT compared to resection or ablation[7]. Resection is associated with a relatively high rate of recurrence[5], with 3-year recurrence frequency above 60% in some series[8]. Recurrence of HCC following resection - at least in cirrhotic patients - is due to de-novo hepatocarcinogenesis in the diseased remnant liver and/or unseen micrometastases. The rationale for LT in the setting of HCC is that it removes not only the cancer but the diseased (and cancer-promoting) liver parenchyma surrounding the tumor(s).

EXPERIENCE WITH LT FOR HCC

Initial experience with LT for HCC as reported in early series was extremely poor[9,10]. Such was the pessimism regarding LT for liver cancer that in many centers HCC was considered a contraindication to transplant. In this era there were no standardized transplant eligibility criteria based on tumor size or number, and imaging ability was limited compared to today. Thus the poor outcomes were likely related to the inclusion of patients with large and/or multifocal tumors, with correspondingly high rates of HCC recurrence after LT. HCC recurrence itself is a leading cause of mortality in this patient population.

Despite the disappointing early experience, there was simultaneous awareness that patients who had small, incidental HCCs found at explant tended to have low rates of recurrence with favorable long-term survival after LT[11]. This in turn led to consideration of LT in patients with limited tumor burden. In 1996 Mazzaferro published his landmark series demonstrating that patients whose pre-LT tumor burden was limited to a single lesion $\leqslant$ 5 cm, or 2 to 3 lesions each $\leqslant$ 3 cm, enjoyed excellent disease-free survival after LT (> 80% at 4 years)[12]. These size parameters have become known as the "Milan criteria" and are widely endorsed as the most common eligibility criteria for LT among patients with HCC.

TUMOR PROGRESSION ON THE TRANSPLANT WAITING LIST

In the United States organ transplantation is regulated by the United Network for Organ Sharing (UNOS). By UNOS classification the Milan criteria include stage T1 (1 tumor < 2 cm) and stage T2 (1 tumor 2-5 cm or 2-3 tumors $\leqslant$ 3 cm). Current UNOS policy allows patients with Milan T2 to receive priority listing for LT[13]. Historically, however, HCC patients pursuing LT still face reduced survival by intention-to-treat analysis[14]. This is due to tumor progression while awaiting LT, resulting in waitlist dropout. For waiting times up to 1 year, historical dropout rates of 10%-30% are encountered, with 5-year survival reduced by as much as 20%[14]. In some UNOS regions, expected waiting time for priority-listed HCC patients exceeds 1 year.

Neo-adjuvant loco-regional therapy (LRT) for HCC is widely utilized by transplant centers internationally. The specific types of LRT available for use have expanded in the last decade, and are discussed later in this manuscript. For patients meeting Milan criteria, the intent of LRT is to serve as bridging therapy to LT by preventing tumor progression and waitlist dropout. For another group of patients who exceed Milan criteria, but fall within expanded criteria allowing a cumulative total diameter for all lesions $\leqslant$ 8 cm, the intent of LRT is "downstaging". Successful downstaging implies that LRT has resulted in tumor shrinkage and/or devitalization (tumors no longer exhibit arterial phase enhancement on imaging), such that upon re-measuring the active tumor burden at some future time point after LRT, the patient falls within Milan criteria.

Advocates of these expanded downstaging criteria - particularly Yao and colleagues at the University of California San Francisco (UCSF) - have reported favorable outcomes for successfully downstaged patients, with a recent paper showing a 56.1% 5-year intention-

to-treat survival for 64 patients assigned to downstaging, not statistically different from a 63.3% 5-year intention-to-treat survival in 488 patients with Milan stage T2[13]. However, expanded downstaging criteria have not been universally accepted and remain controversial in the face of already-present severe organ shortage.

LRT FOR HCC PATIENTS AWAITING TRANSPLANT

To date a post-transplant survival advantage for LRT prior to LT has not been definitively proven[15,16]. However, given what is known about the risk of waitlist dropout, a randomized controlled trial comparing LRT to no LRT in patients awaiting transplant may be difficult to justify. An emerging concept is that tumor biology - as observed by imaging over time - is a more useful surrogate marker of tumor biology than size and number based on an initial imaging study. Patients with HCCs that display radiographic progression over relatively short time periods such as 3-6 mo - without LRT or despite it - are more likely to possess cancers that are inherently aggressive. Such patients are more likely to experience tumor recurrence and diminished survival after LT[17].

Favorable response to LRT - whether used as bridging therapy for Milan criteria, or with downstaging intent for expanded criteria patients - has thus been proposed as a surrogate marker of more favorable tumor biology[13,18-20]. In this paradigm, a mandatory waiting period of 3-6 mo after LRT is required before LT can be offered, in order to observe tumor response to LRT. Presumably, patients whose cancer progressed during the observation period - despite LRT - would not be offered LT. This strategy has been termed "ablate and wait"[21]. The expanded downstaging criteria used and advocated by UCSF requires a minimum 3 mo waiting period after LRT before LT can occur[13], and some UNOS regions (including Region 5 within which UCSF resides) impose a 6-mo delay of the assignment of priority points for listing of Milan stage T2 patients, in order to observe tumor behavior and response to LRT.

A number of different LRT options exist. Transarterial chemoembolization (TACE) and radiofrequency ablation (RFA) have historically been the first and second most commonly utilized neo-adjuvant treatments before LT, respectively[15]. TACE using drug-eluting beads (DEBs) - DEB-TACE - has become more widespread in recent years[22]. Percutaneous ethanol ablation - once common for small tumors - and cryotherapy have declined markedly in use and are not further described here. Other forms of LRT include radioembolization with Yttrium-90 (Y-90), for which emerging literature suggests a favorable efficacy and tolerance[23], and a novel mode of radiation therapy which may be effective as bridging therapy to transplant[24]. The remainder of the manuscript appraises the types of LRT being used as neo-adjuvant therapy before LT, as well as their respective efficacies and roles in various clinical situations.

INTRA-ARTERIAL CHEMOTHERAPY

Traditional TACE involves catheterization - as selectively as possible - of the artery branch(es) supplying the tumor(s) with blood, followed by the infusion of liquid chemotherapy agents into the branch(es). Specific chemotherapy agents different across institutions, but often a mixture of doxorubicin, cisplatin and mitomycin-C is delivered. The liquid chemotherapy is often pre-mixed with ethiodized oil, which serves as both a drug-delivery vehicle as well as a radiopaque marker of where in the liver the mixture has been delivered[25]. The oily nature of the emulsion itself contributes to embolization effect on small vessels, though transiently so. Many centers add embolic particles either to the oily emulsion or as a separate infusion immediately following release of the emulsion[26]. Embolic agents include polyvinyl alcohol particles or Gelfoam. The duration of arterial occlusion is shorter with Gelfoam, with recannulization of flow occurring in about 2 wk. The intended duration of arterial occlusion is not permanent since this would interfere with future chemoembolization if it became clinically desirable. The combination of cytotoxic chemotherapy and embolization achieves varying degrees of tumor necrosis[26,27], but achieving even complete necrosis has not necessarily been predictive of post-LT survival[16].

The outcome of TACE must be assessed with two questions in mind. First, does TACE prior to LT improve survival after LT? And second, is TACE effective as a bridge to LT by preventing tumor progression and waitlist dropout. Both questions are problematic. As mentioned previously, there have been no large prospective trials comparing LRT to no-LRT in patients with HCC awaiting LT. And the evidence to date for pre-transplant TACE does not establish a clear post-transplant survival benefit. The waiting time to LT varies across regions, and a very short duration from TACE to LT does not allow sufficient time for observation of tumor behavior. This in turn will lead to some patients with biologically unfavorable tumors proceeding to LT, likely contributing to increased HCC recurrence and reduced survival. Those limitations notwithstanding, it does appear from a number of studies that TACE is associated with waitlist dropout rates of 3%-13%[18,20,28,29], which is lower than expected based on historical data[14] and supports TACE as an effective bridge to LT. TACE also has a favorable safety profile, and in the case of inoperable disease (non-transplant candidates), is associated with improved survival *vs* supportive care[30].

DEB-TACE is similar to traditional TACE as an intra-arterial therapy for HCC administered selectively in the hepatic arterial circulation. The beads themselves are microspheres impregnated with a chemotherapeutic substance (most commonly doxorubicin), ranging in size from 100 to 700 μm. The amount of delivered doxorubicin is typically 100-150 mg/session[22]. The

proposed advantage of DEB-TACE *vs* traditional TACE is a more concentrated delivery of chemotherapy in the targeted area, and for a longer duration, since traditional TACE results in a more transient drug concentration. This is because there is a delay from release of the oily therapeutic solution and the actual embolization in traditional TACE, causing some release into the systemic circulation (with systemic toxicities, and diminished activity at the intended tumoral site)[31].

The safety of DEB-TACE has been validated in large studies as at least comparable to traditional TACE[31], and the PRECISION-V study showed a statistically significant lower incidence of alopecia, degree of post-treatment aminotransferase elevation, and frequency of decreased left ventricular function with DEB-TACE *vs* conventional TACE[22]. In clinical practice, since there is less induced arterial ischemia with DEB-TACE compared to conventional TACE, the former is an attractive consideration in patients with partially or completely thrombosed portal vein branches, since such patients may not tolerate a new, substantial arterial ischemia. For the same reason, many groups favor DEB-TACE for patients with worse liver function at baseline. In terms of efficacy and survival, there is insufficient data to claim that either TACE or DEB-TACE clearly outperforms the other[22,31]. DEB-TACE has not been widely studied specifically for use as a bridge to transplant, though some published reports suggest its efficacy in this role[32].

RADIOEMBOLIZATION

Transarterial radioembolization (TARE) has emerged as a viable strategy for solid liver tumors. The most commonly used form of TARE for HCC involves Y-90 microspheres delivered intra-arterially. Y-90 has a physical half-life of 64.2 h and decays to stable zirconium-90[33]. A staging visceral angiography with injected technetium-99 is necessary to detect clinically relevant shunting to the gastrointestinal (GI) tract or lung, the latter assessed by measuring lung-shunt percentage on imaging[34]. If shunts to the GI tract cannot be embolized (and closed), or if the lung-shunt fraction is elevated, Y-90 is not offered due to concerns about intestinal and pulmonary toxicity, respectively. If no such problems are encountered, Y-90 microspheres are delivered either to the right or left lobe, usually allowing at least 1 mo before treating the opposite side if bi-lobar disease is present, in order to monitor for toxicity.

Overall tolerance and safety appears comparable to TACE, although the amount of published experience with Y-90 is vastly less than with TACE. Due the hypervascularity of HCCs, radioactive microspheres theoretically flow preferentially - by a factor of 3 to 1[35] - to tumors rather than hepatic parenchyma, limiting toxicity. Nonetheless, post-embolization syndrome following TARE - with nausea, abdominal pain and anorexia with or without fever - occurs with roughly the same frequency as with TACE, though severity may be less[36]. Some unique toxicities of Y-90 therapy must be appreciated. Radiation-induced liver disease (RILD) is a potentially serious sequela of TARE. RILD involves the emergence of varying degrees of liver decompensation with jaundice and ascites occurring 2-8 wk after treatment, with series suggesting a frequency of 4% to as much as 20%[36,37]. The risk of RILD appears to increase significantly with repeated Y-90 administrations[38]. Radiation-induced biliary stricturing is another potential consequence of TARE, though the incidence appears to be less than 10%[39]. As with TACE, care must be taken to avoid inadvertent embolization of the cystic artery, which could cause gall bladder necrosis. Radiation induced pneumonitis and GI ulcerations are rare if standard precautions are undertaken[36], but may occur with unrecognized shunting to lung or bowel.

Efficacy of radioembolization in terms of radiographic response and survival in non-operative candidates appears comparable or possibly superior to TACE[23], acknowledging that the cumulative amount of experience with Y-90 is less. Its utility as a bridge to LT is similarly less defined, but selected series show that TARE is effective in this role[34,40]. Lewandowski published a series comparing TACE (35 patients) to TARE (43 patients) for downstaging of HCC beyond Milan criteria, and reported successful downstaging to Milan T2 was superior with TARE (58% *vs* 31%, $P = 0.023$)[41]. One theoretical concern with Y-90 as a bridge to LT is the risk of radioactivity affecting surgical or pathology team members handling the explanted organ. However the decay properties of Y-90 are such that unless LT happens within 4 wk of TARE, the risks should be trivial.

ABLATION THERAPY

Except for TACE, RFA has been the most widely utilized and reported LRT for patients awaiting LT. RFA involves the insertion of one or more narrow probes - under ultrasound or computed tomography guidance - into a target liver lesion, usually with the patient anesthetized. Occasionally more than one tumor is treated in a given session. The probes are connected to an alternating current that generates heat at their tip, causing thermal injury to tissue. Some technical limitations of RFA involve a relatively long time (16-18 min) to achieve adequate thermal injury to fully ablate a 3-4 cm lesion, as well as the potential loss of heat energy (and thus treatment effect) if large blood vessels are near the treatment zone. In such cases, the vessels act as heat sinks dissipating energy. In view of these limitations, some centers have begun to utilize microwave ablation (MWA). MWA achieves much more rapid heating with shorter treatment time, as well as a larger zone of ablation. However, neither RFA nor MWA is ideal for lesions high in the dome of the liver or near the gall bladder, due the risk of pulmonary insult or gall bladder necrosis, respectively.

Complications of ablation include abdominal pain and anorexia with or without fever, not necessarily different

from the symptoms of post-embolization syndrome. Serious bleeding is possible but uncommon (< 2%), as is the rate of abscess formation, portal vein thrombosis, thoracic injury, and severe liver decompensation[42,43]. The risk of tumoral seeding by ablation probes (2%) and overall mortality (< 1%) is low, and seems comparable between RFA and MWA[43-45].

For very small (≤ 3 cm) HCCs, it is recognized that RFA can achieve complete eradication and is viewed by many as equivalent in efficacy to resection for this scenario[46,47]. Two large series published by Lu *et al*[48] and Mazzaferro *et al*[49] respectively, demonstrated the effectiveness of RFA as a bridge to LT, with very low dropout rates of 6% and 0%, respectively. A large Canadian study reported a higher rate of dropout with RFA (21%) as compared to an untreated cohort (12%), but this was in part driven by longer median waiting time to LT in the RFA cohort (9.5 mo *vs* 5 mo), as well as 9% of RFA-treated patients (*vs* 1% untreated) voluntarily seeking de-listing after achieving complete radiographic response[50]. The role of RFA/MWA for downstaging - at least of larger diameter tumors - is limited in that ablation zones are not ideal to treat tumors > 3-4 cm.

NOVEL EXTRACORPORAL THERAPY

Stereotactic body radiation therapy (SBRT) has emerged as a treatment for solid liver and lung tumors, and is occasionally used for cancer in other sites such as the pancreas, prostate and kidney. SBRT involves highly confocal beams of energy delivered at a narrowly defined site. Prior to treatment, 4-dimensional imaging is used to map the target area as it moves during breathing. Occasionally gold seed fiducials are placed into the target tumor to assist with imaging. Whereas conventional external beam radiation - generally ineffective for HCC - delivers relatively small daily doses over the course of several weeks, SBRT can deliver a much larger dose of radiation per session - usually lasting 30-60 min - such that treatment is completed in 1-5 d. Due to the ability to deliver the radiation in a highly targeted and localized manner, SBRT may have advantages over ablation since it can be used to treat lesions high in the dome of the liver (sparing the lung), near the gall bladder (sparing it), or near large blood vessels (no heat sink effect).

SBRT has been studied in HCC both as a bridge to LT and for inoperable patients. O'Connor *et al*[24] reported in a small study that SBRT (used because patients were deemed ineligible for further standard LRT) was successful as a bridge to LT in 10/10 patients, with none experiencing HCC progression between SBRT and LT[24]. Explant analysis from this series showed a 27% complete necrosis rate in treated tumors, with 75% of the incompletely necrotic tumors measuring smaller than pre-LT imaging size[24]. In two sequential studies using SBRT in 102 patients with Child's class A liver disease and locally advanced HCC, Bujold *et al*[51] reported a median survival of 17 mo[51], which is substantially higher than the median survival of the cohort receiving placebo in the SHARP study of sorafenib, which also was restricted to patients with mostly preserved liver function[52].

Toxicity from SBRT has been limited, and mostly grade 1 or 2 GI toxicity (nausea, vomiting, pain)[24,53], though Bujold's study reported grade 3 toxicity in up to 30%[51]. Rare GI ulcers have occurred following SBRT[53]. The role of SBRT is still evolving, and studies comparing SBRT directly to other forms of LRT for bridging therapy to LT are in progress.

High-intensity focused ultrasound (HIFU) is a novel extracorporal therapy that induces thermal injury to tumors using high frequency sound waves. Experience with HIFU is limited to date, but early experience with HCC patients has suggested a favorable radiographic response rate and safety profile[54]. A recent pilot study from Hong Kong comparing TACE and HIFU as bridging therapy to LT showed comparable degrees of tumor necrosis for both modalities when assessed at explant[55]. While more investigation is needed, the focused, extracorporal nature of HIFU may permit its use in patients with Child-Pugh C liver disease. Reported side effects have included localized bruising and first-and second-degree skin burns on skin overlying treatment zones[54].

CHOOSING THE OPTIMAL LRT FOR HCC IN THE PRE-TRANSPLANT SETTING

An ongoing difficulty for the transplant community is the lack of consensus regarding when/whether to use LRT for HCC prior to LT. There is further lack of consensus regarding which LRT to use for a given tumor. Even within each LRT category there is variation among institutions regarding the specifics of treatment. For example, "TACE" may involve different specific chemotherapeutic agents and/or embolic materials at different centers. And for small lesions, choice of TACE or ablation may come down to institution- or clinician-preference.

Despite these limitations, some general principles may assist decision-making. First, for Milan stage T2 HCC and preserved liver function, TACE has an excellent track record of safety and efficacy as a bridge to LT, with substantial lowering of dropout rates from historical standards[14]. TACE is also effective as a downstaging modality for larger lesions[13], though consideration for DEB-TACE is reasonable if there is portal venous thrombosis and/or decompensated liver function. Y-90 or TACE may be considered for larger (> 4 cm) tumors, the latter only if waiting time to LT is expected to exceed 1 mo.

Ablation (RFA/MWA) continues to be an effective bridge to LT for lesions < 3-4 cm, if the lesion is not located near the dome of the liver (lung), gall bladder or large vessels. For such lesions, ablation or TACE may

be equivalent in efficacy, though explant histological analysis suggests RFA has a higher rate of complete tumor necrosis for very small (< 3 cm) HCCs[56]. For lesions 4-6 cm in sensitive areas such as the dome of the liver or near the gall bladder, SBRT appears to be a safe, targeted therapy with early success reported as a bridging therapy. Lesions these sizes are generally too large for successful ablation. SBRT and novel HIFU may also be compelling considerations for patients with greater liver decompensation, as such patients may not tolerate TACE or TARE. More study is needed and planned.

CONCLUSION

The incidence of HCC has substantially increased in many regions during the past 3-4 decades. For all but very small HCCs, surgery (resection or LT) is necessary for long-term survival or cure. As most HCCs occur in the setting of cirrhosis, resection leaves behind diseased (and presumably prone-to-cancer) tissue, and thus LT appears to strongly out-perform resection in actuarial survival.

Given the risk of tumor progression and waitlist dropout, LRT is routinely offered to patients on the transplant waiting list. TACE and RFA are the most widely studied modalities, and are effective as bridging therapy to LT in appropriate settings. TACE is also used for downstaging in patients whose initial tumor burdens exceed Milan criteria. Other forms of LRT include DEB-TACE, Y-90 and more recently, extracorporal treatments such as SBRT. Each may have a "niche" role in the pre-transplant setting, and ongoing investigation will be critical in the development of widely accepted treatment paradigms to guide the use of LRT in waitlisted patients.

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P- Reviewer: Morioka D, Sugawara Y, Yankol Y
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Transplantation

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5500/wjt.v6.i2.314

World J Transplant 2016 June 24; 6(2): 314-320
ISSN 2220-3230 (online)

Potential approaches to improve the outcomes of donation after cardiac death liver grafts

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Author contributions: Mahboub P wrote the manuscript; Bozorgzadeh A and Martins PN contributed to writing the manuscript.

Supported by University of Massachusetts (FDSP grant to Paulo N Martins).

Conflict-of-interest statement: The authors declare no conflicts of interest.

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Received: July 18, 2015
Peer-review started: July 19, 2015
First decision: September 17, 2015
Revised: December 24, 2015
Accepted: March 24, 2016
Article in press: March 25, 2016
Published online: June 24, 2016

Abstract

There is a growing discrepancy between the supply and demand of livers for transplantation resulting in high mortality rates on the waiting list. One of the options to decrease the mortality on the waiting list is to optimize organs with inferior quality that otherwise would be discarded. Livers from donation after cardiac death (DCD) donors are frequently discarded because they are exposed to additional warm ischemia time, and this might lead to primary-non-function, delayed graft function, or severe biliary complications. In order to maximize the usage of DCD livers several new preservation approaches have been proposed. Here, we will review 3 innovative organ preservation methods: (1) different *ex vivo* perfusion techniques; (2) persufflation with oxygen; and (3) addition of thrombolytic therapy. Improvement of the quality of DCD liver grafts could increase the pool of liver graft's for transplantation, improve the outcomes, and decrease the mortality on the waiting list.

Key words: Biliarycomplications; Donation after cardiac death; Organ preservation methods

Core tip: As the demand for more organs increases, the transplant community searches for new approaches to expand the pool of organs. Recently developed methods to improve the condition of donation after cardiac death (DCD) livers look promising. During the past decade, *ex vivo* machine perfusion method has demonstrated positive results and it is considered as a new potential preservation method for DCD organs. This paper provides an overview of the attempts to ameliorate the quality of DCD liver grafts and transplant outcomes by improving preservation techniques.

Mahboub P, Bozorgzadeh A, Martins PN. Potential approaches to improve the outcomes of donation after cardiac death liver grafts. *World J Transplant* 2016; 6(2): 314-320 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/314.htm DOI:

http://dx.doi.org/10.5500/wjt.v6.i2.314

INTRODUCTION

Liver transplant is considered as the only available treatment for patients with end stage liver disease. Liver transplantation has been performed with success since 1963 and the outcomes continue to improve achieving 1-year graft survival superior to 90%[1,2]. At the same time the demand for liver transplant has increased and many patients die on the waiting list. The organ shortage has led to an increase in the use of grafts with inferior quality such as from donation after cardiac death (DCD) donors, also called non-heart-beating donors. DCD livers undergo additional warm ischemia time (WIT) which is associated with inferior liver function and poor outcome after transplant. Therefore, searching for potential approaches to ameliorate the quality of organs from DCD donors and minimizing injury is of special importance for the transplantation field.

In this paper we discuss about the attempts to ameliorate the quality of DCD liver grafts by improving preservation techniques.

CHARACTERISTICS OF DCD DONORS

DCD donors are characterized by the termination of ventilation and blood circulation before cold flushing of organs[3]. The idea to use DCD liver grafts was reintroduced in the 1990s after achieving success in kidney transplantation[3]. The use of grafts from DCD donors in the United States has exponentially increased from 0.95% in 2000 to 5% in 2010 (1 UNOS).

In general DCD donors are divided into uncontrolled and controlled donation groups. In the uncontrolled group, the organ suffers from prolonged WIT, as the potential donor is dead on arrival or has been undergoing unsuccessful resuscitation. In this group, the organ suffers from long WIT which is a detrimental factor in organ quality. In the controlled group, cardiac arrest is planned and it happens following withdrawal of ventilator in the operating room or intensive unit care[3,4]. It is generally accepted that DCD grafts have less energy stores and undergo more damage during the storage time[5].

Biliary complications are much more common in patients that received grafts from DCD donors (20%-40% compared to 5% in grafts from brain-dead donors)[6]. Post transplant biliary complications could lead to a number of serious complications such as graft loss, high morbidity which requires re-transplant or could result in patient's death[7]. The most critical type of biliary complications are the so-called ischemic-type biliary lesions (ITBL), also called ischemic cholangiopathy, with an incidence varying between 5% and 15%[8,9]. The risk of ischemic cholangiopathy with grafts from DCD donors is 10 times higher than for brain dead donors because of severe warm ischemia suffered by these grafts[6]. The reason why they develop more biliary complications is that bile ducts (cholangiocytes) are more sensitive to ischemia-reperfusion injury than hepatocytes[10]. Many of DCD liver grafts are not used because longer warm or cold ischemia times have been associated with poor outcomes[6,11,12]. Most of the transplant centers accept livers from DCD donors that have a maximum WIT, the period between extubation and cold flushing, of less than 30 min and short cold ischemia time (in general less than 6 h)[11].

OXYGENATED COLD STORAGE (PERSUFFLATION)

Simple cold storage (SCS) is the currently widely used organ preservation method in the clinical setting because of the low cost and simplicity. The idea of CS is to decrease the metabolism level to provide protection from ischemia. However, even at 4 ℃ there is approximately 5% active metabolism in the organ which eventually leads to ATP depletion and accumulation of waste product[13,14]. In order to improve organ preservation method, persufflation (PSF) had been introduced as an alternative method with the capacity of delivering oxygen during cold preservation. PSF has been used in rat livers for the first time between 1980 and 1990 by the Fischer group. They first established the model on rodent liver and continued with large animals (pig) and were able to demonstrate the benefits of PSF by improving the quality of liver grafts[15]. They also showed the feasibility of this method by publishing the outcome of five patients transplanted with persufflated livers[16]. The livers underwent WIT between 20-60 min and they were rejected by all the other transplant centers for transplant. They were flushed with University of Wisconsin (UW) or histidin-tryptophan-ketoglutarate and after arriving to the transplant center were subjected to retrograde PSF (R-PSF) at 18 mmHg for 70-200 min before the implantation. The results were promising and during the two years follow-up period, all the recipients showed good graft function. Later, in a study done by Minor *et al*[17], it was shown that PSF of Wistar rat liver grafts with 18 mmHg of oxygen for 48 h at 4 ℃ could lower the activity of Kupfer cells compared to simple SCS.

Following these preliminary results, the studies were extended to study the effect of PSF in DCD livers. Minor *et al*[18] introduced venous systemic oxygen PSF in DCD rat livers following 30 min of WIT. In a following study, the same group transplanted livers after 24 h PSF preservation, which showed that it improved mitochondrial function, and normalized ATP level[19].

Following the increasing concern on potential reactive oxygen species (ROS) production during PSF, Minor *et al*[20] preserved DCD rat livers for 24 h with R-PSF and compared with the result of the livers that were preserved in UW solution. The ATP level, bile production

and perfusion flow was improved in R-PSF livers. The outcome of this study demonstrated the beneficial role of R-PSF in eliminating ROS and lipid peroxidation production. In another study by Minor *et al*[21] it was also demonstrated that treatment with anti-oxidants such as superoxide dismutase or allopurinol during normothermic R-PSF could eliminate lipid peroxidation and restore the energy level in liver grafts after 60 min of WIT and 60 min of SCS in Euro-Collins solution. They also reported that PSF alone could induce some oxidative damage[21]. Recently in a study done by Lüer *et al*[22], it is shown that pulsatile PSF of DCD rat livers is beneficial in early graft recovery after reperfusion. In this study livers that were procured from male Wistar rats were subjected to 30 min WIT and then 18 h of cold ischemia. Later the grafts (n = 5 each group) were preserved with either nonpulsatile or pulsatile gaseous oxygen PSF. Pulsatile PSF demonstrated better parenchymal preservation, higher nitric oxide levels in perfusate, and decreased portal vein resistance[22].

In the next step, PSF was tested on pig livers, and subsequently to human DCD livers. In 2001 Saad *et al*[23] showed that R-PSF with antioxidant treatment in a transplant model is a promising method in improving the quality of the porcine DCD livers. DCD livers underwent 60 min WIT followed by 4 h SCS in UW solution or R-PSF with antioxidant treatment. In R-PSF group all animals survived, while animals in SCS group died 3 h after transplantation[23]. After successful animal experiments, the first clinical study was started in 2004 in Germany using R-PSF in 5 DCD livers. Liver grafts underwent R-PSF at 18 mmHg at least one hour before transplantation. Evaluation of the histological biopsies taken before and after R-PSF showed that ATP level was enhanced by 2-5 times after R-PSF treatment, and all the patients survived during the two years of observation period with good graft function panel[16].

HYPOTHERMIC MACHINE PERFUSION

Hypothermic machine perfusion (HMP) is considered as one of the alternative preservation methods to SCS which have recently been increased in use for DCD grafts preservation. HMP is a continuous or pulsatile circulation of the cold preservation solution in an organ at 4 ℃-8 ℃, and it has already been shown that HMP can resuscitate DCD liver graft's in different rat models[24-26]. Schlegel *et al*[27] demonstrated that hypothermic oxygenated perfusion (HOPE) in a rat model could impact down regulation of the immune system after transplantation, in addition to protecting against ischemia injury. In this study, using an acute rejection model, livers from the Lewis Rats were used to be transplanted into the Brown Norway Rats. Rat livers underwent one hour HOPE before implantation with or without low dose (0.03 mg/kg) tacrolimus treatment in the recipients during the four weeks of observation. The combination of tacrolimus with HOPE resulted in 100% survival in the recipients without any sign of rejection. As it was mentioned prior, one of the important issues in using DCD liver is to overcome biliary complications, in particular ischemic cholangiopathy related to strictures. In 2013, in a study done by Schlegel *et al*[25], it was demonstrated that HOPE is a sufficient method to protect DCD livers from biliary complications. The rat livers underwent to 30 min warm ischemia and it was followed by 4 h SCS. In the HOPE group, livers underwent one hour HOPE prior to implantation. Subsequently, livers were implanted and the recipients were observed for four weeks. Kupffer cell and endothelial cell activation was reduced. Moreover, cholestasis parameters were also improved in the HOPE group. In another study, Op den Dries *et al*[28] in a DCD pig model indicated the efficacy of oxygenated hypothermic machine perfusion in decreasing and limiting arteriolonecrosis injury of the peribiliary vascular plexus of the bile ducts. After 30 min of warm ischemia, the livers were preserved by SCS or oxygenated hypothermic machine perfusion using dual perfusion machine for 4 h. Next step was liver reperfusion for two hours at 37 ℃ with oxygenated autologous blood to simulate transplantation. Studying the bile duct histology disclosed reduced arteriolonecrosis of the peribiliary vascular plexus in the livers that were subjected to HMP perfusion *vs* SCS.

The feasibility of HMP study on human livers of brain dead donors was performed by Guarrera *et al*[29] at Columbia University. They used dual perfusion to perfuse 20 livers and successfully transplant them. They reported reduced early graft dysfunction, peak transaminases and improved renal function[29]. The first use of HMP for DCD livers was reported in 2014 by Dutkowski *et al*[30]. Eight DCD livers with median of 38 min WIT were included. Liver grafts underwent 1-2 h HOPE with perfusion pressure at 10 ℃, 3 mmHg. After transplantation the grafts revealed good hepatic function and no evidence of ITBL. Using HMP in other organs such as kidney is more common. There have been several clinical trials done on kidney HMP and it has become routine to use this method to preserve the human kidney in some part of Europe and some states in United States. Cold static storage is still the most common method of preservation in liver since cannulation and perfusion is more complicated in liver, and currently there is no Food and Drug Administration approved liver perfusion machine for clinical use.

SUBNORMOTHERMIC MACHINE PERFUSION

Another new potential method to replace SCS is subnormothermic (SNP) machine perfusion. Olschewski *et al*[31] presented that SNP perfusion is more beneficial in DCD rat liver which were subjected to one hour warm ischemia and reperfused at body temperature. Berendsen *et al*[32] established a rat liver transplant model. In this study the livers underwent 3 h of SNP perfusion at 21 ℃ with Williams Medium E solution

after one hour of WIT[32]. The survival rate was 83.3% in a one month observation period. In another study perforemd by the same group, they perfused 7 human discarded DCD livers at 21 ℃ for 3 h with oxygenated Williams Medium E[33]. This study found that oxygen uptake and ATP content was improved with an increase in bile production, and better bile quality. They suggested that SNP perfusion is effective in improving DCD livers quality and hepatobiliary cellular parameters.

NORMOTHERMIC MACHINE PERFUSION

Normothermic machine perfusion (NMP) is one of the innovative organ preservation techniques. NMP consists of a pulsatile flow of oxygenated perfusion solution in the organ which supports cellular metabolism at body temperature, restores the energy content of the organ and washes out waste products prior to the reperfusion in the recipient body. Another advantage of this method is to provide the opportunity of assessing the organ viability prior to implantation. In 2001, Friend *et al*[34] published a paper in which they described maintaining viability of DCD livers for a minimum of 24 h by applying NMP. After 60 min of WIT the liver grafts were stored for 24 h in UW solution or were immediately subjected to NMP. To mimic the anastomosis time, the livers were not perfused for 45 min after flushing with cold preservation solution. After 45 min, livers were reperfused for another 24 h. The continuous bile production, lower resistance in portal flow, reduced alanine transaminase level in the NMP group suggested that the quality of preservation can be enhanced by NMP perfusion.

op den Dries *et al*[35] was the first group to report the feasibility of this method in human DCD livers. They perfused 4 DCD discarded livers for 6 h using a dual perfusion system. The perfusion fluid consisted of packed blood cells with fresh frozen plasma to provide a sufficient support for high metabolism activity at 37 ℃. Reduced lactate level to the normal value, bile production, and well preserved hepatocytes and biliary sinusoids suggested that NMP is beneficial in improving the quality of DCD livers. One year later the same group published a study on criteria of assessing the graft viability during *ex vivo* NMP perfusion[36]. They investigated whether bile production and the quality of the produced bile during NMP would be a reliable biomarker for viability assessment. Twelve discarded DCD livers with median cold storage of 6.5 h were included and subjected to 6 h NMP at 37 ℃ with plasma and red blood cells. Liver grafts were divided into two groups; high bile production (more than 30 g in 6 h, and low bile production (less than 20 g). Higher bilirubin and bicarbonate concentration in the bile samples and lower hepatic necrosis in the high bile production group suggested that bile production might be a potential biomarker to assess the organ viability during warm perfusion. In a recent case report, Watson *et al*[37] from Addenbrooke's Hospital revealed the effect of Normothermic perfusion on a DCD liver graft before implantation. The liver graft was retrieved from a 57-year-old donor. Circulatory arrest occur 150 min after stopping of life-supporting treatment and the graft underwent 5 h cold storage. Later the graft was perfused at 37 ℃ for 132 min with a plasma free solution. During the first 74 min of perfusion, the lactate was decreased from 7.2 to 0.3 mmol/L. after implantation the liver biochemistry was normal and during 6 mo posttransplant observation, there was no evidence of cholangiopathy[37].

GRADUAL REWARMING MACHINE PERFUSION

Minor *et al*[38] for the first time introduced the concept of thermally controlled oxygenated rewarming (COR) of the liver grafts prior to reperfusion. In this study, Porcine livers were subjected to 18 h SCS and then were perfused 90 min by COR perfusion, HMP and SNP. In the COR group, during the first part of the perfusion temperature was stabilized at 8 ℃ and then was gradually enhanced to 12 ℃, 16 ℃, and 20 ℃ after 30 min, 45 min and 60 min, respectively. The perfusion pressure was kept at 4 mmHg in the portal side and at 25 mmHg at the hepatic artery side. In order to mimic the anastomosis time, the liver grafts were not perfused and were kept for 30 min in room temperature and then were reperfused with autologous blood for 4 h. The liver in the COR group demonstrated increased ATP, decreased lipid peroxidation, enzyme leakage and improved bile production. Minor *et al*[39] suggested that starting reoxygenation in a low temperature could reduce oxidative stress injury during reperfusion, and improve mitochondrial function[40]. Following the previous study Westerkamp *et al*[41] investigated COR in a rat DCD model. In this study, the rat DCD livers were subjected to SCS at 4 ℃ for 6 h and then subjected to COR, HMP or SNP. After 45 min mimic anastomosis time, they were reperfused 2 h with red blood cells and Williams Medium E solution. In the control group, livers were immediately reperfused at 37 ℃. Reduced transaminase enzymes level and lipid peroxidation level, superior mitochondrial function, higher bile production, improved bile quality and better preserved bile duct epithelium was observed in the COR group. The COR represented superior liver function compare to the SCS groups but comparable to the HMP and the SNP group.

ABDOMINAL REGIONAL PERFUSION

The main concept of abdominal regional perfusion is to limit deleterious effect of warm ischemia in DCD organs by the abdominal organ perfusion with continuous flow. Abdominal regional perfusion is being done *via* cannulation of femoral artery and vein using cardio pulmonary bypass machine or extracorporeal membrane oxygenation machine. For the first time regional perfusion was performed by a Spanish surgeon at 1989[42]. The perfusion is being used in two categories

Table 1 Disadvantages of the different methods

Preservation methods	Disadvantages
Persufflation	Not able to assess the viability of the grafts
Hypothermic perfusion	High cost, not able to assess the viability of the graft
Subnormothermic perfusion	High cost, clinical challenging, not able to assess the viability of the graft
Normothermic perfusion	High cost, clinical challenging, the blood supply, the risk of losing the graft in the case of emboli in the system
Gradual rewarming	High cost, clinical challenging, need an accurate machine to be able to change the temperature and pressure
Abdominal regional perfusion	Ethical challenging
Thrombolytic therapies	Risk of severe bleeding

as hypothermic or normothermic perfusion[43]. One group from West forest University describes perfusing of six DCD livers with hypothermic regional perfusion which was performed at 22 ℃. In this study they showed good initial graft survival[44]. The hospital clinic in Barcelona started using normothermic regional perfusion protocol on human category 2 DCD donors. The recipients were subjected to a median 45 mo follow-up. One year graft survival was 73% while patient survival rate was 81%. In another study a group from La coruna in Spain included category 2 DCD liver donors, they subjected 7 donors to hypothermic regional perfusion and 10 donors to normothermic regional perfusion. The results demonstrated high biliary complication in the recipients (25%) with low rate of five years graft survival[45].

THROMBOLYTIC THERAPIES (TISSUE PLASMINOGEN ACTIVATOR)

Hashimoto *et al*[46] suggested that the higher incidence in biliary complications of DCD livers may be related to microthrombi in the peri-biliary plexus. In this study they included 22 patients and assessed the effect of tissue plasminogen activator (TPA) injected into the hepatic artery of donor's during back table. Fourteen recipients out of 22 developed excessive post reperfusion bleeding and 2 patients developed ITBS. The TPA level was investigated in all the patients to find out if there was a correlation between the TPA level and excessive bleeding. They found that TPA level in the patients with bleeding was comparable with those who did not develop bleeding. The patients with excessive bleeding had history of higher previous laparotomy done in the past and higher body mass index, which might be associated with incidence of massive bleeding. In another study Seal *et al*[47] recently showed that TPA treatment in DCD liver grafts decreases ITBSs occurrence and improves one- and three-year graft survival after transplant. TPA injection was delivered into the hepatic artery during liver transplant in 85 patients and compared to 33 patients who did not undergo TPA treatment. They reported lower occurrence rate of ITBL (16.5% *vs* 33.3%) and lower intrahepatic constriction in the group that received TPA treatment (3.5% *vs* 21.2%).

THE DISADVANTAGES OF DIFFERENT PRESERVATION METHODS

The disadvantages of each method are listed in the Table 1.

CONCLUSION

Because of exponential increase in the demand of liver grafts and high mortality on the waitlist, the interest of expanding the suitable organs for transplant has been increased. The optimized use of DCD liver grafts, different *ex-vivo* preservation interventions have been proposed achieving high rates of success. There is enough evidence that these new techniques have potential to improve graft function. Now, it is time for randomized controlled trials and a cost-effective analysis to determine if these techniques will become standard clinical practice.

ACKNOWLEDGMENTS

The authors would like to thank Stefanie Parker for proof-reading and editing.

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P- Reviewer: Balaban YH, Kruel CRP, Kita K, Marino IR, Sugawara Y
S- Editor: Gong XM **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Transplantation

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DOI: 10.5500/wjt.v6.i2.321

World J Transplant 2016 June 24; 6(2): 321-330
ISSN 2220-3230 (online)

MINIREVIEWS

First line *vs* delayed transplantation in myeloma: Certainties and controversies

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Author contributions: Brioli A performed bibliographical research and wrote the paper.

Conflict-of-interest statement: The author declares no conflicts of interests.

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Received: June 28, 2015
Peer-review started: July 5, 2015
First decision: September 17, 2015
Revised: March 22, 2016
Accepted: April 7, 2016
Article in press: April 11, 2016
Published online: June 24, 2016

Abstract

Since the middle of 1990s autologous stem cell transplantation has been the cornerstone for the treatment of young patients with multiple myeloma (MM). In the last decade the introduction of novel agents such as immunomodulatory drugs (IMiDs) and proteasome inhibitors (PI), has dramatically changed the therapeutic scenario of this yet incurable disease. Due to the impressive results achieved with IMiDs and PI both in terms of response rates and in terms of progression free and overall survival, and to the toxicity linked to high dose therapy and autologous stem cell transplantation (ASCT), a burning question nowadays is whether all young patients should be offered autotransplantation up front or if this should be reserved for the time of relapse. This article provides a review of the data available regarding ASCT in MM and of the current opinion of the scientific community regarding its optimal timing.

Key words: Autologous stem cell transplantation; Immunomodulatory drugs; Proteasome inhibitors; High dose therapy; Multiple myeloma

Core tip: Autologous stem cell transplantation (ASCT) is the cornerstone for the treatment of young multiple myeloma patients. This review summarizes the current knowledge on ASCT, with a special focus on the role of ASCT in the era of novel agents for multiple myeloma treatment.

Brioli A. First line *vs* delayed transplantation in myeloma: Certainties and controversies. *World J Transplant* 2016; 6(2): 321-330 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/321.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.321

INTRODUCTION

Multiple myeloma (MM) is the second most common

hematologic malignancy, accounting for approximately 13% of all blood neoplasm and for approximately 1% of all cancers. The number of new cases diagnosed every year is of approximately 86000 worldwide[1]. MM is mainly a disease of the aging population, however young individuals below 65 years of age can also be affected[1].

Traditionally MM patients have been divided in two groups, based on their eligibility and fitness to receive high dose therapy (HDT) and autologous stem cell transplantation (ASCT). Fit patients, usually younger than 65-70 years of age, were offered HDT (with doses ranging from 200 to 100 mg/m^2 based on age and clinical conditions) and ASCT, while conventional treatment with lower doses of chemotherapy (mostly Melphalan) and steroids was given to elderly or unfit patients[2-9].

In the last decade major advances in the management of MM have been made thanks to the introduction of novel agents such as immunomodulatory drugs [the immunomodulatory drugs (IMiDs), such as thalidomide, lenalidomide and pomalidomide] and proteasome inhibitors [the proteasome inhibitors (PI) bortezomib and carfilzomib][10-15]. The introduction of these drugs as part of the frontline treatment in both transplant eligible and non-eligible patients translated into a markedly increased rate of complete remission (CR), time to progression (TTP), progression-free survival (PFS) and overall survival (OS)[11,13,16-18]. In patients ineligible to ASCT, the addition of bortezomib to the conventional melphalan and prednisone (MP) treatment translated into a rate of CR of 30%, with an OS at 5 years of 56.4 mo[19,20]. These impressive results, comparable to the rate of CR and OS achieved with ASCT, have raised the question whether autologous transplant is nowadays still needed to treat MM patients or if it should be replaced by new drug containing regimens with or without chemotherapy. In this latter case ASCT would be used as a salvage treatment at the time of progression in patients initially treated with novel agents. This review will focus on the current role of ASCT for the treatment of MM patients.

UP-FRONT TRANSPLANTATION

High dose melphalan supported by ASCT for the treatment of fit MM patients was first developed in the 1980s, and it has been considered the standard of care for this group of patients since the middle of 1990s[21,22]. The infusion of harveste and criopreserved autologous stem cells, first introduced in the relapsed-refractory setting, proved to be able to reduce the prolonged myelosuppression caused by high doses of melphalan[23,24]. In consideration of the good results seen in this subset of patients, ASCT was translated in the newly diagnosed setting, and also in this group of patients HDT ASCT demonstrated its superiority in comparison to conventional chemotherapy[4,5]. At present 7 randomised trials have compared ASCT with conventional chemotherapy, and results largely confirm the benefit of a transplant treatment approach (Table 1)[4,5,9,25-28]. The majority of the studies demonstrated that treatment with ASCT was associated with a longer PFS[4,5,9,25-27]; conversely, the benefit in terms of OS was less clear[4,5,9]. This finding can be partly explained by the fact that patients initially treated with only chemotherapy were later rescued with ASCT, thus providing a rational for reserving ASCT at a later time point in patient's history[29]. Similar results were shown in a meta-analysis of 2411 patients, in which a benefit in terms of PFS, but not of OS, was observed[30].

The introduction of novel agents in the induction phase before and in a consolidation or maintenance phase after ASCT, has further improved the outcomes of MM patients, increasing response rates, PFS and OS (Table 2). The combination of thalidomide and dexamethasone (TD) or of thalidomide with conventional chemotherapy has significantly increased the rate of responses compared to chemotherapy alone[10,17,31-33]. TD incorporated into double ASCT was able to improve PFS and OS (median PFS 48 mo, OS 65% at 5 years) compared to standard chemotherapy with vincristine, adriamycin and dexamethasone[10,31].

Bortezomib in the context of ASCT gave even more impressive results[16,34-36], with the best combinations being those of bortezomib plus dexamethasone and an IMiDs[13,37,38]. The combination of bortezomib, thalidomide and dexamethasone incorporated into ASCT resulted in a PFS of 68% at 3 years[13], and a OS that reached 82% at 2 years[37].

Even more interesting seems the combination of bortezomib and dexamethasone with lenalidomide (VRD) followed by ASCT. A phase Ⅰ/Ⅱ study investigating this combination in newly diagnosed MM patients reported impressive results, with an overall response rate of 100% and an estimated PFS and OS at 18 mo of 75% and of 97% respectively. This results have however to be carefully interpreted and confirmed, considering the short follow up that at the time of reporting of only 21 mo[38].

The high rate of good quality responses seen with the incorporation of PI and IMiDs as induction before, and consolidation and maintenance after ASCT translated into an increase of both PFS and OS; in consideration of these results, and of the toxicity associated with HDT and ASCT, a burning question nowadays is whether new treatments alone, without the use of upfront ASCT, would be sufficient to treat young MM patients[39]. In this scenario it is worth noting that the majority of patients enrolled in clinical trials that were not treated with ASCT upfront could still receive it at the time of relapse. Furthermore impressive results were seen with the introduction of novel agents in the treatment of MM patients not suitable for ASCT.

Table 1 Phase Ⅲ clinical trials of chemotherapy *vs* transplantation

Ref.	Publication year	Random	Patients *n*	ORR (%)	CR (%)	PFS/EFS	OS
Attal *et al*[4]	1996	ASCT	100	81	[1]22	28 mo	57 mo
IFM90		CCT	100	57	5	18 mo	44 mo
				P < 0.001	*P* < 0.001	*P* = 0.01	*P* = 0.03
Child *et al*[5]	2003	ASCT	200	86	44	32 mo	54 mo
MRC VII		CCT	201	48	8	20 mo	42 mo
				P = NR	*P* < 0.001	*P* < 0.001	*P* = 0.04
Fermand *et al*[25]	1998	ASCT	91	78	57	39 mo	64.6 mo
MAG90		CCT	94	58	20	13 mo	64 mo
Barlogie *et al*[28]	2006	ASCT	261	93	17	17%	38%
S9321		CCT	255	90	15	14%	38%
						At 7 yr	At 7 yr
Fermand[27]	2005	ASCT	94	62	36	37 mo	79 mo
MAG95		CCT	96	58.5	20	16 mo	43 mo
Bladé *et al*[26]	2005	ASCT	81	82	30	42 mo	66 mo
PETHEMA		CCT	83	83	11	33 mo	61 mo
					P = 0.002		
Palumbo *et al*[9]	2004	ASCT	95	72	[1]25	28 mo	58 mo
MMSG		CCT	99	66	6	16 mo	42 mo
					P = 0.002	*P* < 0.001	*P* < 0.001

[1]≥ nCR. Only statistical significant *P* is reported. CCT: Conventional chemotherapy; ASCT: Autologous stem cell transplantation; ORR: Overall response rate; CR: Complete remission; nCR: Near CR; PFS: Progression free survival; EFS: Event free survival; OS: Overall survival; NR: Not reported; IFM: Intergroupe Francophone du Myèlome; MRC: Medical Research Council; PETHEMA: Programma Para El Estudio y Tratamiento De Las Hemopatìas Malignas; MAG: Myèlome Autogreffe.

RATIONAL FOR DELAYED TRANSPLANTATION: NEW DRUGS COMBINATIONS WITH OR WITHOUT CHEMOTHERAPY FOR PATIENTS NOT CANDIDATE TO ASCT

The advent of new drugs has dramatically changed the outcomes not only of young MM patients, but also, and maybe even more impressively, those of older transplant ineligible patients. Already the implementation of thalidomide into the classic combination of MP was able to improve patients outcomes compared to MP alone[40]. The addition of bortezomib to MP led to even more impressive results, increasing the response rate of elderly MM patients to rates previously seen only in patients that received ASCT. Patients treated with Bortazomib, Melphalan and Prednisone (VMP) showed a TTP of 24 mo and a 3- and 5-year OS of 68.5% and 46%, respectively. The addition of bortezomib to MP was able to increase the OS of patients of 13 mo[19,20,41].

Another interesting combination is the one of lenalidomide and dexamethasone. The combination of lenalidomide and dexamethasone was first evaluated both in young and elderly MM patients, identifying the association of lenalidomide with low dose dexamethasone (Ld) as the combination to bring forward in further trials[11]. This combination has been proved to be extremely beneficial in the elderly population. A continuous treatment with lenalidomide and dexamethasone was found to be superior not only to MP plus thalidomide, but also to the same regimen given for a fixed number of cycles (18 cycles); continuous Ld significantly reduced the risk of death (HR = 0-78; *P* = 0.02) and the authors speculate that for the first time a regimen without chemotherapy can be considered as a standard of care for the treatment of MM patients[42]. The knowledge that ASCT can be given also as a salvage treatment, together with the data coming from the aforementioned trials resulted in the treatment strategy comprehensive of upfront ASCT now being questioned by some centres[43].

DELAYED TRANSPLANTATION

The best timing of ASCT, whether it should be given as an upfront treatment or as salvage therapy at the time of relapse, was already a burning question before the novel agents era. From 1990 to 1995, Fermand *et al*[25] randomly assigned 185 patients to receive early ASCT or conventional chemotherapy with vincristine, melphalan, cyclophosphamide and prednisone (VMPC). In this latter group ASCT was reserved for the time of relapse. Although median event free survival was longer for patients treated with early ASCT (39 mo *vs* 13 mo) the median OS was not significantly different between the two groups (64.6 mo *vs* 64 mo, *P* = 0.92), and 90% of the patients randomised to the VMPC arm were able to receive the planned delayed ASCT at the time of relapse[25].

Several analyses, summarised in Table 3, have investigated the role of ASCT as a salvage therapy for MM[29,44-51]. These works are not always comparable, due to the different nature of the works (both prospective and retrospective) and to the fact that ASCT was

Table 2 Improved outcomes with the introduction of novel agents in the upfront treatment of multiple myeloma

Ref.	Publication's year	Therapy	Patients *n*	⩾ VGPR (%) preASCT	⩾ VGPR (%) postASCT	⩾ PR %	CR/ nCR %	PFS/EFS OS
Thalidomide								
Rajkumar *et al*[32]	2006	TD *vs* D	200	63 *vs* 41 (⩾ PR)	NR			NR
Cavo *et al*[10]	2009	TD *vs* VAD	270	30 *vs* 15	68 *vs* 49			PFS 51% *vs* 31% at 4 yr OS 69% *vs* 53% at 5 yr
Barlogie *et al*[17]	2006	TT2 + Thal *vs* TT2	668	NR	62 *vs* 43			EFS 56% *vs* 44% at 3 yr OS 65% *vs* 65% at 5 yr
Lokhorst *et al*[33]	2010	TAD *vs* VAD	402	32 *vs* 15	49 *vs* 32			EFS 34 mo *vs* 22 mo OS 73 mo *vs* 60 mo
Lenalidomide								
Richardson *et al*[38]	2010	VRD	35			100	57	NR
Palumbo *et al*[56]	2014		402					
		MPR	202			NR	NR	PFS 22.4 mo *vs* 43 mo
		vs						
		HDM	200			NR	NR	OS 65.3% *vs* 81.6%
		Maintenance R	198			78	23	PFS 41.9 mo *vs* 21.6 mo
		vs						
		No maintenance	204			77	19	OS 79% *vs* 88%
McCarthy *et al*[66]	2012	Lenalidomide *vs* placebo	460					PFS at 3 yr 66% *vs* 39% OS at 3 yr 88% *vs* 80%
Attal *et al*[67]	2012	Lenalidomide *vs* placebo	614					PFS at 4 yr 43% *vs* 22% OS at 4 y 73% *vs* 75%
Bortezomib								
Harousseau *et al*[16]	2010	®VD *vs* VAD	482	38 *vs* 15	54 *vs* 37			36 m *vs* 27 m
Sonneveld *et al*[34]	2012	®Induction PAD + maint VEL *vs* induction VAD + maint Thal	626	NR	75 *vs* 61			46% *vs* 42% at 3 yr
Cavo *et al*[13]	2010	®VTD *vs* TD induction and consolid	480	62 *vs* 28	82 *vs* 64			68% *vs* 56% at 3 yr
Rosiñol *et al*[37]	2012	®VTD *vs* TD	202	29 *vs* 14 (CR)	59 *vs* 40 (CR)			82% at 2 yr (OS)
Moreau *et al*[35]	2011	®VD *vs* vtD	199	49 *vs* 39	74 *vs* 58			30 mo *vs* 26 mo
Leleu *et al*[36]	2013	VTd-ASCT + consolid VTd *vs* VTd-ASCT	217	After treatment: 83 *vs* 64				TTP: 62% *vs* 29% at 4 yr

VGPR: Very good partial response; TTP: Time to progression; PFS: Progression free survival; NR: Not reported; Thal: Thalidomide; TD: Thalidomide dexamethasone; VAD: Vincristine adriamycin dexamethasone; TAD: Thalidomide adriamycin dexamethasone; MPR: Melphalan prednisone lenalidomide; VTD: Bortezomib thalidomide dexamethasone; VD: Bortezominb dexamethasone; PAD: Adriamycin bortezomib dexamethasone; vtD: Reduced doses bortezomib thalidomide dexamethasone; R: Lenalidomide; VRD: Bortezomib lenalidomide dexamethasone; OS: Overall survival; ASCT: Autologous transplantation; nCR: Near CR; HDM: High dose melphalan; Consolid: Consolidation; Maint: Maintenance.

Table 3 Major studies of delayed autologous stem cell transplantation (for randomised trials only data regarding delayed autologous stem cell transplantation are reported)

Ref.	Publication's year	Patients *n*	Type of trial	Median interval between diagnosis or first ASCT and delayed ASCT	Previous ASCT	ORR (%)	PFS (mo)	OS (mo)
Cook *et al*[49]	2011	106	Retrospective	19 mo (relapse from first transplant)	Yes	63%	NR	37
Jimenez-Zepeda *et al*[51]	2012	81	Retrospective	39 mo (relapse from first transplant)	Yes	97.4%	16.43	53
Sellner *et al*[44]	2013	200	Retrospective	NR	Yes	80.4%	15.2	43.2
Cook *et al*[46]	2014	89	Prospective	2.7 yr	Yes	83%	19	80.3% at 3 yr
Gertz *et al*[29]	2000	64	Prospective	NR	No	97%	11.4	19.6
Michaelis *et al*[45]	2013	187	Retrospective	32 mo	Yes	68%	5% at 5 yr	29% at 5 yr
Shah *et al*[68]	2012	44	Retrospective	30 mo	Yes	90%	12.3	31.7
Kumar *et al*[48]	2012	112	Prospective	> 12 mo	No	32% (⩾ VGPR)	16 (TTP)	73.4% at 4 yr
Dunavin *et al*[47]	2013	65	Retrospective	17.7 mo	No	NR	23 (TTP)	63% at 5 yr

VGPR: Very good partial response; TTP: Time to progression; PFS: Progression free survival; ASCT: Autologous stem cell transplant; OS: Overall survival; NR: Not reported; ORR: Overall response rate.

in some cases given as a salvage treatment after a previous ASCT[44-46], whilst in others patients received ASCT after relapsing from a treatment not including transplantation[29,47,48].

One of the biggest records is the one published by Sellner *et al*[44], in which 200 MM patients retreated with ASCT at the time of relapse were retrospectively analysed. In the study a prognostic score was created, based on the International Staging System (ISS) at the time of relapse and on the duration of response after the first ASCT. The analysis showed that the biggest benefit of salvage ASCT was achieved in those patients with a low ISS (ISS 1) and with a first PFS longer than 18 mo. Another interesting finding of the study was that about 50% of the patients presented at the time of relapse with cytogenetic features of high risk, such as the presence of del(17p), t(4;14) or amp(1q), and that these patients had a worst outcome as compared to patients that relapsed with standard risk features[44]. These findings are of primary importance in the decision of when to perform an ASCT (upfront or at relapse), taking into account that patients may relapse with a more aggressive disease, and that cytogenetic abnormalities known to confer a dismal outcome are seen more often in patients in advanced stages of disease, probably as the result of an increasing biological risk and clonal selection[52-54].

Most of the studies available were published before IMiDs and PI became available for upfront treatment. In the era of novel agents two studies have retrospective analysed the role of early *vs* delayed ASCT[47,48] and one study prospectively evaluated a second ASCT after relapse from a previous one[46]. One study reported the outcomes of 290 patients treated with IMiDs based therapy (thalidomide or lenalidomide) and that received early (within 12 mo of diagnosis) or late ASCT; PFS was similar irrespective of when ASCT was performed (early or late) and no significant difference could be observed in OS, with both groups experiencing a 4-year OS of 73%[48]. In a similar study Dunavin *et al*[47] retrospectively reviewed the outcome of 167 patients treated with novel agent-based therapy (IMiDs or PI) and receiving early or delayed ASCT. The 5-year OS from diagnosis was similar in the two groups (63% both in early and late ASCT, $P = 0.45$), in accordance with the data reported by Kumar *et al*[48]. The English group prospectively evaluated the role of salvage ASCT after relapse from a previous one; patients relapsing after ASCT were randomised between treatment with a second ASCT or chemotherapy with cyclophosphamide (Cy). With a median follow-up of 31 mo, although patients randomised to a second ASCT experienced a longer PFS compared to patients treated with Cy (19 *vs* 11 mo for ASCT and Cy respectively, $P < 0.0001$) no difference in terms of OS could be seen. It also has to be noted that the comparator chemotherapy arm, comprehensive of only weekly Cy, might not be the standard of care in a time when multiple drugs, such as third generation IMiDs, second generation PI, spindle kinase inhibitor or monoclonal antibodies are available for the treatment of relapsed MM.

NEW DRUGS IN THE CONTEXT OF UP-FRONT VS DELAYED TRANSPLANTATION: PHASE Ⅲ CLINICAL TRIALS

As already stated the advent of new drugs has dramatically changed the therapeutic scenario of MM patients. Not only an induction treatment comprehensive of new drugs significantly increased the rate of high quality responses and improved survival outcomes[11,13,16,34], but the manageable toxicity of these compounds make them suitable for a long term and continuous treatment[42,55]. In the above mentioned phase Ⅰ/Ⅱ VRD trial, a post hoc landmark analysis showed that the risk of progression after one year was low irrespective of whether patients had received or not an ASCT and that in patients who did not wish to undergo transplantation, responses increased prolonging therapy from 4 to 8 cycles[38].

The impressive results obtained with first line treatment comprehensive of IMiDs and PI prompt the investigation of upfront vs delayed transplantation in the context of specifically designed phase Ⅲ randomised trials.

The Italian Gruppo Italiano Malattie Ematologiche dell'Adulto conducted a phase Ⅲ clinical trial aimed at comparing melphalan, prednisone and lenalidomide (MPR) *vs* two courses of HDT with melphalan (melphalan 200 mg/m^2). All patients had previously received an induction treatment with four courses of Lenalidomide and low dose dexamethasone (Ld). With a median follow-up of 51.2 mo the results showed a clear advantage of the ASCT arm both in terms of PFS (43 *vs* 22 mo, $P < 0.001$) and of OS (82% *vs* 65% at 4 years, $P = 0.02$)[56]. Another factor that might have influenced the outcome of the study was that 41% of the patients randomised in the late transplant arm did not receive the planned salvage ASCT[56]. High dose melphalan (HDM) after 4 cycles of induction with Ld was also compared to cyclophosphamide, lenalidomide and dexamethasone (CRD). Similarly to what already seen with the MPR treatment, HDM was superior to CRD in terms of PFS (27 mo *vs* not reached for CRD and HDM, respectively, $P = 0.012$), whilst no advantage was seen in terms of OS (estimated 3-year OS 81% *vs* 84% for CRD and HDM, respectively, $P = 0.891$)[57]. A pooled analysis the two trials showed that in newly diagnosed MM patients, HDM followed by ASCT significantly improved PFS and OS in comparison to MPR or CRD. Patients with favourable baseline conditions, such as a good baseline performance status (PS) (Karnofsky PS $\geqslant$ 80%), a low ISS (ISS 1), the absence of high-risk cytogenetic abnormalities [del(17p), t(4;14), t(14;16)] and those that had achieved at least a very good partial response

after induction had the most significant benefit in terms of OS[58].

The reported trials seem to favour upfront ASCT, however a possible caveat of these studies is the not-optimal induction treatment, with the rate of complete responses reported after consolidation (with MPR or HDM) that where lower than those reported at the same time point after other chemotherapy-free induction regimens, such as bortezomib-thalidomide-dexamethasone[13,37,56]. The most promising induction combinations to be tested in the context of upfront *vs* delayed transplantation are triplet combinations including two novel agents or a novel agent and a chemotherapeutic drug associated with Dexamethasone[13,34,37,38]. Two multicentre randomised phase Ⅲ trials are currently ongoing, evaluating the role of upfront *vs* delayed ASCT in the context of a new drug based therapy. The European Myeloma Network (EMN) on one side and the Intergroupe Francophone du Myélome (IFM) in association with the Dana-Farber Cancer Institute (DFCI) on the other, are conducting two trials aimed at assessing the role of ASCT in comparison to a novel agent based consolidation. The EMN02 trial randomises transplant eligible newly diagnosed MM patients, after an induction with 4 cycles of bortezomib, cyclophosphamide and dexamethasone, to receive a consolidation therapy with 4 cycles of VMP or with ASCT to support one or two cycles of HDM. Patients are further randomised to a second consolidation treatment with VRD *vs* observation; all patients will receive maintenance treatment with lenalidomide. The IFM/DFCI 2009 trial compares VRD with or without transplantation in a subset of patients similar to those included in the EMN02 study. As for patients in the EMN02 study, patients enrolled in the IFM/DCFI 2009 trial will receive maintenance lenalidomide. Both trials are currently closed to recruitment and definitive results with a long follow up results are eagerly awaited.

CONCLUSION

In the era of novel agents the appropriate timing for performing ASCT, whether upfront or at relapse, is still a burning question. If on one hand it is true that early ASCT improves PFS rates, on the other hand it is associated with a higher toxicity compared to a treatment with novel agents[56]. It has to be also acknowledged that, whilst almost all randomized studies showed longer PFS for early ASCT, the benefit on OS was not uniformly reported[25,56-58]. The lack of advantage observed in some cases in terms of OS is mainly do to the effective salvage therapy nowadays available, and to the possibility for patients to receive ASCT later in their disease history as a salvage treatment. For this reason some centres nowadays recommend ASCT only for those patients with high-risk features, whilst for standard risk patients a treatment option reserving ASCT for the time of relapse is considered acceptable[59-61]. In this contest it has to be emphasised, in patients for whom a delayed ASCT may be considered, the extreme importance of early stem cell collection and cryopreservation; an early stem cell collection is particularly important in those patients receiving lenalidomide based treatments[62,63].

Despite being a feasible option for carefully selected patients, delayed ASCT has some important caveats: Not only a significant percentage of patients might not be able to receive HDM at the time of relapse, due to the worsening of their clinical conditions[56], but also a worst outcome could be expected due to the higher rate of adverse cytogenetic features in more advance disease phases[44]. Furthermore it has to be noted that reliable cost effectiveness data comparing early ASCT *vs* the continuation of a novel agent based therapy are currently not available[64].

Based on the available data the recent guidelines from the American Society for Blood and Marrow Transplantation recommend performing ASCT early in disease history (within 12 mo)[64], and there is a global consensus strongly in favour of upfront ASCT[21,65]. Results of ongoing phase Ⅲ studies are eagerly awaited to answer the burning question regarding the optimal timing of ASCT in young MM patients and whether, in the era of novel agents, HDM is still a need in order to treat MM.

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P- Reviewer: Saeki K **S- Editor**: Gong XM
L- Editor: A **E- Editor**: Liu SQ

World Journal of Transplantation

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DOI: 10.5500/wjt.v6.i2.331

World J Transplant 2016 June 24; 6(2): 331-335
ISSN 2220-3230 (online)

MINIREVIEWS

State of deceased donor transplantation in India: A model for developing countries around the world

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Author contributions: Abraham G, Vijayan M, Yuvaraj A, Nair S and Shroff S wrote the manuscript; Gopalakrishnan N, Sundarrajan S and Amalorpavanathan J provided the data and reviewed the manuscript.

Conflict-of-interest statement: None declared by the authors.

Manuscript source: Invited manuscript

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Received: January 13, 2016
Peer-review started: January 15, 2016
First decision: February 29, 2016
Revised: May 11, 2016
Accepted: May 31, 2016
Article in press: June 2, 2016
Published online: June 24, 2016

Abstract

Renal replacement therapy (RRT) resources are scarce in India, with wide urban-rural and interstate disparities. The burden of end-stage renal disease is expected to increase further due to increasing prevalence of risk factors like diabetes mellitus. Renal transplantation, the best RRT modality, is increasing in popularity, due to improvements made in public education, the deceased donor transplantation (DDT) programme and the availability of free and affordable transplant services in government hospitals and certain non-governmental philanthropic organizations. There are about 120000 haemodialysis patients and 10000 chronic peritoneal dialysis patients in India, the majority of them waiting for a donor kidney. Shortage of organs, lack of transplant facilities and high cost of transplant in private facilities are major barriers for renal transplantation in India. The DDT rate in India is now 0.34 per million population, among the lowest in the world. Infrastructural development in its infancy and road traffic rules not being strictly implemented by the authorities, have led to road traffic accidents being very common in urban and rural India. Many patients are declared brain dead on arrival and can serve as potential organ donors. The DDT programme in the state of Tamil Nadu has met with considerable success and has brought down the incidence of organ trade. Government hospitals in Tamil Nadu, with a population of 72 million, provide free transplantation facilities for the underprivileged. Public private partnership has played an important role in improving organ procurement rates, with the help of trained transplant coordinators in government hospitals. The DDT programmes in the southern states of India (Tamil Nadu, Kerala, Pondicherry) are advancing rapidly with mutual sharing due to public private partnership providing vital organs to needy patients. Various health insurance programmes rolled out by the governments in the southern states are effective in alleviating financial burden for the transplantation. Post-transplant immunological and pathological surveillance of recipients

remains a challenge due to the scarcity of infrastructure and other facilities.

Key words: Deceased donor transplantation; Kidney; India; Developing countries

Core tip: Deceased donor transplantation (DDT) has been increasing in India, especially in the southern states due to proactive policies of the state governments and public private partnership. With the goal of achieving maximum organ harvesting from potential organ donors and universal access to transplant services, small steps of improvement have been made. The DDT program in India has to keep progressively expanding to cater to the end-stage renal disease affected population of India.

Abraham G, Vijayan M, Gopalakrishnan N, Shroff S, Amalorpavanathan J, Yuvaraj A, Nair S, Sundarrajan S. State of deceased donor transplantation in India: A model for developing countries around the world. *World J Transplant* 2016; 6(2): 331-335 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/331.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.331

INTRODUCTION

India is the third largest economy in the world, by purchasing power parity. However, the gross domestic product (GDP) spent on healthcare is only 4%, with merely 1.3% spent by the public sector[1,2]. GDP per capita for India is United States $1630.8[3]. India faces tremendous challenges in providing basic healthcare facilities for its population, as the major percentage of the population lives below the poverty line. There has been a shift in disease epidemiology in India, with non-communicable diseases on the rise. Chronic kidney disease (CKD) is of increasing prevalence in India. CKD which is asymptomatic in early stages, puts greater financial burden on the stakeholders at the later stages. There are urban-rural and interstate disparities in the provision of renal replacement therapy (RRT), due to lack of skilled nephrologists, transplant surgeons and poor government support[4]. The CKD registry of India found that diabetic nephropathy was the commonest cause (31%) of CKD[5]. About 43% of the CKD patients had a monthly family income of < rupees 5000 (United States $78.26) and RRT has an enormous financial impact on these patients. The majority of end-stage renal disease (ESRD) patients die within months of diagnosis as RRT is unaffordable to them. With risk factors like diabetes, hypertension and obesity showing an increasing trend, the financial strain of supporting RRT services is going to be enormous. Healthcare in India is provided by the public and private sector. In the southern states such as Tamil Nadu, Kerala, Puducherry, Karnataka, Andhra Pradesh, government sponsored health insurance schemes have ensured that tertiary care hospitals provide maintenance haemodialysis, transplantation and follow-up either at very subsidized rate or free of cost and hence they cater to the lower socio economic sections of the society. RRT in private sector is unaffordable to the great majority of ESRD patients in India.

Renal transplantation is the best choice of RRT. India is a pluralistic country in terms of religion and no religion opposes saving a fellow human's life through organ donation. The first successful live renal transplant in India was done at Christian Medical College, Vellore in 1971. The Transplantation of Human Organs Act of 1994 was an initial step in promoting legal organ transplantation in India. Over the past 4 decades, the transplantation rate has shown a steady increase, though still much lower than in developed countries.

CURRENT STATE OF DDT IN INDIA

Deceased donor transplantation (DDT) is increasing in India, due to steps taken in both public and private sector, especially in the southern states. Healthcare spending is increasing partly because of revenue generation[6]. Currently the deceased donor transplantation rate is 0.34 per million which was previously 0.08 per million population in 2013[7]. As shown in Figure 1 and Table 1, a total of 1150 organs from 411 donors have been done harvested in India in 2014, comprising of 720 kidneys, 354 livers, 54 hearts, 16 lungs, 5 pancreas and 1 intestine, out of which 417 organs were harvested in Tamil Nadu. Figure 2 shows that the DDT program has steadily increased in 3 years. The generic immunosuppressive medications and induction molecules being manufactured in India have served as cost cutting measures to support multi-organ transplantation. In the government setup, transplantation services are offered at a free or subsidized cost. The cost of DDT could vary enormously in the private sector depending upon whether they are non-profit organizations or run for a profit. The DDT program in Tamil Nadu has brought down the incidence of organ trade[8].

The main barrier to DDT in India is a shortage of harvested organ pool. In India, each year an estimated 137572 people die in road traffic accidents, and about 70% of them are declared brain dead, from whom organs can be harvested[9]. Tamil Nadu state, with a population of 72 million, has developed a model approach to this problem which is being emulated by other states. Rounds of consultation have been held between the involved stakeholders and government officials to tackle the challenges of ensuring a transparent and efficient transplant program which provides practical guidelines for organ harvesting and transplantation. Government orders issued in this regard have greatly benefitted the DDT program in Tamil Nadu. There is a central coordinator for transplantation in the state of Tamil Nadu who is in charge of the donor list for

Table 1 Deceased organ donors in different states of India in 2014

State	Population	No. of donors	Organ donation rate
Tamil Nadu	72138958	136	1.9
Kerala	33387677	58	1.7
Maharashtra	112372972	52	0.5
Andhra Pradesh	84665533	52	0.6
Karnataka	61130704	39	0.6
Gujarat	60383626	28	0.5
Delhi-NCR	16753235	20	1.2
Puducherry	1244464	13	10.4
Uttar Pradesh	199581477	7	0.04
Chandigarh	1054686	6	5.7

deceased donor transplantation. This list has potential recipients from both public and private hospitals. As per the waiting list, organs are distributed whether it is private or public hospitals.

Public private partnership has been utilized to improve organ harvesting rates from potential organ donors. A total of 2028 transplantations from 677 donors have been done in Tamil Nadu between October 2008 and June 2015, comprising 1201 kidney transplants, 621 liver transplants, 135 heart transplants, 67 lung transplants and 4 pancreas transplants[10]. In addition to the above, 1006 corneal transplants, 616 heart valves, 17 skin donations, 2 small bowel and 1 blood vessels transplant were done in this period. Female donors constituted only 1/5th of the donor pool. Donors comprised from 21 to 50 years of age.

FREE AND AFFORDABLE TRANSPLANTATION IN GOVERNMENT RUN HOSPITALS

In India, government hospitals provide healthcare free of cost or at a subsidized cost for the underprivileged sections of the society. In Government General Hospital (GGH), Chennai, renal transplantation has been regularly performed since 1987. Pre transplant dialysis, work-up of recipient and donor and transplant surgery are provided free of cost. Life-long post-transplant immunosuppression and follow up is provided free. Initially, only living related donor transplantation was done. Though the first deceased donor renal transplantation was done in 1996,the program picked up momentum only in 2008 when the Government of Tamil Nadu gave an impetus and made it one of its "flagship" programs. So far, 172 deceased donor renal transplantations have been done at GGH.

Successful deceased donor transplant program at GGH has achieved the following**.** Access to renal transplantation has improved. Economic and social barriers of transplantation have been broken. The problem of shortage of organs has been taken care of, at least to a small extent. Procurement and supply of less expensive generic immuno-suppressive by the government

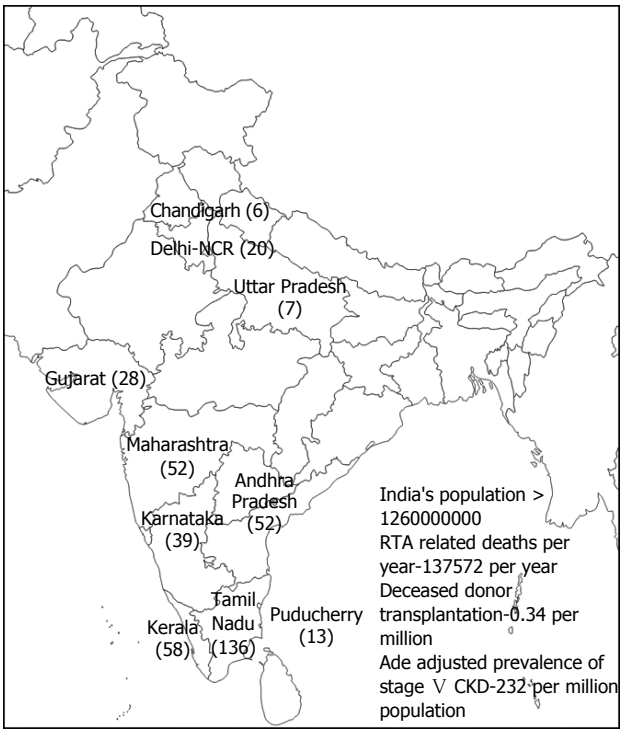

Figure 1 Map of India showing deceased organ donors in different states of India in 2014. CKD: Chronic kidney disease; RTA: Road traffic accidents.

curtails expenditure significantly. Continuous training of nephrologists, urologists, nursing staff and technicians in renal transplantation is creating a trained work force. This program has shown the benefits of "public - private" partnership. Grief counselors at Madras Medical college are provided by Multi Organ Harvesting and Networking (MOHAN) foundation. MOHAN foundation (http://www.mohanfoundation.org/) is a philanthropic non-governmental organization that aims to promote organ donation and transplantation. According to Indian law, a transplant coordinator must be present at every hospital where organ transplantation is being done. The role of the transplant coordinator is to grieve with the family and motivate the family members to get involved in the DDT program, thereby saving lives. MOHAN Foundation, through their educators, has trained 813 transplant coordinators. The MOHAN Foundation signed a memorandum of understanding with the Government General Hospital, Chennai, in 2010 to place its transplant coordinators there. Their presence has made a tremendous difference to the deceased organ donation program in the hospital. When a trained transplant coordinator counsels and spends time with grieving family, conversion figure in getting "yes for donation" is 65% in most hospitals.

THE ROAD AHEAD FOR DECEASED DONOR TRANSPLANTATION IN INDIA

In 2013, there were 137572 road traffic accidents in India. If we convert 50% of this figure into a prospective

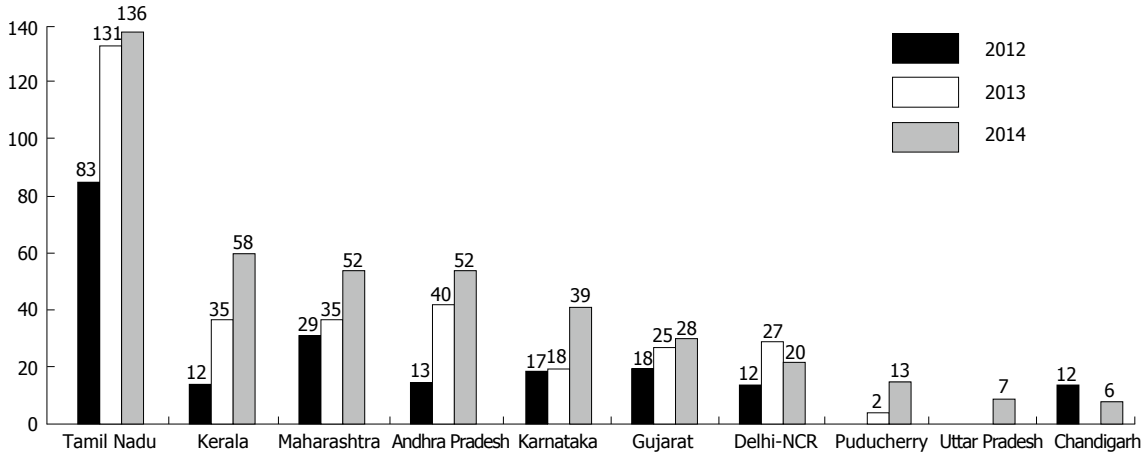

Figure 2 Number of renal transplants done in different states and union territories of India in 3 years (2012-2014).

organ donor pool, we will have more than 65000 donors supplying vital organs including 130000 kidneys yearly to be transplanted for the majority of prevalent end stage renal disease patients who require renal transplantation. The authors have also used poisoned organ donors from snake envenomation leading to brain death which also forms a donor pool of tens of thousands of organ donors in the country. With economic growth and increase in transplant centers to 166 in 2013, more centers undertake organ transplantation since last 2 years in India. It is believed that there are 120000 prevalent patients on maintenance haemodialysis and 10000 prevalent patients on chronic peritoneal dialysis in India, majority of them waiting for a deceased organ donation.

There are number of drawbacks in the DDT scenario in India. Ensuring optimal and prolonged function of the graft in the recipients is a great challenge. A complement dependent cytotoxicicity cross matched technique is the predominant test to detect the compatibility between the donor and recipient. Human leukocyte antigen (HLA) matching is not done in DDT as the large majority of the patients waiting for the organ are not typed to look for HLA mismatch. Advanced immunological facilities for screening and matching are available only in the private sector and those available in a few flagship government hospitals charge a fee for the tests. Flow cytometry and luminex based platforms for testing for HLA based antibodies are not utilized in DDT, because of the lack of facilities. Hence immunological risk is not assessed regularly in recipients waiting for transplants and the long term outcome is unknown as there is a lack of dependable registries to capture the data of graft and patient survival. What we need is to find out the immunological risk to stratify the waiting patients into high, intermediate and low immunological categories. In order to augment the results of the transplantation, we require the following immunological platforms: Cell based assays, flow cytometry based assays, solid phase assays-enzyme linked immunosorbent assay, flowcytometry and microparticle based assays.

Scarcity of nephropathological services in many parts of the country in evaluating graft dysfunction on a speedy basis is another limiting factor of the DDT program. There is a lack of knowledge of prior sensitization in the recipient as panel reactive antibodies are not evaluated in the great majority of recipients. Therapeutic drug monitoring of calcineurin inhibitors and mTOR inhibitors are fraught with inaccurate results due to lack of standardization. The prospective transplant programs should take this into consideration as a next step in promoting trouble free survival of the graft by allocation according to sensitization, avoiding HLA mismatches, careful monitoring and follow up with skilled transplant team. This can only be accomplished with robust support from the respective state governments who should set up a central 24 × 7 laboratory facility which can be cost beneficial to the stakeholders in the long run.

CONCLUSION

The DDT program in India is steadily increasing due to positive steps taken by some state governments. However, organ harvestation rates from potential donors can be further increased. Emphasis must be placed on road safety, less frequent road traffic accidents and higher organ harvestation rates from potential donors. Public awareness on this is on the increasing trend. Public private partnerships have had a positive impact on the DDT program. We foresee in the next 2 decades, India will emerge as the largest deceased donor transplantation in the world. This model of public private partnership in one of the largest developing economies can be emulated by other developing countries in South Asia and African continent.

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P- Reviewer: Chkhotua A, Cantarovich F, Sureshkumar KK
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DOI: 10.5500/wjt.v6.i2.336

World J Transplant 2016 June 24; 6(2): 336-346
ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Basic Study

Role of cytomegalovirus on the maturation and function of monocyte derived dendritic cells of liver transplant patients

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Author contributions: Karimi MH, Shariat A and Yaghobi R contributed equally to this work; Karimi MH and Yaghobi R designed the research; Shariat A performed the research; Shariat A and Yaghobi R analyzed the data; Karimi MH, Shariat A and Yaghobi R wrote the paper; all the authors contribute to the paper.

Institutional review board statement: The study was reviewed and approved by Shiraz University of Medical Sciences.

Informed consent statement: The study was reviewed and approved by Shiraz University of Medical Sciences.

Conflict-of-interest statement: The authors declare no conflicts of interest regarding this manuscript.

Data sharing statement: No data were created so no data are available.

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Received: October 27, 2015
Peer-review started: October 31, 2015
First decision: November 30, 2015
Revised: March 18, 2016
Accepted: April 21, 2016
Article in press: April 22, 2016
Published online: June 24, 2016

Abstract

AIM: To study the impact of association between cytomegalovirus (CMV) pathogenesis with dendritic cell (DC) maturation and function was evaluated in CMV reactivated liver transplanted patients in comparing with non-reactivated ones, and healthy controls.

METHODS: Monocyte derived dendritic cells (MoDCs) was generated from collected ethylenediaminetetraacetic acid-treated blood samples from patient groups and controls. In these groups, expression rates and mean fluorescent intensity of DC markers were evaluated using flowcytometry technique. Secretion of cytokines including: interleukin (IL)-6, IL-12 and IL-23 were determined using enzyme-linked immunosorbent assay methods. The gene expression of toll-like receptor 2 (TLR2), TLR4 and IL-23 were analyzed using in-house real-time polymerase chain reaction protocols.

RESULTS: Results have been shown significant decreases in: Expression rates of MoDC markers including CD83, CD1a and human leukocyte antigen DR (HLA-DR), the mean fluorescence intensitys for CD1a and HLA-DR, and secretion of IL-12 in CMV reactivated compared

with non-reactivated liver transplanted patients. On the other hand, significant increases have been shown in the secretions of IL-6 and IL-23 and gene expression levels of TLR2, TLR4 and IL-23 from MoDCs in CMV reactivated compared with non-reactivated liver transplanted recipients.

CONCLUSION: DC functional defects in CMV reactivated recipients, such as decrease in expression of DC maturation markers, increase in secretion of proinflammatory cytokines, and TLRs can emphasize on the importance of CMV infectivity in development of liver rejection in transplanted patients.

Key words: Cytomegalovirus; Dendritic cells; Liver transplantation

Core tip: Cytomegalovirus (CMV) can interfere with maturation and antigen-presenting function of dendritic cell (DC). This interference with DC function could promote viral spread by paralyzing the adaptive immune system. CMV with DC infection induces inflammatory cytokines and activation of the interferon pathway in transplanted patients. DCs undergo lytic viral cycles, can induce late gene expression of CMV, release of infectious virus, and stimulating of T-cell responses resulted to allograft rejection.

Karimi MH, Shariat A, Yaghobi R, Mokhtariazad T, Moazzeni SM. Role of cytomegalovirus on the maturation and function of monocyte derived dendritic cells of liver transplant patients. *World J Transplant* 2016; 6(2): 336-346 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/336.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.336

INTRODUCTION

Liver transplantation is the definitive treatment of choice for patients with end-stage liver disease[1]. Graft rejection and infection remains major complications post liver transplantation[2]. In liver recipients, cytomegalovirus (CMV) is the most determinative viral infectious pathogen cause of morbidity and mortality post-liver engraft and associate with diminished graft survival[3]. This ubiquitous viral infection in immunocompromised transplant patients belongs to family *Herpesviridae*, subfamily *Betaherpesvirinae*, genus human herpes virus 5, species *Cytomegalovirus*[4]. CMV primary infection results in life-long residence of the virus in the host, and reactivates in immunocompromised individuals frequently. Reactivation of CMV infection and development of related severe diseases and syndromes are common in solid organ recipients may lead to severe complications following transplant, such as acute rejection[5,6]. CMV may lead also to higher rates of bacterial and fungal infections in transplant recipients[7]. In transplant patients, CMV infection causes both direct effects, reflecting cell destruction and indirect effects, such as acute or chronic rejection[8]. Primary CMV infection induced immune related proinflammatory response that was maintained during latency. This continuous activation of the immune system may play a role in the acceleration of chronic diseases and pathogenesis of chronic allograft rejection[9,10].

$CD14^+$ monocytes and/or myeloid progenitor cells are site of CMV latency and are capable of harboring quiescent viral genomes[11]. Monocyte represents a key cell type in the CMV pathogenesis, since mostly represent as an important cellular reservoir for latent virus[12,13]. A number of studies have shown that CMV infection in monocytes is non-permissive and cellular differentiation is prerequisite for CMV replication[11]. CMV replication can be reactivating in latently infected monocytes related to differentiation dependent manner[11]. The dendritic cells (DCs) generated from CMV infected monocytes. CMV infected monocyte derived DCs (CMV-MoDCs) have an altered phenotype and functional defects[14]. DCs are determinative initiators of cellular immunity against CMV infection[1]. DCs also act with superiority over other antigen-presenting cells (APCs) in stimulating T-lymphocyte responses and maintaining protective antiviral immunity[15].

CMV can interfere with maturation and antigen-presenting function of DCs and also disturb both innate and adaptive immunity[14,16]. This interference with DC function could promote viral spread by paralyzing the adaptive immune system[17]. CMV with DC infection induces many hallmarks of innate immunity, such as the production of inflammatory cytokines and activation of the interferon pathway in transplanted patients. This induction is rapid and can promote without requirement of CMV reactivation. DCs undergo lytic viral cycles, can induce late gene expression of CMV, release of infectious virus, and stimulating of T-cell responses resulted to allograft rejection[8].

Therefore, in this study the impact of association between CMV pathogenesis with DC maturation and function was evaluated in CMV reactivated liver transplant patients in comparing with non-reactivated ones and healthy controls.

MATERIALS AND METHODS

Patients and samples

Ten liver transplanted patients who admitted at Transplant Center of Namazi Hospital, Shiraz, Iran were enrolled in this study between years 2012 and 2014. These patients divided to two groups including: 5 patients with CMV reactivation and rest of them without CMV reactivation. Therefore, CMV reactivation was confirmed in these transplanted patients using antigenemia protocol. The CMV antigen positive cells were counted and positive results are reported as one or more CMV pp65 antigen infected cell per 50000

Table 1 Underlying diseases in liver transplanted patients

Underlying diseases	Patients (n = 10)
PSC	2
Hypercholesterolemia	1
Cryptogenic cirrhosis	2
Hepatitis C virus infection	1
Hepatitis B virus infection	1
Wilson disease	1
NASH	1
Autoimmune hepatitis	1

PSC: Primary sclerosing cholangitis; NASH: Non-alcoholic steato-hepatitis.

Table 2 Average means fluorescence intensity for surface markers of monocyte derived dendritic cells in cytomegalovirus reactivated, non-reactivated liver transplanted recipients, and healthy individuals

Surface marker	CMV reactivated patients	CMV non-reactivated patients	Healthy controls of MoDCs
	MFI ± SE	MFI ± SE	MFI ± SE
CD83	30.15 ± 1.06	32.3 ± 2.3	29.2 ± 2.5
CD86	100.5 ± 3.1	103 ± 4.5	83 ± 6.7
CD1a	47 ± 1	80 ± 3	53 ± 13
HLA-DR	49.3 ± 5.4	73.6 ± 6.5	55.8 ± 4.9

The data are the means ± SE. MFI: Mean fluorescence intensity; HLA-DR: Human leukocyte antigen DR; CMV: Cytomegalovirus; MoDC: Monocyte derived dendritic cell.

white blood cells (WBCs). In all 5 patients with CMV reactivation 5 to 7 CMV pp65 antigen infected cell was found per 50000 WBCs, with a mean of 6 ± 1.01 cells/50000 WBCs. Underlying diseases for studied liver transplanted patients have been shown in Tables 1 and 2. The 20 mL ethylenediaminetetraacetic acid (EDTA)-treated blood samples were collected from each evaluated transplant recipients.

Also, 20 mL EDTA-treated blood samples were collected from 5 healthy volunteers as healthy controls. The age range (20-50 years old) and male to female ratio were similar in studied transplanted patient groups and healthy controls. This study was in accordance with the ethical standards of Shiraz University of Medical Sciences Committee (the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki). The informed consent was obtained from each studied transplanted patients. The immunosuppressive conditioning regimen used in liver transplanted patients was previously described[18].

CMV antigenemia assay

CMV antigenemia protocol was performed on the EDTA-treated blood samples to determine viral reactivation by evaluation of the presence of lower matrix pp65 antigen in polymorph nuclear cells using the CMV Brite Turbo kit (IQ Products, Groningen, Netherlands) according manufacturer instruction as previously described[19].

Generation of MoDCs

Leukocytes were isolated from 20 mL EDTA-treated blood samples collected from each liver transplanted patients with and without CMV reactivation using gradient centrifugation through Lymphodex (Inno-train, Germany), the cells from interphase were collected. $CD14^+$ monocytes were isolated by positive selection using a MACS system (Miltenyi Biotech, Bergisch Gladbach, Germany), according to the manufacturer's protocol.

Monocytes were cultured in six-well cell culture plates in RPMI medium (Invitrogen, United States) supplemented with 4 mmol/L L-glutamine (Life technologies, United States), 100 IU/mL penicillin (Life technologies, United States), 10% heat-inactivated fetal bovine serum (Life technologies, United States), 1% sodium pyruvate (Bioidea, Iran), 1% non-essential amino acid (Life technologies, United States), 1000 IU/mL recombinant human granulocyte macrophage-colony stimulating factor (R and D Systems, United Kingdom) and 500 IU/mL recombinant human interleukin-4 (IL-4; R and D Systems, United Kingdom) in a 37 ℃ 5% CO_2 humidified incubator. Every 3 d 200 μL of the medium was exchanged with fresh medium and cytokines. For mature cells, maturation was induced on day 5 by adding 1000 IU/mL recombinant human tumor necrosis factor-α (TNF-α; R and D Systems, United Kingdom) and allowing maturation to proceed for 48 h.

Analysis of MoDC markers

Maturated MoDCs were harvested and stained with fluorescently labeled monoclonal antibodies including: PE-anti-CD14, FITC-anti-CD83, FITC-anti-CD86, FITC-anti-CD1a and FITC-anti-HLADR (eBiosciences, United States). Cell suspension was mixed with antibody solution followed by incubation in the dark at 4 ℃ for 45 min. Cells were suspended in phosphate buffer saline and data acquisition for 10000 events was performed using flowcytometry (Becton Dickinson, San Jose, United States). FlowJo software (Flexera Company, United States) was used to analyze the expression rate and mean fluorescence intensity (MFI) of studied DC markers.

Measurement of cytokine levels

The IL-6, IL-12 and IL-23 cytokines released from MoDCs in culture supernatant were measured using the commercial human enzyme-linked immunosorbent assay Ready-SET-Go kits (eBioscience, United States) according to the manufacturer's protocols.

The gene expression of cytokines and toll-like receptors

Total RNA was extracted from MoDCs using RNX plus (CinnaGen, Iran). RNA samples were reverse transcribed using Reverse Transcriptase (Vivantis, Malaysia) and random hexamer as previously described[20]. An amount of 1 μg total RNA was used to produce cDNA. The primers that were used to analyze the gene transcripts including: TLR-2 (NM_003264.3), TLR-4 (NM_003266.3),

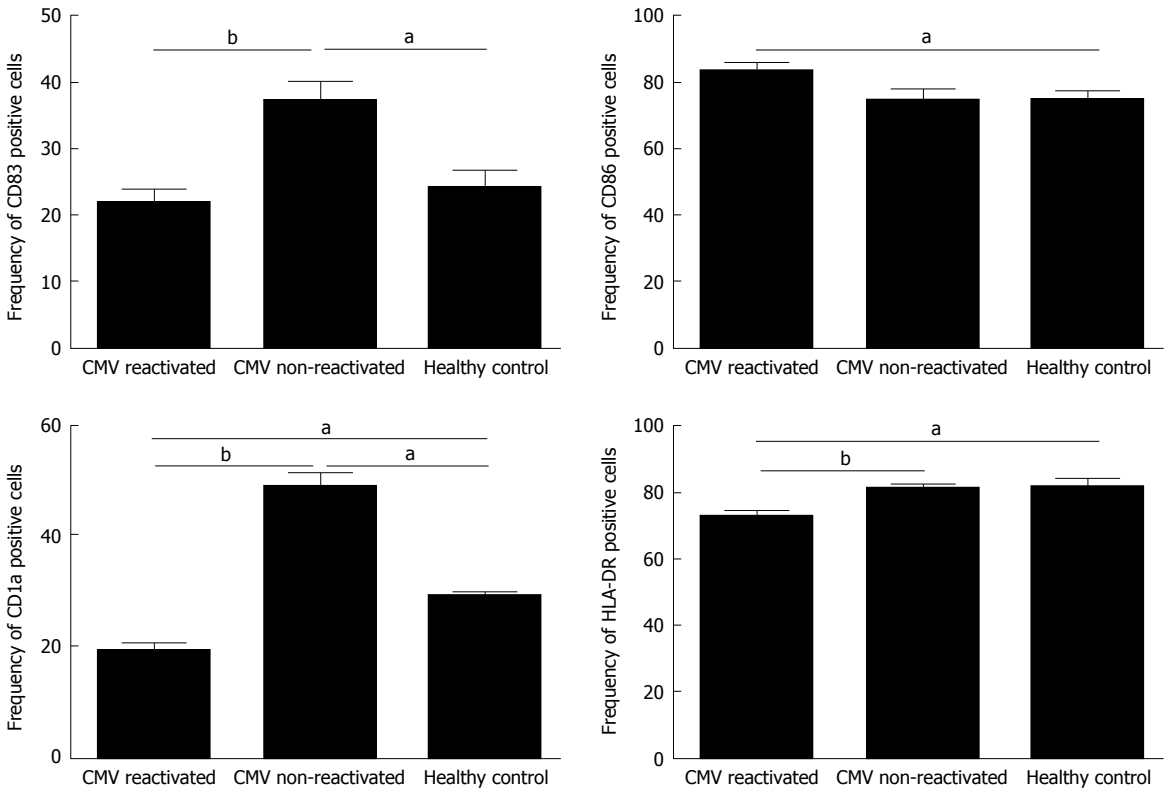

Figure 1 The expression rates for surface monocyte derived dendritic cell markers of CD83, CD86, CD1a and human leukocyte antigen DR in cytomegalovirus reactivated patients, cytomegalovirus non-reactivated recipients, and healthy control. The expression rates of CD83, CD1a and HLA-DR were significantly decreased in CMV reactivated patients *vs* non-reactivated recipients. Any significance is indicated [a]*P* < 0.05, [b]*P* < 0.01. The data are the means ± SE. HLA-DR: Human leukocyte antigen DR; CMV: Cytomegalovirus.

IL-23 (NM_016584.2) and β-actin (NM_001101.3)[18]. The mRNA expression levels of the IL-23, TLR2 and TLR4 genes were finally determined in MoDCs of liver transplanted patients with and without CMV reactivation compared with healthy controls using in-house-real time polymerase chain reaction (PCR) protocols as previously described[18].

The PCR reaction was carried out in a final volume of 20 μL containing: 10 μL SYBR green Premix by Ex taq (Takara, Japan), 0.4 μL SYBR Green Dye, 0.8 μL forward and 0.8 μL reverse primers (8 pmol), 6 μL H_2O and 2 μL cDNA template. The thermal cycling profile was the same for each primer set and consisted of an initial denaturation at 95 ℃ for 2 min, followed by 40 amplification cycles of 95 ℃ for 30 s and 65 ℃ for 20 s using Step One Plus Real-Time instrument (ABI, Step One Plus, United States). The mean Ct value of target genes in each sample was normalized using β-actin gene Ct value to give a ΔCt value. This was then normalized to healthy control (ΔΔCt), and finally the $2^{-\Delta\Delta Ct}$.

Statistical differences between studied groups were evaluated using non-parametric tests of version 15 of SPSS software (Chicago, United States). The sample analysis was also analyzed using version 5 of Graph Pad Prism software (United States). The $2^{-\Delta\Delta Ct}$ value was calculated using Livak method for analysis of the expression level of studied genes. The *P*-value of < 0.05 was considered as significant.

RESULTS

Expression of MoDC markers in patient groups and controls

The expression rate (*P* = 0.02) and MFI (*P* = 0.04) of CD86 were both significantly increased in CMV reactivated patients in comparing with healthy controls with median value of 82% *vs* 75%, respectively (Figures 1 and 2). The expression rate of CD1a was significantly decreased in CMV reactivated patients in comparing with healthy controls with median value of 18% *vs* 30%, respectively (*P* = 0.01) (Figure 3). The expression rate of human leukocyte antigen DR (HLA-DR) was significantly decreased in CMV reactivated patients in comparing with healthy controls with median value of 72% *vs* 84%, respectively (*P* = 0.01) (Figure 4).

The expression rate of CD83 was significantly increased in CMV non-reactivated patients in comparing with healthy controls with median value of 40% *vs* 21%, respectively (*P* = 0.02) (Figure 1). The MFI of CD86 was significantly higher in CMV non-reactivated patients than that in healthy control (*P* = 0.04) (Figure 2). The expression rate of CD1a was significantly raised in CMV non-reactivated patients than that in healthy controls with median value of 50% *vs* 30%, respectively (*P* = 0.01) (Figure 1). The MFI of HLA-DR was significantly raised in CMV non-reactivated liver transplanted patients than that in healthy controls (*P* =

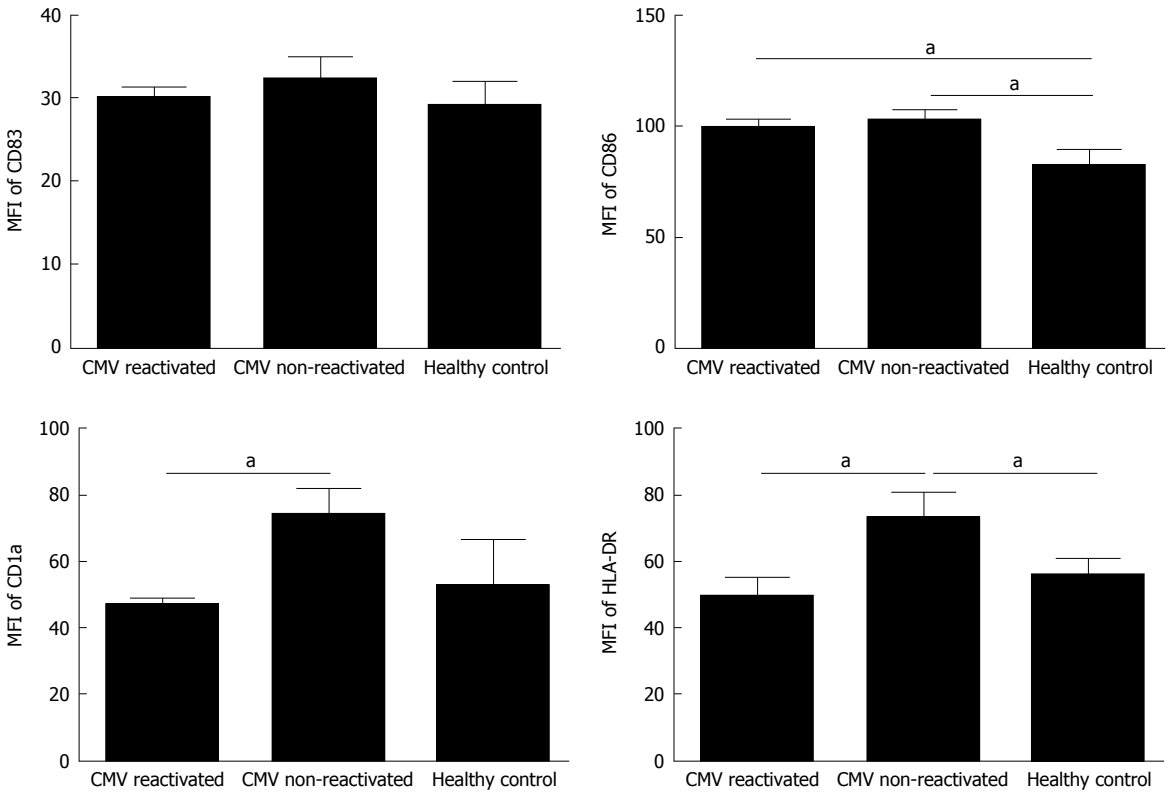

Figure 2 Mean fluorescence intensity for surface monocyte derived dendritic cell markers of CD83, CD86, CD1a and human leukocyte antigen DR in cytomegalovirus reactivated patients, cytomegalovirus non-reactivated recipients, and healthy control. The MFI of CD1a and HLA-DR were significantly decreased in CMV reactivated patients *vs* non-reactivated recipients. Any significance is indicated [a]*P* < 0.05. The data are the means ± SE. MFI: Mean fluorescence intensity; HLA-DR: Human leukocyte antigen DR; CMV: Cytomegalovirus.

0.03) (Figure 5).

Expression rate of CD83 was significantly decreased in CMV reactivated compared with non-reactivated liver transplanted patients with median value of 22% *vs* 40%, respectively (*P* = 0.007) (Figure 1). The expression rate (*P* = 0.007) and MFI (*P* = 0.02) of CD1a was significantly lower in CMV reactivated compared with non-reactivated patients with median value of 18% *vs* 50%, respectively (Figures 1 and 2). The expression rate (*P* = 0.007) and MFI (*P* = 0.03) of HLA-DR was significantly decreased in CMV reactivated patients compared with non-reactivated recipients with median value 72% *vs* 80%, respectively (Figures 1 and 2).

Cytokine secretions by MoDCs in patient groups and controls

The secretion of IL-6 from MoDCs of CMV reactivated patients was significantly increased in comparing with healthy control with mean of 334.6 ± 2.2 pg/mL *vs* 312 ± 1.08 pg/mL (*P* = 0.009) (Figure 6A). The secretion of IL-12 from MoDCs of CMV reactivated patients was significantly decreased in comparing with healthy control with mean of 1.26 ± 0.04 pg/mL *vs* 1.54 ± 0.03 pg/mL (*P* = 0.01) (Figure 6B). The IL-23 secretion level from MoDCs of CMV reactivated patients was significantly increased in comparing with healthy control with mean of 13.53 ± 0.09 pg/mL *vs* 13.1 ± 0.1 pg/mL (*P* = 0.02) (Figure 6C).

The secretion of IL-6 from MoDCs of CMV non-reactivated patients was significantly decreased in comparing with healthy control with mean of 261.2 ± 3.72 pg/mL *vs* 312 ± 1.08 pg/mL (*P* = 0.006) (Figure 6A). Also, secretion of IL-12 from MoDCs of CMV non-reactivated patients was significantly increased in comparing with healthy control with mean of 1.98 ± 0.03 pg/mL *vs* 1.54 ± 0.03 pg/mL (*P* = 0.009) (Figure 6B). The secretion of IL-23 from MoDCs of CMV non-reactivated patients was significantly decreased in comparing with healthy control with mean of 8.77 ± 0.19 pg/mL *vs* 13.1 ± 0.1 pg/mL (*P* = 0.008) (Figure 6C).

The secretion of IL-6 from MoDCs was significantly higher in CMV reactivated patients than that in CMV non-reactivated ones with mean of 334.6 ± 2.2 pg/mL *vs* 261.2 ± 3.72 pg/mL (*P* = 0.005) (Figure 6A). The secretion of IL-12 from MoDCs was significantly lower in CMV reactivated patients compared with non-reactivated ones with mean of 1.26 ± 0.04 pg/mL *vs* 1.98 ± 0.03 pg/mL (*P* = 0.007) (Figure 6B). The secretion of IL-23 from MoDCs was significantly higher in CMV reactivated patients than that in CMV non-reactivated ones with mean of 13.53 ± 0.09 pg/mL *vs* 8.77 ± 0.19 pg/mL (*P* = 0.007) (Figure 6C).

Cytokine and TLR gene expression by MoDCs in patient groups and controls

The IL-23, TLR2, and TLR4 mRNAs was expressed 5.2

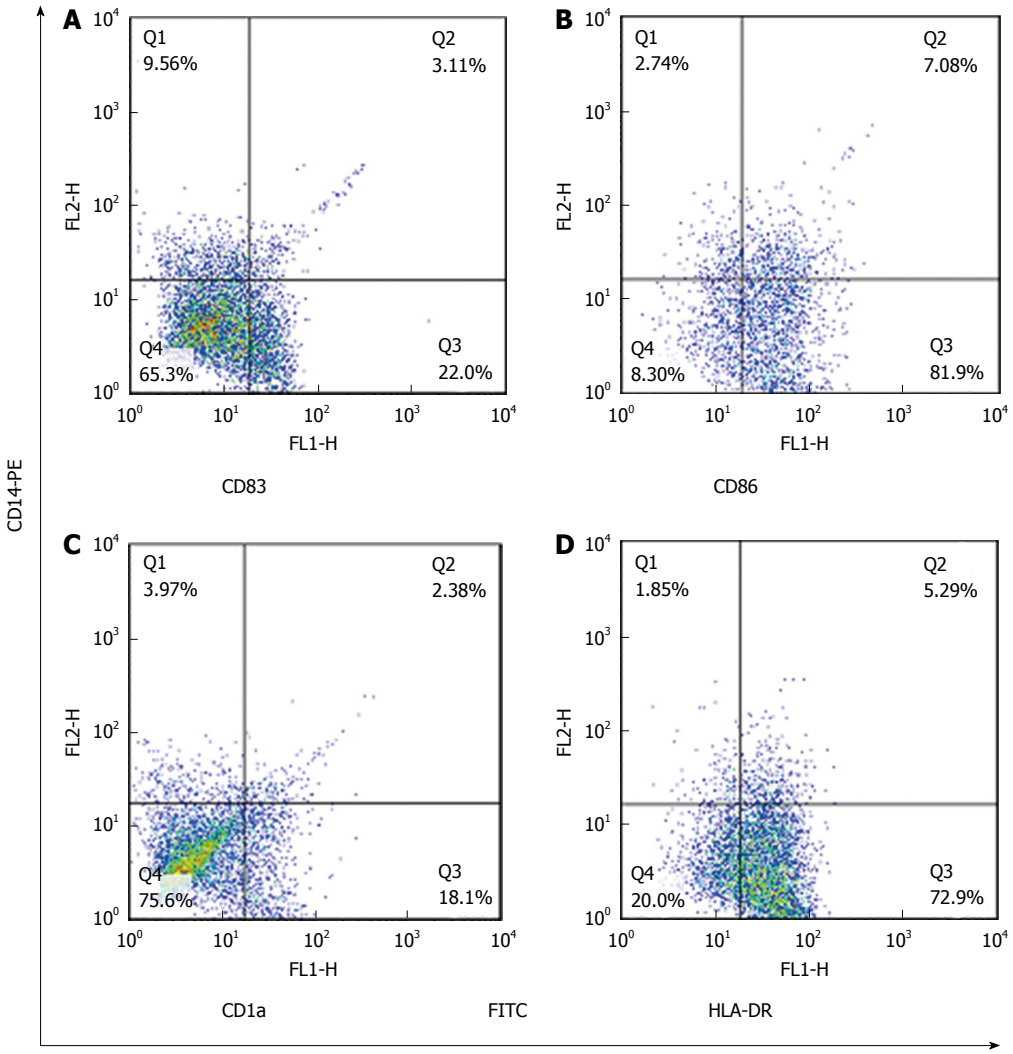

Figure 3 The expression rate of monocyte derived dendritic cell markers in cytomegalovirus reactivated patients was examined by dual-color cytometry. Expression of surface markers: CD83 (22%) (A), CD86 (82%) (B), CD1a (18%) (C) and HLA-DR (72.9%) (D) on MoDCs in CMV reactivated patients. CD14-PE, phycoerythrin-conjugated CD14, CD83-FITC, fluorescein isothiocyanate-conjugated CD83, CD86-FITC, fluorescein isothiocyanate-conjugated CD86, CD1a-FITC, fluorescein isothiocyanate-conjugated CD1a, HLADR-FITC, fluorescein isothiocyanate-conjugated HLA-DR. HLA-DR: Human leukocyte antigen DR; CMV: Cytomegalovirus; MoDC: Monocyte derived dendritic cell.

(P = 0.005), 3.6 (P = 0.007), and 4.3 (P = 0.009) folds significantly more in CMV reactivated patients compared with healthy controls, respectively (Figure 7).

The gene expression level of IL-23 was the same in both CMV non-reactivated patients and healthy controls (P = 0.6) (Figure 7A). The TLR2 mRNA expression was significantly decreased in CMV non-reactivated patients in comparing with healthy controls (P = 0.02) (Figure 7B). The IL-23, TLR2, and TLR4 mRNAs was expressed 4.7 (P = 0.007), 5.4 (P = 0.005), and 2.8 (P = 0.01) folds significantly more in CMV reactivated compared with non-reactivated patients, respectively (Figure 7).

DISCUSSION

DCs have superiority over other APCs in viral infections to stimulate T-cell responses and maintaining protective antiviral immunity[15]. DCs are critical initiators of cellular immunity against viruses especially CMV[1]. CMV as a determinative human pathogen can cause fatal complications and promote rejection in transplanted recipients[21]. CMV infection can also increase the rate of immunosuppression with interfering the maturation and function of DCs post-transplantation[16,22]. Therefore, in this report, the effects of CMV reactivation compared to non-reactivation were elucidated on maturation and function of DCs in liver transplanted recipients and healthy controls.

CMV and DC maturation was interested of related researchers in earlier studies[16,22]. Down-regulation of major histocompatibility complex (MHC) class Ⅰ and CD86 and CD83 costimulatory molecules on immature DCs and inhibition of DC maturation was indicated following *in vitro* CMV infection of DCs[16]. CMV targets DCs and alters their functions by interfering with MHC-Ⅱ biosynthesis and maturation, as well as with the expression and function of related endocytic proteases[23]. CMV-infected DCs displayed abnormal

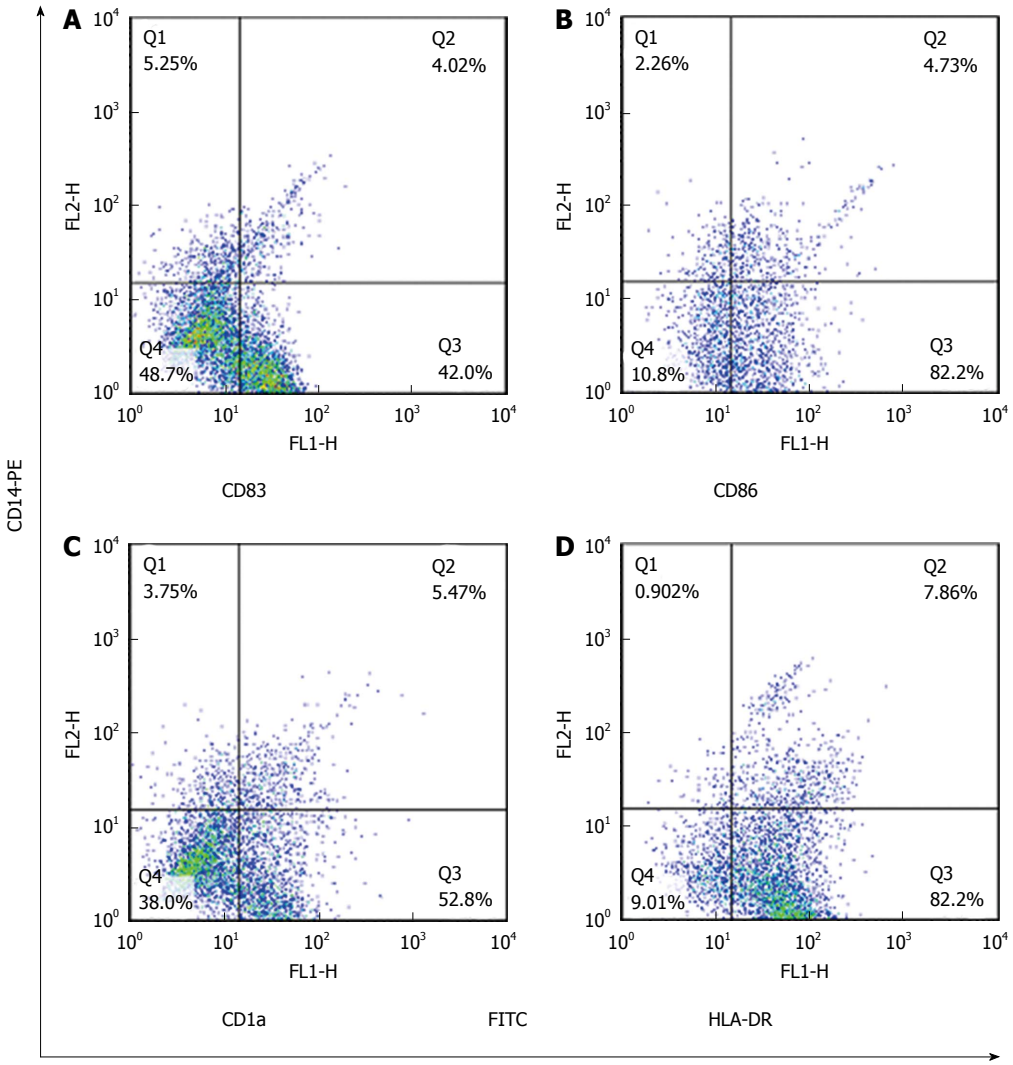

Figure 4 The expression rate of monocyte derived dendritic cells markers in cytomegalovirus non-reactivated patients was examined by dual-color cytometry. Expression of surface markers: CD83 (42%) (A), CD86 (82%) (B), CD1a (52.8%) (C) and HLA-DR (82%) (D) on MoDCs in CMV non-reactivated patients. HLA-DR: Human leukocyte antigen DR; CMV: Cytomegalovirus; MoDC: Monocyte derived dendritic cell.

phenotypic characteristic and stable expression of CD83 as a maturation marker[24,25]. Infectivity of CMV in MoDCs resulted in maturation of the surviving cells, up-regulation of the CD86, and down-regulation of MHC-Ⅰ and Ⅱ[21,26]. In this study, results also demonstrated the down-regulation of CD83, CD1a and HLA-DR molecules on MoDCs in CMV reactivated compared to non-reactivated liver transplanted patients. The MFIs of CD1a and HLA-DR were significantly down-regulated in CMV reactivated patients compared to non-reactivated ones. On the other hand CMV-mediated up-regulation of CD86 and down-regulation of CD1a and HLA-DR molecules were found in CMV reactivated patients compared to healthy controls. Therefore CMV interference with maturation of DCs promotes viral spread by paralyzing the adaptive immune system[17]. Especially, in transplanted recipients, CMV-infected DCs are less capable of developing antiviral activated APCs, this may lead to impaired immune responses not only against CMV, but most likely also against other invading microorganisms.

Stimulation of toll-like receptors (TLRs) on DCs activates signal transduction pathways lead to induction of a range of antimicrobial genes and inflammatory cytokines[11,27]. TLR signaling pathways trigger a series of interactions among specific intracellular mediators that ultimately result in the release of nuclear factor-κB (NF-κB) from its related endogenous inhibitors[11]. Earlier reports emphasized that markedly up-regulation of TLR2 and TLR4 responsible for early activation of alloimmune T-cells favoring to acute renal and also liver allograft rejection[28-30]. TLR2 was recently identified as a cell surface receptor activates secretion of inflammatory cytokine response to CMV infection[4,11]. *In vitro* stimulation of TLR2 by CMV resulted in NF-κB activation and cytokine secretion[31,32]. CMV was also able to activate TLR4 and mediate cytokine secretion in human monocytic cells[33]. Similarly, *TLR2* and *TLR4* gene expression by MoDCs was significantly increased in studied CMV reactivated liver transplanted patients compared to non-reactivated ones and healthy controls.

Pathological processes associated with CMV reac-

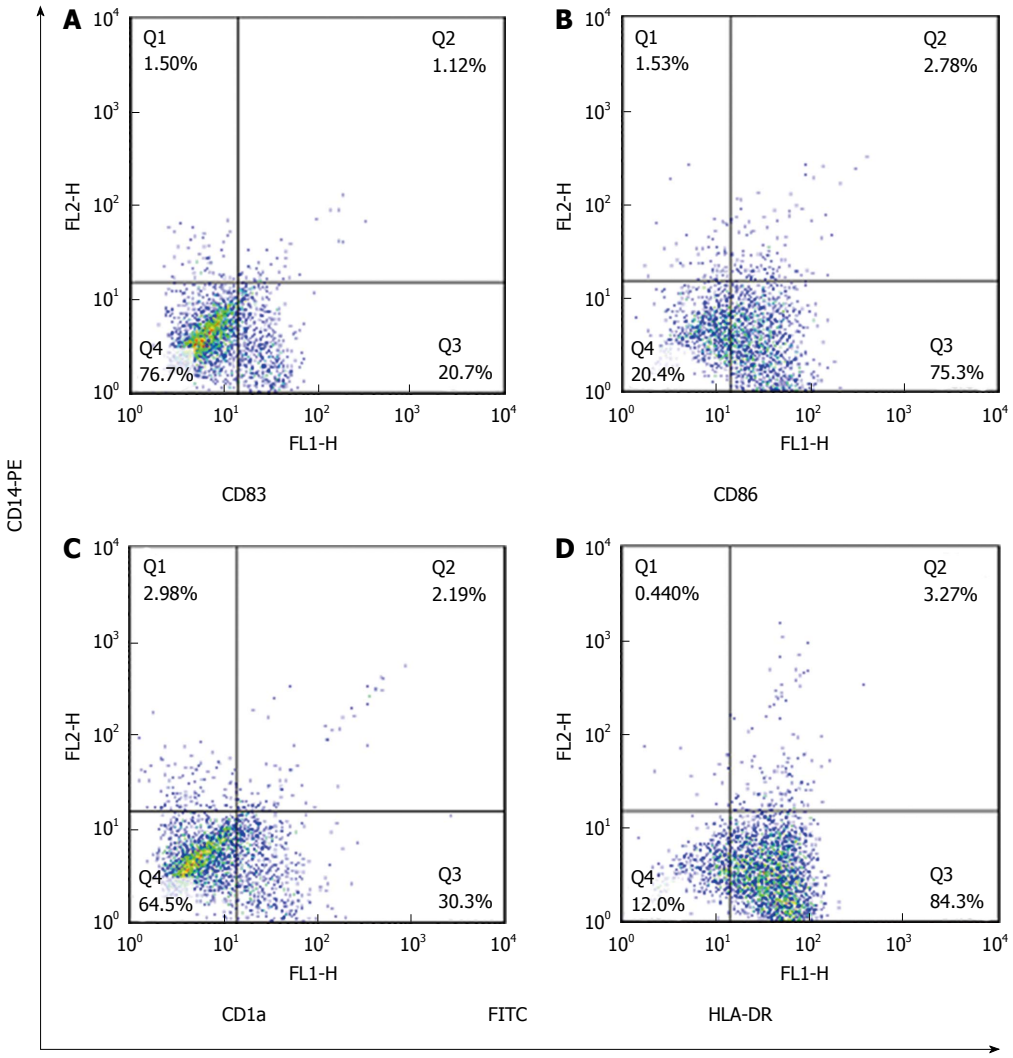

Figure 5 The expression rate of monocyte derived dendritic cell markers in healthy control was examined by dual-color cytometry. Expression of surface markers: CD83 (20.7%) (A), CD86 (75.3%) (B), CD1a (30%) (C) and HLA-DR (84%) (D) on MoDCs in healthy control. HLA-DR: Human leukocyte antigen DR; MoDC: Monocyte derived dendritic cell.

tivation appear to be mediated by the release of inflammatory cytokines[11]. Following CMV infection DCs produce no IL-12 and only low levels of TNF-α[16]. Down-regulation of IL-12 production impairs the antiviral mechanisms of T cells and NK cells in patients with active CMV infection[34]. Similar to previous reports, results of the present study revealed that secretion of IL-12 by MoDCs was significantly decreased in CMV reactivated liver transplanted patients compared to CMV non-reactivated ones and healthy controls. But, Th17 cell linage and related cytokines like IL-17 and IL-23 have determinative role contribute to the mechanisms of allograft rejection[35-39]. IL-6 is also essential for differentiation of IL-17-producing human Th cells[40]. IL-6 and subsequent signaling pathways are important for activation and differentiating DCs. Activation and concomitant production of these cytokines also appear to be essential for reactivation and replication of CMV in infected patients such as transplant recipients[21]. Similarly, IL-6 and IL-23 secretion and expression by MoDCs are significantly higher in CMV reactivated in comparing with non-reactivated patients and healthy controls.

In conclusion, results of this study highlight the fact that, CMV and DCs contractions promote different pathways including: Interference with the maturation and expression of DC markers (CD83, CD1a and HLA-DR), IL-12 decreasing and IL-6 and IL-23 elevation from MoDCs and also increase of the mRNA expression levels of *TLR2*, *TLR4* and *IL-23* genes in MoDCs of CMV reactivated liver transplanted patients. These pathways can implicate in the development of acute or chronic allograft liver rejection.

ACKNOWLEDGMENTS

The study was financially supported using a grant from Iran National Science Foundation. We also thanks to Transplant Research Center of Namazi Hospital, Shiraz, Iran for Lab support.

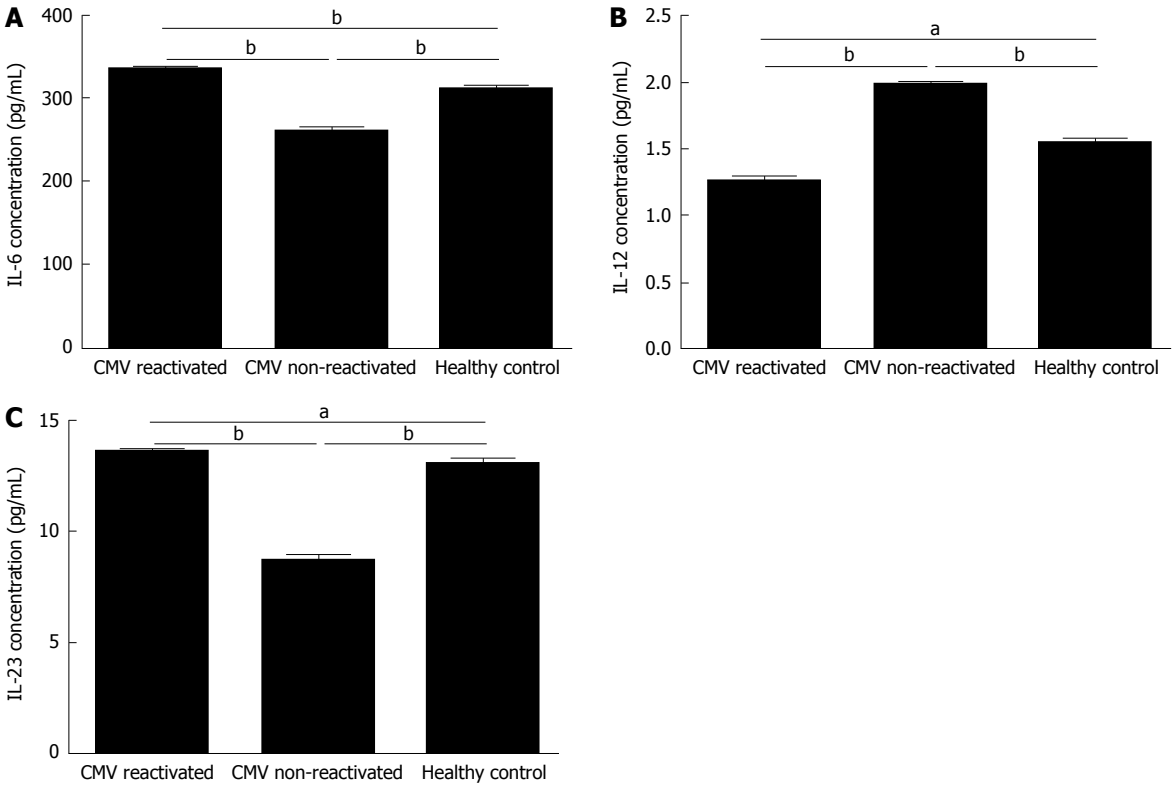

Figure 6 Levels of cytokines secreted by monocyte derived dendritic cells in cytomegalovirus reactivated patients, non-reactivated recipients, and healthy controls. The IL-6, IL-12 and IL-23 concentrations in the supernatants were measured by enzyme-linked immunosorbent assay. IL-6 and IL-23 secretions were significantly increased in CMV reactivated patients compared to non-reactivated recipients and healthy controls (A and C). IL-12 secretion was significantly decreased in CMV reactivated patients compared to non-reactivated ones and healthy controls (B). Any significance is indicated [a]$P < 0.05$, [b]$P < 0.01$. The data are the means ± SE. CMV: Cytomegalovirus; IL: Interleukin.

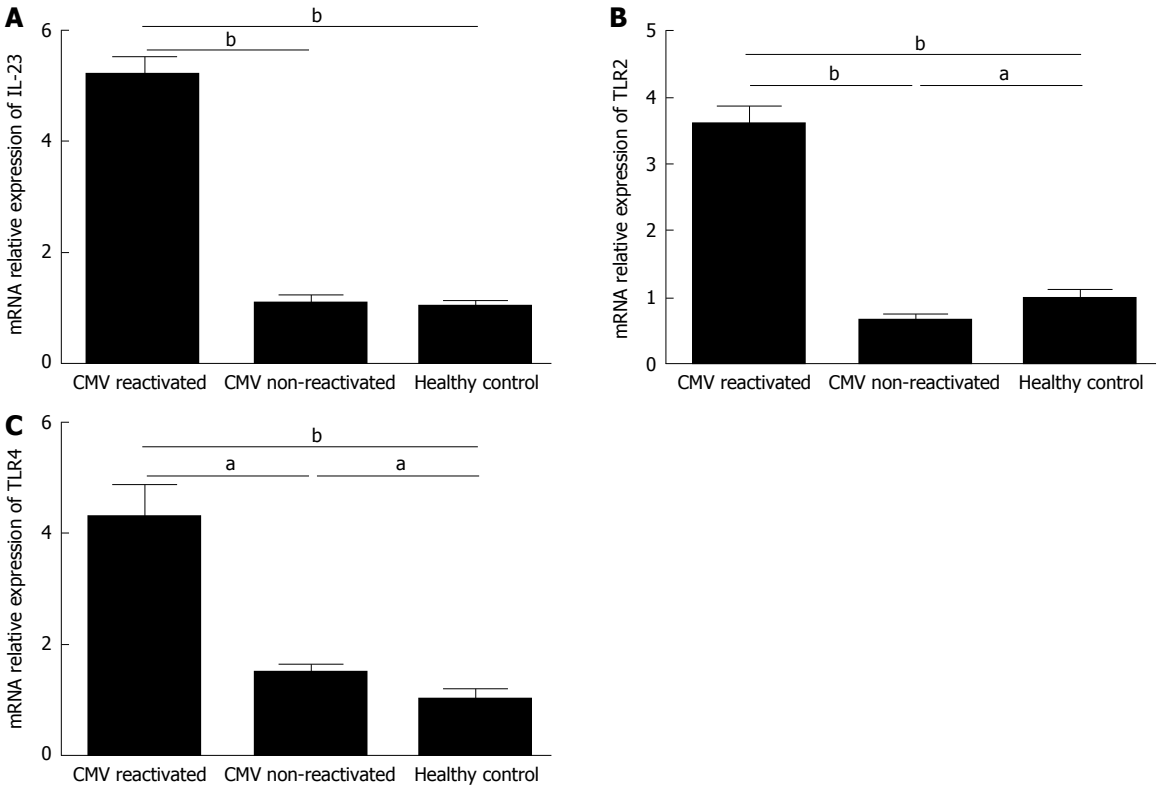

Figure 7 In cytomegalovirus reactivated patients, non-reactivated recipients, and healthy controls, mRNA relative expressions of interleukin-23 (A), toll-like receptor 2 (B) and toll-like receptor 4 (C) were determined by real-time polymerase chain reaction protocols. The gene expression levels of IL-23, TLR2 and TLR4 were significantly increased in CMV reactivated patients in comparing with non-reactivated ones and healthy controls. Any significance is indicated [a]$P < 0.05$, [b]$P < 0.01$. The data are the means ± SE. CMV: Cytomegalovirus; IL: Interleukin; TLR: Toll-like receptor.

COMMENTS

Background

Cytomegalovirus (CMV) is the most determinative viral infectious pathogen cause of morbidity and mortality post-liver engraft and associate with diminished graft survival. CMV infected monocyte derived dendritic cells (CMV-MoDCs) have an altered phenotype and functional defects. DCs are determinative initiators of cellular immunity against CMV infection. CMV can interfere with maturation and antigen-presenting function of DCs and also disturb both innate and adaptive immunity. DCs undergo lytic viral cycles, can induce late gene expression of CMV, release of infectious virus, and stimulating of T-cell responses resulted to allograft rejection. However, association between CMV pathogenesis with DC maturation and function in CMV reactivated liver transplant patients was not yet evaluated.

Research frontiers

CMV can interfere with maturation and antigen-presenting function of DCs and also disturb both innate and adaptive immunity. This interference can promote viral spread by paralyzing the adaptive immune system. CMV with DC infection induce the production of inflammatory cytokines and activation of the interferon pathway in transplanted patients.

Innovations and breakthroughs

This is the first study evaluating the interferance between CMV reactivation with maturation and antigen-presenting function of DCs in Iranian liver transplanted patients.

Applications

Interference of CMV and DCs can promote viral spread by paralyzing the adaptive immune system and induce the production of inflammatory cytokines and activation of the interferon pathway in transplanted patients. Results of this study highlight the fact that, CMV and DCs contractions promote different pathways including: Interference with the maturation and expression of DC markers and cytokines in MoDCs of CMV reactivated liver transplanted patients. These pathways can implicate in the development of acute or chronic allograft liver rejection.

Terminology

CMV infected MoDCs have an altered phenotype and functional defects. DCs are determinative initiators of cellular immunity against CMV infection. DCs also act with superiority over other antigen-presenting cells in stimulating T-lymphocyte responses and maintaining protective anti-CMV immunity.

Peer-review

The manuscript entitled, "Role of cytomegalovirus on the maturation and function of monocyte derived dendritic cells of liver transplant patients", by Karimi *et al*, demonstrated the functional defects of dendritic cells in CMV reactivated liver transplant recipients when compared to those without CMV reactivation or healthy norms demonstrating the differences in cytokine concentrations and expressions. This is very detailed investigation of cytokines of monocyte derived dendritic cells.

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World J Transplant 2016 June 24; 6(2): 347-355
ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Single-lung transplantation in emphysema: Retrospective study analyzing survival and waiting list mortality

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Author contributions: All authors contributed equally to design, case retrieval and analysis; Borro JM drafted the manuscript; all authors reviewed it and contributed to the final approved version.

Institutional review board statement: This study was approved by the institutional review board of Galicia (Registre code 2012/235).

Informed consent statement: Patients were required to give informed consent to the study.

Conflict-of-interest statement: We have no financial relationships or conflicts of interest to disclose.

Data sharing statement: No additional data are available.

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Received: November 28, 2015
Peer-review started: November 30, 2015
First decision: December 28, 2015
Revised: January 31, 2016
Accepted: March 9, 2016
Article in press: March 14, 2016
Published online: June 24, 2016

Abstract

AIM: To performed remains a subject of debate and is the principal aim of the study.

METHODS: This retrospective analysis included 73 patients with emphysema (2000-2012). The outcomes of patients undergoing single-lung transplantation (SL) (n = 40) or double-lung transplant (DL) (n = 33) were compared in a Cox multivariate analysis to study the impact of the technique, postoperative complications and acute and chronic rejection on survival rates. Patients were selected for inclusion in the waiting list according to the International Society of Heart Lung Transplantation criteria. Pre and postoperative rehabilitation and prophylaxis, surgical technique and immunosuppressive treatment were similar in every patients. Lung transplantation waiting list information on a national level and retrospective data on emphysema patient survival transplanted in Spain during the study period, was obtained from the lung transplantation registry managed by the National Transplant Organization (ONT).

RESULTS: Both groups were comparable in terms of gender and clinical characteristics. We found significant differences in the mean age between the groups, the DL patients being younger as expected from the inclusion criteria. Perioperative complications occurred in 27.6% SL *vs* 54% DL (P = 0.032). Excluding perioperative mortality, median survival was 65.3 mo for SL and 59.4 mo for DL (P = 0.96). Bronchiolitis obliterans and overall 5-year survival were similar in both groups. Bacterial

respiratory infection, cytomegalovirus and fungal infection rates were higher but not significant in SL. No differences were found between type of transplant and survival (P = 0.48). To support our results, national data on all patients with emphysema in waiting list were obtained (n = 1001). Mortality on the waiting list was 2.4% for SL *vs* 6.2% for DL. There was no difference in 5 year survival between 235 SL and 430 DL patients transplanted (P = 0.875).

CONCLUSION: Our results suggest that SL transplantation in emphysema produce similar survival than DL with less postoperative complication and significant lower mortality in waiting list.

Key words: Lung transplantation; Single-lung; Survival; Complications; Emphysema; Double-lung

Core tip: This is a retrospective and comparative study of 2 cohort of patients with advanced-stage emphysema who were performed uni or bilateral lung transplant. The results of this study support the realization of single-lung transplantation in most of the cases of emphysema because it is technically simpler, it has less risk of surgical sutures, and finally it has less immediate postoperative complications. Single and double lung transplantation has a similar long-term survival. Moreover, if a second transplant is needed in the long-term, the contralateral transplantation has the same initial transplant survival if the patient remains in a similar clinical situation. Survival Spanish national register does not show difference between the two techniques too, supporting the results of our relatively small series. This strategy of performing single lung transplantation in most of the cases of emphysema would encourage and enhance the use of donors thanks to the twining procedure, and would decrease mortality in the waiting list as shown in the National Transplant Organization patients analysed. Proper pre and postoperative prophylaxis and postoperative early extubation protocol is essential to achieve good results.

Borro JM, Delgado M, Coll E, Pita S. Single-lung transplantation in emphysema: Retrospective study analyzing survival and waiting list mortality. *World J Transplant* 2016; 6(2): 347-355 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/347.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.347

INTRODUCTION

The number of solid organ transplantations performed in Spain has steadily increased in the last 20 years from around 1300 in 1989 to over 4000 in 2009[1]. This has been possible thanks to a greater number of donors, achieved as a consequence of a set of organizational measures, known as the "Spanish Model", directed by the Spanish National Transplant Organization (ONT)[1]. However, this increase in organ donation does not meet all lung transplantation requirements and, as in the rest of the world, organ availability remains the main limitation[2]. The latest data available indicate that 238 lung transplantations, including combined transplantations, were performed in Spain in 2012. Only 48% of candidates on the waiting list were transplanted during this same year[2]. The median time on the waiting list was 163 d in 2011 (interquartile range: 65-303). Waiting list mortality in the same period was 4.6%. Moreover, 2.7% were taken off the waiting list due to clinical worsening[3].

Advanced-stage pulmonary emphysema is the most common indication for lung transplantation[4]. In 2011, 29.5% of advanced emphysema patients were in need of this intervention, 26.2% of whom had chronic obstructive pulmonary disease (COPD) type 1, and 3.3% α1-antitrypsin deficiency[4]. The question remains whether single or double-lung transplantation should be performed[5-9]. Single-lung transplantation (SL) has the advantage of making optimal use of available organs, but some studies indicate better outcomes and survival in patients with double-lung transplant (DL) compared to SL recipients[8,10]. However, SL is often performed in older and retransplanted patients, who generally present with more comorbidities[11]. One of the main problems in SL is native lung hyperinflation[12,13], but minimally invasive techniques such as video-assisted thoracoscopic surgery (VATS)[14] or bronchoscopic lung volume reduction[15,16] are now available and have shown good results. An appropriate clinical protocol implementing these recent developments might help narrow the gap in survival rates of single and DL patients reported in the literature.

Our first goal was to evaluate our group's 10-year experience in lung transplantation in patients with emphysema, in order to assess and compare survival and outcomes of SL and DL recipients. We further compared and assessed our results against national data available in the Spanish Lung Transplant Registry (RETP), in order to explore the impact of SL or DL on patient survival and waiting list progress, and discuss the consequences of the different approaches.

MATERIALS AND METHODS

Local (single-center) data

We conducted a retrospective study of the records of patients transplanted in our center between 2000 and 2012. This study was approved by the Clinical Research Ethics Committee of the Galician Healthcare Authorities overseeing Complejo Hospitalario Universitario A Coruña (CHUAC). All procedures were performed in compliance with Spanish regulations and the Declaration of Helsinki.

Spanish national data

Further assessment of our data required comparison

Table 1 Demographic, clinical characteristics, and complications of patients in the single-center series *n* (%)

	Study cohort *n* = 73	Single-lung *n* = 40	Double-lung *n* = 33	*P*-value
Age in years (mean ± SD)	54.9 ± 7.1	57.3 ± 6.1	51.9 ± 7.3	0.001
Gender (male)	62 (85%)	33 (82.5%)	29 (87.9%)	0.520
Underlying disease				0.940
α1-antitrypsin deficiency	18 (24.6%)	10 (25%)	8 (24.2%)	
Chronic obstructive pulmonary disease	55 (75.3%)	30 (75%)	25 (75.2%)	

with Spanish national registry data. The 7 centers performing lung transplantation in Spain started around the same time; the economic and social status of the population attended is similar in all of them, and all use similar techniques and postoperative care protocols.

Lung transplantation waiting list information (1999 to 2012) on a national level was obtained from the ONT. All Spanish transplantation teams pool their data in the RETP that began its activity in 2001, the first year of complete data availability. Follow up information from patients transplanted between 2001 and 2012 was selected.

General care protocol for lung transplant recipients

Patients were selected for inclusion in the waiting list according to the International Society of Heart Lung Transplantation criteria[17]. Between 2000 and 2003, DL was preferred in emphysema patients < 60 years of age, and SL was reserved for those > 60 years or with comorbidities. However, in view of the good clinical results with SL, we decided in 2003 that SL would be the preferred approach in all cases, including α1-antitrypsin deficiency. According to our protocol, the patient performs physical and respiratory exercises during the waiting period. Postoperative tracheostomy is used when necessary due to prolonged intubation. In addition, SL candidate receive antifungal prophylaxis with weekly amphotericin B lipid complex *via* aerosol. Patients with a history of recurrent infections also receive tobramycin before surgery[18].

The surgical technique used by our group has not undergone substantial changes since our program began in 1999, and is similar to the recently described[19]. Ventilation difficulties during the immediate postoperative period are critical in SL in emphysema[20]. Patients are routinely extubated in the surgery room, or within the following 6 h whenever possible. This is possible in most cases, but patients requiring assisted ventilation after surgery also benefit from an optimized care protocol, including ventilation with 2 respiratory units.

Immunosuppressive treatment is described elsewhere[18], and consisted of triple therapy including basiliximab for induction, oral or intravenous cyclosporine, azathioprine and decreasing doses of corticosteroids in all cases. Cyclosporine and/or azathioprine were switched to tacrolimus and/or mycophenolate after repeated acute rejection or persistent rejection. All patients received antibacterial prophylaxis with amoxicillin and clavulanic acid, modified after transplantation according to the postoperative cultures of bronchial aspirate. In addition, all patients received antiviral prophylaxis with valganciclovir for 3-6 mo depending on their Cytomegalovirus (CMV) serology risk; antifungal prophylaxis with fluconazole, followed by amphotericin B lipid complex as described elsewhere[21], and trimethoprim with sulfamethoxazole on alternate days were prescribed to prevent infection by *Pneumocystis carinii.*

Hyperinflation during the long-term postoperative period was treated with surgical or bronchoscopic volume reduction[22].

Statistical analysis

Transplanted patients (in our center and on the national waiting list) were classified into 2 groups: SL or DL transplantation. Combined transplants were not included.

The total of 73 patients in this single-center analysis (see RESULTS) allows for the detection of a hazard ratio (HR) $\geqslant$ 2.6 with a confidence of 95%, and a statistical power of 80%, assuming 56% of censored measurements (percentage of patients alive after 5 years) and 54.7% of exposed patients (percentage of patients receiving DL transplantation). A retrospective analysis of the single-center data was performed comparing the demographic characteristics of the SL and DL groups, followed by univariate analysis to compare the qualitative variables, using the χ^2 test and student *t*-test for quantitative ones. Kaplan-Meier survival curves were compared using the log-rank test. The impact of the type of transplant, infections (cytomegalovirus or bacterial), and acute and chronic rejection on patient survival was determined in the local setting using a Cox multivariate analysis. Statistical analyses were performed with SPSS 16.0. The statistical analyses were review by Professor Salvador Pita, head of biostatistics department and paper coautor.

RESULTS

A total of 280 patients were transplanted in CHUAC between 2000 and 2012, of whom 73 had a previous diagnosis of advanced-stage pulmonary emphysema: 40 underwent SL and 33 received DL. Both groups were comparable in terms of gender and clinical characteristics (Table 1). We found significant differences in the mean age between the groups, the DL patients being younger (Table 1), as expected from the inclusion criteria.

The average preoperative forced expiratory volume in 1 s (FEV_1) was 22.89% ± 6.95% (range 12%-49%). The median follow-up of the series was 67.4 mo (range: 0-156.5 mo; interquartile range: 22.4-96.4). Perioperative medical and surgical complications (hemothorax, lung edema, broncho-vascular sutures problems) were reported in 11 patients (27.6%) in the SL group,

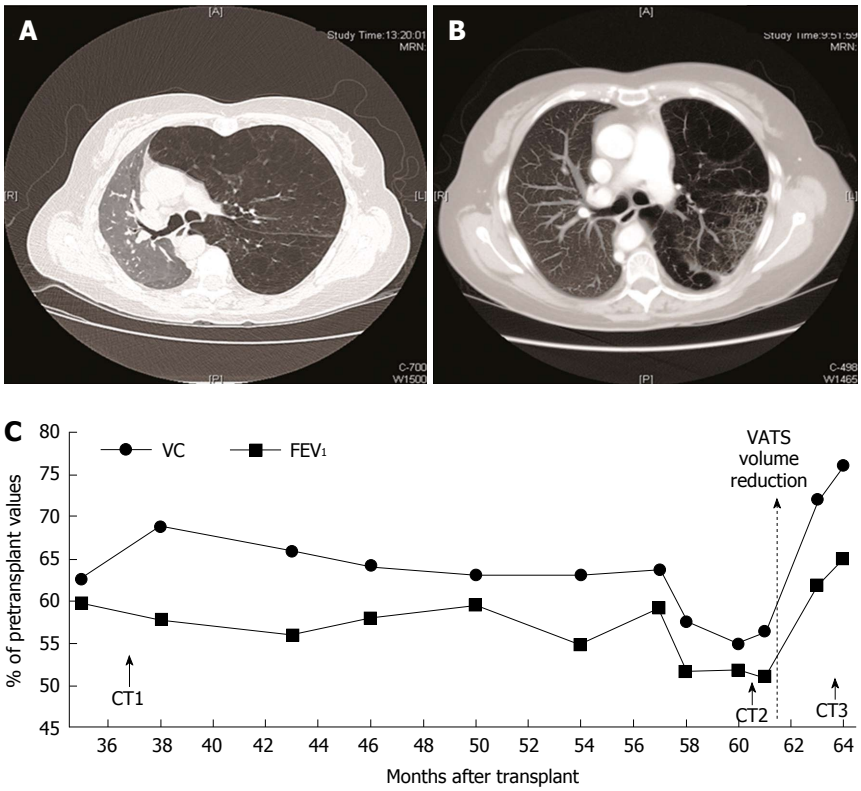


Figure 1 Results of patient undergoing volume reduction surgery by video-assisted thoracoscopic surgery. A: CT before surgery (CT2); B: CT after surgery (CT3); C: Spirometric values recovered after surgery. CT: Computed tomography; VATS: Video-assisted thoracoscopic surgery.

Table 2 Survival probability according to type of transplant

Time until transplantation	Single-lung		Double-lung	
	No. at risk	Survival probability	No. at risk	Survival probability
1 yr	34	85.0%	26	78.8%
2 yr	31	77.5%	24	72.7%
3 yr	29	72.5%	22	66.7%
4 yr	23	57.5%	20	60.6%
5 yr	20	52.4%	17	51.5%

Log-rank test = 0.001; P = 0.976.

compared to 18 subjects (54%) in the DL group, which was significantly higher (P = 0.032).

Complications reported during follow-up were similar in both groups (Table 1). Rejection episodes were treated with steroid boluses, combined with a modification of the patient's immunosuppressive treatment when deemed necessary.

Clinically significant hyperinflation of the native lung was detected in 3 SL patients during the follow-up period. This was diagnosed by worsening respiratory function, decreased %FEV_1 compared to initial post-transplant values, and confirmed by high-resolution computed tomography (CT). Transbronchial biopsy was performed to rule out other possible causes of functional deterioration, including bronquiolitis obliterative sindrome (BOS).

Volume reduction surgery by VATS was performed in 2 patients. The first case experienced a significant improvement in functional capacity (Figure 1), recovering pretransplant spirometric values, leading to a clear improvement in the patient's quality of life. The second patient died 3 wk after the intervention due to sepsis caused by lung infection. The third patient underwent video-assisted bronchoscopic volume reduction. Endobronchial valves were placed in 3 segmental bronchi of the right upper lobe without incidents. Postoperative CT imaging showed atelectasis at this site[22]; the patient experienced clinical improvement from stage 3 to 2 in the Medical Research Council dyspnea functional scale[23], improved exercise tolerance and better quality of life, with no significant changes in spirometry. Lung volume reduction by bullae resection was performed in a fourth patient during the transplantation procedure without incidents.

Postoperative mortality (within 30 d after surgery), was significantly higher in DL: 4 patients (5.57%) died in the immediate postoperative period, compared to 2 (2.73%) in the SL group. Regarding survival, 85% of SL patients were alive one year after the intervention, 72.5% 3 years later, and 52.4% after 5 years, and in DL, 78.8%, 66.7% and 51.5% respectively (Figure 2). Survival probabilities are shown in Table 2. There were no significant differences between the 2 survival curves (P = 0.976). Multivariate regression analysis revealed that the type of transplant performed, single or double-lung, was not related to survival (P = 0.802), while age and having COPD as the underlying disease did not reach statistical significance (Table 3). Univariate and

Table 3 Cox regression analysis to predict mortality adjusting for different variables

	B	SE	Wald	*P*-value	HR	95%CI HR
Age in years	0.040	0.024	2.780	0.095	1.041	0.993-1.091
Gender (female)	0.475	0.391	1.481	0.224	1.609	0.748-3.459
Underlying disease (COPD)	0.737	0.407	3.278	0.070	2.088	0.941-4.651
Type of transplant (double-lung)	-0.086	0.345	0.063	0.802	0.917	0.466-1.804

COPD: Chronic obstructive pulmonary disease; B: Regression coefficient; SE: Standard error; HR: Hazard ratio.

Table 4 Univariate and multivariate analysis of postoperative complications in relation to type of transplant *n* (%)

	Single-lung *n* = 40	Double-lung *n* = 33	Univariate analysis	Multivariate analysis[1]	
			P-value	*P*-value	OR (95%CI)
Complications during follow-up					
Bronchiolitis obliterans syndrome	22 (55.0)	16 (48.5)	0.579	0.475	0.7 (0.2-1.9)
Acute rejection episodes	18 (45.0)	18 (54.5)	0.417	0.397	1.6 (0.6-4.4)
Bacterial respiratory infections	27 (67.5)	22 (68.8)	0.910	0.597	0.7 (0.2-2.3)
CMV infection/disease	19 (47.5)	9 (27.3)	0.077	0.104	0.4 (0.1-1.2)
Fungal infections	12 (30.0)	8 (24.2)	0.583	0.807	0.9 (0.3-2.7)

[1]Multivariate logistic regression analysis adjusted for age, gender and underlying disease. CMV: Cytomegalovirus.

Table 5 Waiting list status of patients diagnosed with chronic obstructive pulmonary disease, emphysema or α1-antitrypsin deficiency

		Final waiting list status				Total
		Active	Excluded	Deceased	Transplanted	
SL	No.	25	25	9	284	343
	Total SL %	7.3%	7.3%	2.6%	82.8%	100.0%
DL	No.	35	51	40	532	658
	Total DL %	5.3%	7.8%	6.1%	80.9%	100.0%
Total	No.	60	76	49	816	1001
	Total %	6.0%	7.6%	4.9%	81.5%	100.0%

Lung transplantation waiting list, Spain 1999-2012. SL: Single-lung; DL: Double-lung.

multivariate analyses of postoperative complications, presented in Table 4, show that the occurrence of complications was not related to the type of transplant, even after adjustment for age, gender and underlying disease.

The analyses of waiting-list national data included a total of 1001 patients with emphysema, COPD or alfa-1 antitrypsin deficiency: 343 were SL patients and 658 DL (Table 5).

Patient progress in the waiting list differed ($P = 0.068$) depending on the type of transplant awaited: 83% of those waiting for SL were transplanted, compared to 81% of DL waitlisted. In contrast, waiting list mortality was higher in DL group (6.1% *vs* 2.6%) (Table 5). In addition, time on the waiting list at national level was longer in the DL than in the SL group ($P < 0.001$), explained by the fact that patients in the SL group were transplanted earlier (Figure 3). Notably, no significant difference was found in survival-time curves between DL and SL in patients with COPD, emphysema or α1-antitrypsin deficiency in the RETP ($P = 0.875$), shown in Figure 4.

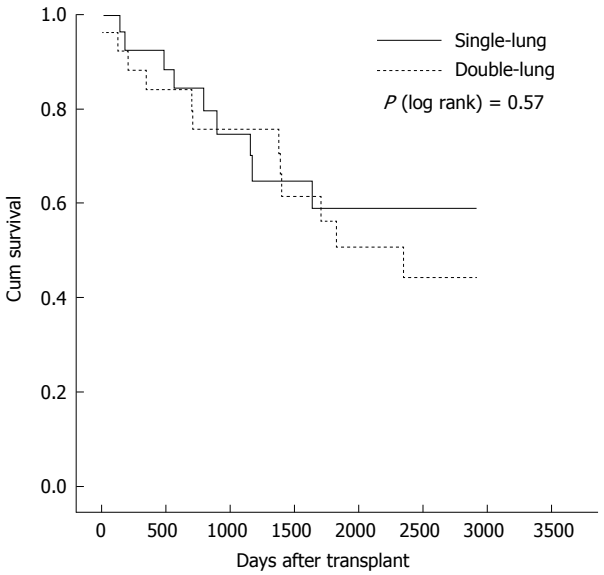


Figure 2 Survival curves of emphysema patients transplanted with 1 or 2 lungs (single-center series), transplants performed between 2000 and 2012.

DISCUSSION

Several previous studies have compared follow-up results of DL *vs* SL transplantation for emphysema, and the general conclusion has been that survival rates were better in the former case, at least in youger patients[8,10,11]. However, comparison groups were not homogeneous and confounding factors were often present. Cassivi *et al*[10] reported 5-year survival rates of 66.7% in DL recipients, *vs* 44.9% in SL transplanted patients, but most of the younger patients with α1-antitrypsin deficiency had received 2 lungs. We considered the question of age, as Thabut *et al*[8] concluded in their study that patients aged over 60 years may not have a

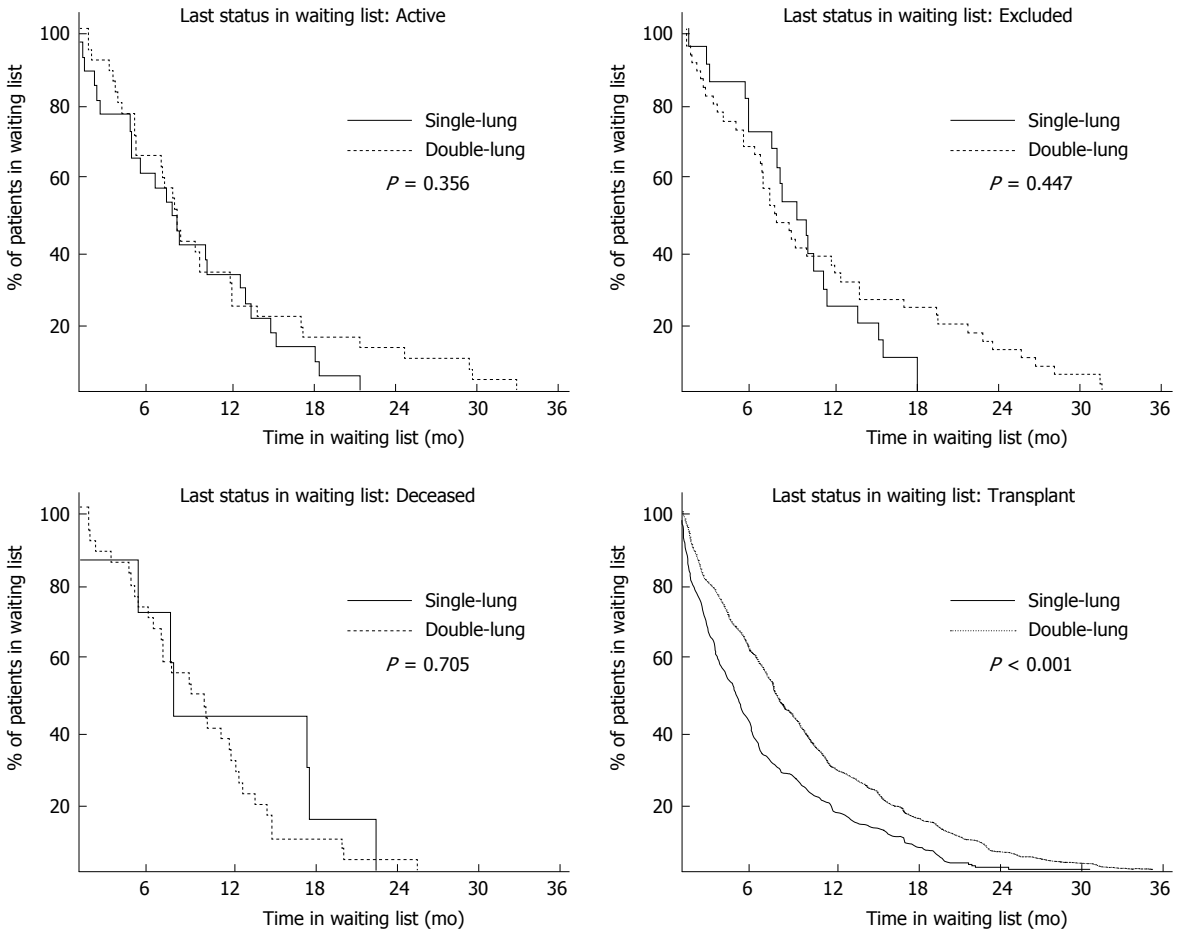


Figure 3 Time on lung transplantation waiting list according to final status. Patients diagnosed with COPD, emphysema or α1-antitrypsin deficiency. National Registry of Donation and Transplantation, Spain 1999-2012. Log rank test used for survival curves comparison. COPD: Chronic obstructive pulmonary disease.

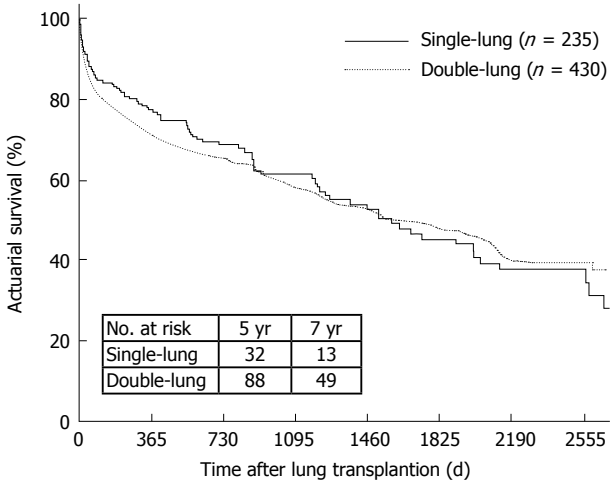
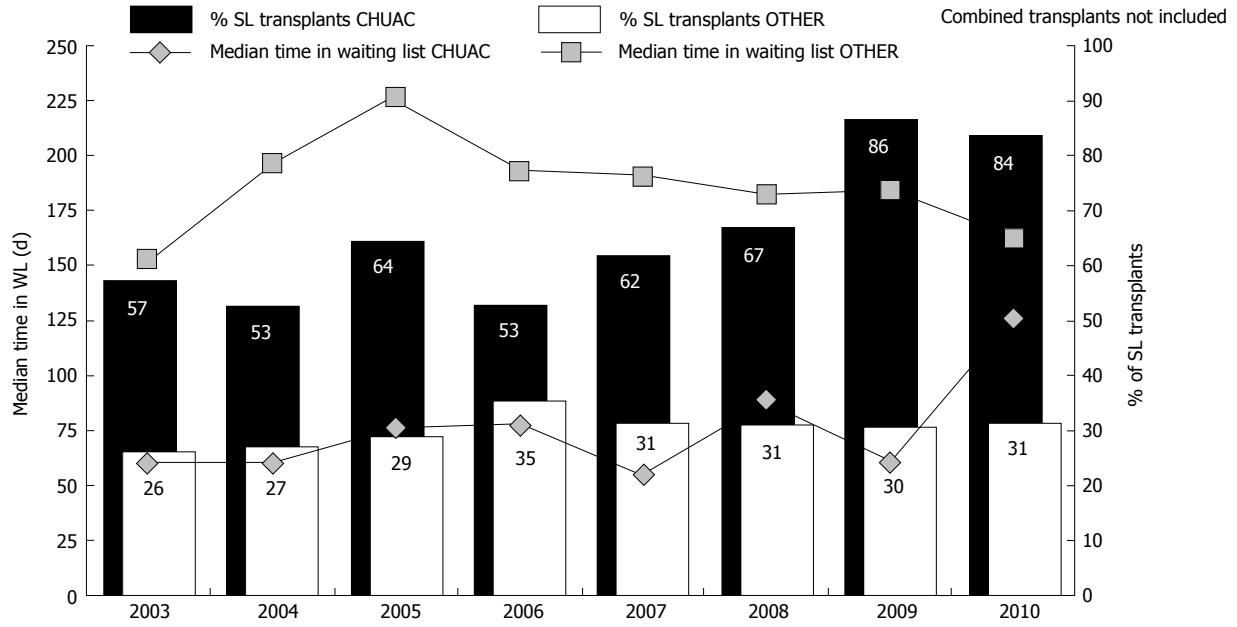


Figure 4 Post-lung transplantation survival of patients with chronic obstructive pulmonary disease, emphysema or α1-antitrypsin deficiency. Data from the Spanish Lung Transplant Registry, transplants performed between 2001 and 2012.

survival benefit after receiving both lungs, but younger patients presented better survival rates after bilateral lung transplantation[8]. Our single-center series is small compared to those of other reports exploring this topic, with the obvious limitations this brings, but on the other hand, this means that our protocols and surgical techinques were totally homogenous. Multicenter studies are based on large databases that can be inaccurate or incomplete, and details that would have allowed to investigate the mechanisms responsible for greater survival after DL are often missing. For this reason, Thabut *et al*[8] were unable to differentiate causes of death. Our study shows no differences in terms of complications and survival in DL and SL patients. The benefits of a slightly better long-term survival reported in previous studies for DL recipients could be cancelled out by higher waiting list mortality, if DL is the preferred approach. Our results might be influenced by our preoperative prophylaxis protocol aimed at prevention of native lung colonization, early extubation (frecuentely extubated in the surgical room in SL), appropriate management of ventilation complications during the early postoperative period, and long-term management of hyperinflation. Our aim was to analyse fully comparable groups, and this was achieved in general terms, as can be observed in the demographic and clinical characterization of our series. Although the SL group was older than the DL group, our results showed no difference between the study groups in terms of long-term mortality, nor was morbidity higher in the SL group, as suggested in previous studies. Many authors



		2003	2004	2005	2006	2007	2008	2009	2010
CHUAC	Median	60	60	76	77	55	89	60.5	126
	p25	19	14	35	7	20	29	19	63
	p75	167	135	151	183	108	165	178	261
	%SL	57	53	64	53	62	67	86	84
OTHER	Median	153	196	227	193	191	182	184	162
	p25	58	78	74	73	72	75	61	57
	p75	269	356	408	384	381	309	394	332
	%SL	26	27	29	35	31	31	30	31

Figure 5 Median time on waiting list in our hospital (Complejo Hospitalario Universitario A Coruña) and other Spanish hospitals performing lung transplantation. (Spanish national data; OTHER) in the context of the percentage of single-lung transplants (SL) performed from 2003 to 2010. Preference for SL reduces the median number of days on the waiting list. CHUAC: Complejo Hospitalario Universitario A Coruña.

have advocated bilateral transplantion[7,10-12,24], arguing that native lung hyperinflation may be responsible for poorer results after SL transplantation. In our series, lung volume reduction surgery was performed in 4 patients with generally good results, and increasing numbers of centers are implementing techniques such as VATS and endobronchial valves[25-28]. In the present clinical setting, the possibility of appropriately controlling this complication is high, and, moreover, SL is technically simpler, anatomically less aggressive, and involves shorter total ischemia time[5], which would explain the lower perioperative morbidity and mortality rates in our series. Furthermore, recent techniques such as normothermic *ex vivo* lung perfusion systems may allow the conservation and transplant of lungs in optimal conditions, likely improving the present results[29,30]. Another possible benefit of SL is the treatment of BOS[31], currently the main limiting factor for survival during follow-up. The therapeutic strategy in SL-transplanted patients is retransplantation in the contralateral side, which has shown lower morbidity and mortality in various series, compared to retransplantation in patients with a previous bilateral transplant[32].

The results of our single-center series suggest that SL and DL transplantation have similar outcomes in terms of survival, but SL recipients presented fewer complications. Although this is a small series, our findings correlate with the data from the national registry. further showing lower mortality on the waiting list, we believe SL transplantation would be the preferable option in the context of organ shortages and waiting lists, as suggested elsewhere[6,9,33,34]. Figure 5 shows how our preference for SL has clearly reduced the median number of waiting list days, compared to the rest of Spanish hospitals performing this procedure. In addition, our policy of performing preferably SL has led to the implementation of the "twinning procedure", 2 SL performed simultaneously from 1 donor[35]. Twinning in the same hospital has been shown to be feasible with adequate planning, permitting better use of donors and reducing waiting list time and mortality. Our analysis of the data from the Spanish Registry indicates that patient progress on the waiting list is influenced by the type of transplant awaited. Patient survival is associated with the extra waiting time for DL, and advanced patients presenting would clearly benefit from shorter waiting

times. Munson *et al*[34] advocate shortening waiting list times with an optimized allocation program and, specifically, the performance of SL, arguing that bilateral transplantation maximizes the total number of life-years gained post-transplant only when waiting lists are short or if the local survival benefits of DL compared with SL are large[34].

Our study, being small, retrospective and single-center, has several limitations and therefore conclusions drawn must be equally limited. However, it offers a comparison of outcomes of both transplantation possibilities in emphysema, while excluding the possible confounders occurring in multicenter studies, such as different care protocols used in the various hospitals, different surgical tecniques, surgical teams or the lack of complete, validated data, among others. In these conditions, we have observed that the survival of both groups is similar. The postoperative complications in our group, with mortality rates twice those of DL transplant recipients, are consistent with longer and more complex interventions requiring double the number of sutures that increase the risk of complications.

In conclusion, our results suggest that SL could be the best option in the present clinical scenario, and we believe that this should be the treatment of choice in most of emphysema patients. This approach, in our experience, has no impact on patient survival or complications, may alleviate donor organ shortage, and contributes to decreasing morbidity and mortality on the national waiting list. In addition, this approach also allows contralateral transplantation, if needed to solve future complications[36].

ACKNOWLEDGMENTS

The authors thank the staff of the Spanish National Transplant Organization for their collaboration, providing national data and sharing comments and opinions.

COMMENTS

Background

Advanced-stage pulmonary emphysema is the most frequent indication for lung transplantation. However, whether single-lung transplantation (SL) or double-lung transplant (DL) transplantation should be performed remains a subject of debate.

Research frontiers

It is known that the quality of life in single lung transplantation is usually similar to double lung. Therefore, the shortage of organs and the consequent waiting list mortality encouraged to make unilateral transplantation is emphysema patients since it would reduce waiting list times, if they can provided that long-term survival were similar for both.

Innovations and breakthroughs

These study results show similar survival for uni and bilateral lung transplantation in patients with emphysema, with less immediate postoperative morbidity and mortality. Survival Spanish national register does not show difference between the two techniques too. Likewise, mortality in national waiting list is significantly higher in the group of both lungs. Only infections are more prevalent in the group-lung without statistical significance.

Applications

The results obtained reinforce their decision that single lung transplant is the transplantation of choice in most cases of emphysema without bacterial or fungal colonization.

Peer-review

This article is interesting and has a good potential.

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P- Reviewer: Hilmi I, Nosotti M, Salvadori M **S- Editor**: Qiu S
L- Editor: A **E- Editor**: Liu SQ

World Journal of Transplantation

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5500/wjt.v6.i2.356

World J Transplant 2016 June 24; 6(2): 356-369
ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Impact of body mass index on outcomes of 48281 patients undergoing first time cadaveric liver transplantation

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Author contributions: Ayloo S and Molinari M designed the research, analyzed the data, created tables and graphics; Hurton S and Cwinn M reviewed the literature, revised the manuscript.

Institutional review board statement: This study was performed using data extracted from UNOS Standard Transplant Analysis and Research (STAR) Files collected in the United States. Local Institutional Review Board approval was not required.

Informed consent statement: Since this study was performed using data extracted from UNOS Standard Transplant Analysis and Research (STAR) Files, acquisition of patients consent statements was neither feasible nor required.

Conflict-of-interest statement: All the Authors have no conflict of interest related to the manuscript.

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Received: October 25, 2015
Peer-review started: October 27, 2015
First decision: December 28, 2015
Revised: January 6, 2016
Accepted: March 9, 2016
Article in press: March 14, 2016
Published online: June 24, 2016

Abstract

AIM: To investigate possible disparities in perioperative morbidity and mortality among different body mass index (BMI) groups and to simulate the impact that these differences might have had on the cohort of patients undergoing cadaveric liver transplantation (LT).

METHODS: All adult recipients undergoing first time LT for benign conditions and receiving a whole graft from brain-dead donors were selected from the united network of organ sharing registry. From January 1994 to June 2013, 48281 patients satisfied the inclusion criteria and were stratified by their BMI. The hypothesis that abnormal BMIs were independent predictors of inferior outcomes was tested with univariate and multivariate regression analyses.

RESULTS: In comparison to normal weight recipients, underweight and morbidly obese recipients had increased 90-d mortality (adjusted OR = 1.737; 95%CI: 1.185-2.548, P = 0.005) (adjusted OR = 1.956; 95%CI: 1.473-2.597, P = 0.000) respectively and inferior patients' survivals (adjusted HR = 1.265; 95%CI: 1.096-1.461, P = 0.000) (adjusted HR = 1.157; 95%CI: 1.031-1.299, P = 0.013) respectively. Overall, patients' 5-year survival were 73.9% for normal-weight, 71.1% for underweight, 74.0% for overweight, 74.4% for class Ⅰ obese, 75.0% for class Ⅱ obese and 71.5% for class Ⅲ obese recipients. Analysis of hypothetical exclusion of underweight and morbidly obese patients from the pool of potential LT candidates would have improved the overall survival of the entire cohort by

2.7% (95%CI: 2.5%-3.6%).

CONCLUSION: Selected morbidly obese patients undergoing LT for benign conditions had 5-year survival rates clinically comparable to normal weight recipients. Impact analysis showed that exclusion of high-risk recipients (underweight and morbid obese patients) would not significantly improve the overall survival of the entire cohort of patients requiring LT.

Key words: Obesity; Impact analysis; Survival; Liver transplantation; Body mass index

Core tip: Obesity has become a prevalent condition in many part of the world. Yet, evaluation of its impact on patients requiring liver transplantation is limited. Analysis of united network of organ sharing data of 48281 patients undergoing first time cadaveric liver transplantation has shown that, 5-year survival rates for selected underweight and morbidly obese patients were clinically comparable to normal weight recipients as 5-year survival for class Ⅲ obese recipients was 71.5% *vs* 73.9% for normal weight patients. Impact analysis showed that exclusion of morbidly obese and underweight recipients would not significantly improve the overall survival of the entire cohort of patients undergoing liver transplant.

Ayloo S, Hurton S, Cwinn M, Molinari M. Impact of body mass index on outcomes of 48281 patients undergoing first time cadaveric liver transplantation. *World J Transplant* 2016; 6(2): 356-369 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/356.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.356

INTRODUCTION

Since 1980, the incidence of obesity in the adult population has more than doubled in many countries[1,2]. Obesity might cause a spectrum of disorders such as non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NASH) that can lead to cirrhosis and hepatocellular carcinoma[3]. Data from the United States have shown that during the last decade, the indication for liver transplantation (LT) for NASH has risen from 1.2% to 9.7%. Currently it represents the third most common cause of liver failure but it is expected to be the leading indication by year 2025 if the current trends of obesity remain unchanged[4].

Some studies have reported that obese recipients have worse outcomes than normal weight counterparts[4-7]. However, some other investigators did not find any significant differences[8,9]. One of the shortcomings of these studies is the lack of adjustment for known effect modifiers such as coexisting comorbidities. Therefore, the controversy around the issue whether obesity itself is an independent predictor of poorer outcomes after LT still remains. In vision of these conflicting results, we reviewed the outcomes of a large cohort of adult patients who underwent LT in the United States with the intent of assessing if abnormal body mass index (BMI) was an independent predictor for patients' and grafts' survival after adjusting for clinical and demographic characteristics selected a priori. Secondary outcomes of this study were to investigate possible disparities in perioperative morbidity and mortality among different BMI groups and to simulate the impact that these differences might have had on the cohort of patients undergoing LT.

MATERIALS AND METHODS

Data of this study were extracted from United Network for Organ Sharing (UNOS) Standard Transplant Analysis and Research (STAR) Files that included socio-demographic and clinical variables of every donor and recipient of solid organ transplants performed in the United States during the period between January 1, 1994 and June 30, 2013. For each recipient, BMI was calculated using the formula: Weight (kg)/height (m)$^{[2]}$. The World Health Organization definitions were used to classify recipients in six categories: Underweight (BMI < 18.5), normal weight (BMI 18.5-24.9), overweight (BMI 25-29.9), class Ⅰ obese (BMI 30-34.9), class Ⅱ obese (BMI 35-39.9) and class Ⅲ obese patients (BMI $\geqslant$ 40)[10]. Data for different BMI classes were not adjusted for ascites because the volume of peritoneal fluid drained during LT was not recorded in the STAR Files.

Every adult (age $\geqslant$ 18 years) undergoing a LT was considered a potential candidate without restriction of race, citizenship or UNOS region where surgery was performed. Recipients who underwent LT for known primary or secondary liver malignancies (*e.g.*, hepatocellular carcinoma, cholangiocarcinoma, neuroendocrine metastases, *etc.*) and recipients who had a malignancy found in their explanted livers were excluded to avoid confounders related to the neoplastic nature of their disease. Other exclusion criteria were transplants using grafts harvested from living or non-heart beating donors, split grafts, multivisceral or redo transplants and transplant performed across ABO incompatible blood groups as those recipients had increased risk of non-functioning grafts, perioperative morbidity and mortality. Additional exclusion criteria were missing data on recipients' weight or height, lack of records on short and long-term outcomes, or the presence of variables that were deemed implausible for an adult recipient[11]. Cutoffs for these variables were: Recipient height $\leqslant$ 120 cm or $\geqslant$ 240 cm, recipient weight $\leqslant$ 30 kg or $\geqslant$ 250 kg, BMI $\leqslant$ 13 or $\geqslant$ 80, cold ischemia time $\leqslant$ 1 h or $\geqslant$ 24 h and warm ischemia time $\leqslant$ 10 min or $\geqslant$ 120 min. No imputations of missing data were performed and recipients who had more than 10% of omitted information were excluded.

For the purpose of this study, variables included in the final analysis were recipients' age at the time of transplant, sex, ethnicity, primary cause of liver disease, height and weight or BMI when available, presence of renal failure requiring hemodialysis before surgery, history of diabetes (type Ⅰ or Ⅱ), presence of chronic obstructive pulmonary disease (COPD), hypertension, model for end-stage liver disease score after its implementation in 2002 and beforehand when serum creatinine, bilirubin and INR were available for calculation, perioperative complications, perioperative mortality and overall patients' and grafts' survival. Donors' variables included age, gender, height and weight or BMI if available, primary cause of death and ethnicity. Intraoperative variables included warm ischemia time measured in minutes and cold ischemia times measured in hours.

Recipient overall survival was estimated by calculating the difference between the date of transplantation and the date of death from any cause. Censoring was used for recipients who were still alive at the end of the time interval of this study or who were alive at the time of their last available follow-up or at the time of re-transplantation.

Graft survival was calculated by the difference between the date of transplantation and the date of recipient death or the first date that recorded graft failure or the date when the recipient underwent a redo LT. Perioperative adverse events leading to death were grouped in the following categories: Hemorrhagic (*e.g.,* intraoperative or postoperative bleeding), vascular (either arterial or venous thrombosis), biliary (anastomotic strictures or leaks), infections, acute cellular rejection, cerebrovascular complications (ischemic or hemorrhagic strokes) and primary graft non function defined as irreversible graft function requiring emergency liver replacement within the first 2 wk after LT. The remaining less common complications were categorized as others or unknown if the cause of death was not reported in the UNOS files.

Primary outcomes of this study included patient and graft survival, and secondary outcomes were perioperative causes of morbidity and mortality stratified by recipients' BMI groups.

Late causes of death (≥ 12 mo after LT) were grouped in the following categories: Infections, cardiopulmonary (*e.g.,* ischemic cardiomyopathy, embolism, insufficiency), renal failure, cerebrovascular events (ischemic or hemorrhagic strokes), malignancies (any type of cancer), graft failure (*e.g.,* recurrent disease or chronic rejection), and hemorrhagic (any cause). The remaining infrequent causes of death were grouped together under the category named "other", and if there was no recorded cause of death, patients were entered in the group named "unknown". This study was conducted and reported according to recommendations from the STROBE statement[12] and did not require approval by the local ethic review board.

Statistical analysis

Sample size of this retrospective analysis was fixed. All variables of first time cadaveric LTs performed over a 19-year period in the United States had been captured in an electronic healthcare database prospectively maintained by UNOS and provided to the authors upon their request.

The cohort was described using estimates of central tendency (means, medians) and spread (standard deviation, interquartile range) for continuous data and frequency and percentages for categorical data.

Etiologies of end stage liver disease (ESLD) were grouped as follows: Hepatitis C virus (HCV), alcohol, alcohol and HCV, other viral hepatitis in combination with HCV, primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), congenital or metabolic diseases (*e.g.,* alpha-1-antitrypsin deficiency, Budd-Chiari syndrome, hemocrhomatosis, polycystic liver disease, *etc.*), NASH, hepatitits B virus (HBV), autoimmune, acute liver failure and other rare conditions.

The primary end points were overall patient and graft survivals stratified by recipients' BMI at time of transplant. Kaplan-Meier method was used to calculate survival estimates and analyzed with two-sided log-rank test, with the hazard ratio and two-sided 95%CIs. All hazard ratios (HR) and adjusted HR (AHR) involving patients' and grafts' survivals are reported with normal weight recipients as the reference group. The median follow-up time for both patients' and grafts' survival were estimated by means and medians of the reverse Kaplan-Meier method. Multiple clinically relevant two-way interactions were evaluated in the multivariable Cox model and included in the final model if significant at a P-value < 0.05. The proportional hazard assumption of the final adjusted model was tested visually by plotting the scaled Schoenfeld residuals of time and BMI, the main predictor of interest. Departure from linearity was assessed by plotting scatterplot smooth curves through residuals[13,14]. Time-dependent covariates such as recipients' age, which allowed for a change in the hazard ratio over time, were considered and used in the model when appropriate.

To account for the cohort effects, all analyses were adjusted for year of transplantation. Univariate and multivariate Cox regression analyses were performed to test the null hypothesis that recipients' BMI was a predictor of patients' and graft survival. Only pre-transplant characteristics were used in the models and all confounders entered in the regression models were selected a priori as they had been shown to be correlated with patients' and grafts' survival in earlier studies: Year of transplantation, patients' and recipients' characteristics (age, gender, BMI), recipients' comorbidities (renal insufficiency, diabetes, COPD, hypertension), primary indication for LT, warm and cold ischemia times.

Secondary outcomes were perioperative morbidity and mortality. For these analyses, proportions were compared using the χ^2 test and continuous variables

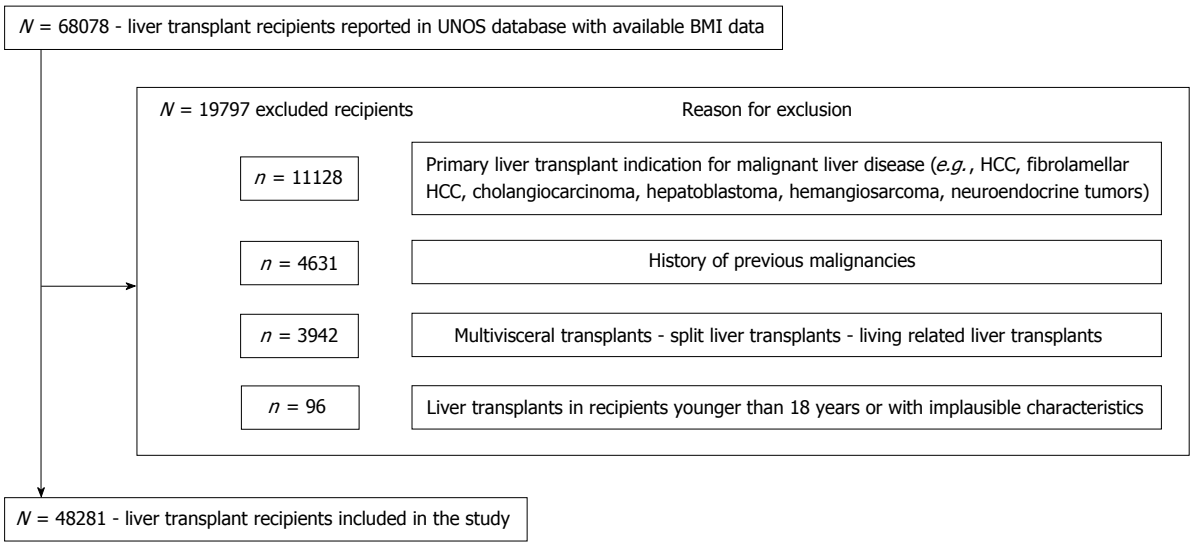


Figure 1 Flowchart of all the include and exclude patients in this study. BMI: Body mass index; UNOS: United Network for Organ Sharing; HCC: Hepatocellular carcinoma.

were compared using ANOVA test across multiple BMI groups. Perioperative mortality was calculated during the index admission, at 30, 60 and 90 d and at 1-year post LT. Unadjusted and adjusted risk estimates of perioperative mortality were calculated as odds ratios (OR) and adjusted OR (AOR) with 95%CI using logistic regression analysis. Risk estimates were adjusted for patients' and donors' BMI (six categories: Underweight, normal weight, overweight, class Ⅰ obese, class Ⅱ obese and class Ⅲ obese), recipients' and donors' age (six categories: 18-45, 46-55, 56-65, 66-75, ≥ 76), recipients' and donors' sex, year of transplantation, recipients' comorbidities (four categories: Renal insufficiency requiring dialysis, diabetes type Ⅰ and Ⅱ, COPD, hypertension), warm and cold ischemia time, and primary indication for LT (twelve categories: HCV, Alcohol and HCV, HCV and other viral hepatitis, Alcohol, HBV, PSC, PBC, NASH, autoimmune, acute liver failure, congenital or metabolic disease, other).

All statistical analyses were performed using IBM SPSS Statistics version 20 (SPSS Inc. Chicago, IL, United States). Statistical significance was identified by two-tailed *P*-values of less than 0.05 and 95%CI.

Impact analysis of the potential benefit of allocating grafts to patients with BMIs that had the longest survival and lowest perioperative mortality risk was performed using estimates of central tendency and 95%CI. Microsoft® excel 2008 was used to calculate the overall number and 95%CI of preventable perioperative deaths and the number and 95%CI of life-years that could have been saved by allocation grafts to low-risk recipients.

RESULTS

Donors and recipients characteristics

Among 68078 LT recipients recorded in the UNOS registry, a total of 48281 (70.9%) met eligibility criteria (Figure 1). Of these, 914 (1.89%) were underweight, 14529 (30%) had normal BMI, 16724 (34.6%) were overweight and 16114 were obese (33.3%). Within the group of obese recipients, 9944 (61.7%) were class Ⅰ obese, 4438 (27.5%) class Ⅱ and 1732 (10.3%) satisfied class Ⅲ criteria (Table 1). Demographic and clinical characteristics of the donors are summarized in Table 2.

Primary outcomes

Overall survival: During the study period, 16689 patients (34.6%) died while 31539 were alive or censored. Median overall survival for the entire cohort was 12.7 years (95%CI: 12.5-12.9). Normal weight patients had the longest median survival (13.1 years; 95%CI: 12.6-13.6 years) while the shortest survival was observed in class Ⅲ obese recipients (11.3 years; 95%CI: 10.3-12.3) and underweight patients (11.5 years; 95%CI: 10.4-12.7) (Table 3).

Kaplan-Meier functions, stratified by recipients' BMI, are reported in Figure 2. Logrank test showed a significant survival difference across BMI groups ($P = 0.004$) and pairwise comparisons showed that underweight ($P = 0.034$) and class Ⅲ obese patients ($P = 0.001$) experienced significant lower survivals compared to normal weight counterparts.

At multivariate cox regression analysis, after adjusting for recipients' and donors' characteristics (age, gender, BMI, primary cause of end-stage liver disease, comorbidities), cold and warm ischemia times and year of transplantation, underweight status (AHR = 1.265; 95%CI: 1.096-1.461; $P = 0.001$) and class Ⅲ obesity (AHR = 1.157; 95%CI: 1.031-1.299; $P = 0.013$) remained significant predictors for shorter survival in comparison to normal weight recipients (Table 4). On the other hand, being overweight appeared to have modest protective effect (AHR = 0.908; 95%CI: 0.864-0.954; $P = 0.000$).

Table 1 Demographic and clinical characteristics of the study population

Variable	Total number of patients (*n* = 48281) (100%)	WHO BMI classification						*P* value
		Underweight (*n* = 914) (1.9%)	Normal weight (*n* = 14529) (30.0%)	Overweight (*n* = 16724) (34.6%)	Obese recipients			
					Class Ⅰ (*n* = 9944) (20.5%)	Class Ⅱ (*n* = 4438) (9.2%)	Class Ⅲ (*n* = 1732) (3.5%)	
Age in years, median (25^{th}, 75^{th})	53 (46, 59)	50 (40, 57.2)	52 (44, 58)	53 (46, 58)	53 (46, 58)	53 (46, 58)	52 (47, 58)	0.000
Gender, *n* (%)								
Male	30250 (62.7)	407 (44.5)	8573 (59.0)	11336 (67.8)	6489 (65.3)	2609 (58.8)	836 (48.3)	0.000
Female	18.031 (37.3)	507 (55.5)	5956 (41.0)	5388 (32.2)	3455 (34.7)	1829 (41.2)	896 (51.7)	
Recipient living status, *n* (%)								
Alive	27552 (57.1)	496 (54.3)	8040 (55.4)	9534 (57.1)	5825 (58.6)	2642 (59.6)	1015 (58.6)	0.000
Dead	16689 (34.6)	346 (37.9)	5123 (35.3)	5804 (34.7)	3344 (33.7)	1471 (33.2)	601 (34.7)	
Lost at follow-up	3987 (8.3)	72 (7.9)	1348 (9.3)	1370 (8.2)	765 (7.7)	317 (7.2)	115 (6.6)	
Race, *n* (%)								
Non-hispanic white	36809 (76.2)	664 (72.6)	10850 (74.7)	12820 (76.7)	7687 (77.3)	3475 (78.3)	1313 (75.8)	0.000
Non-hispanic black	3962 (8.2)	92 (10.1)	1237 (8.5)	1277 (7.6)	815 (8.2)	371 (8.4)	170 (9.8)	
Hispanic	5535 (11.5)	73 (8.0)	1534 (10.6)	2020 (12.1)	1191 (12.0)	500 (11.3)	217 (12.5)	
Asian	1446 (3.0)	78 (8.5)	782 (5.4)	415 (2.5)	131 (1.3)	30 (0.7)	10 (0.6)	
Other	529 (1.1)	7 (0.7)	126 (0.8)	192 (1.1)	120 (1.2)	62 (1.3)	22 (1.2)	
Recipient BMI, median (25^{th}, 75^{th})	27.05 (23.8, 31.1)	17.63 (17.0, 18.1)	22.73 (21.2, 23.9)	27.26 (26.0, 28.5)	32.01 (30.3, 33.3)	36.85 (35.8, 38.1)	42.24 (41.1, 44.2)	0.000
Primary indication for liver transplantation, *n* (%)								
HCV	13838 (28.7)	176 (19.3)	3538 (24.4)	5248 (31.4)	3101 (31.2)	1302 (29.3)	473 (27.3)	0.000
Alcohol	8111 (16.8)	163 (17.8)	2543 (17.5)	2909 (17.4)	1686 (17.0)	622 (14.0)	188 (10.9)	
Idiopathic	5073 (10.5)	77 (8.4)	1179 (8.1)	1656 (9.9)	1270 (12.8)	628 (14.2)	263 (15.2)	
Alcohol + HCV	3601 (7.5)	44 (4.8)	1033 (7.1)	1370 (8.2)	762 (7.7)	310 (7.0)	82 (4.7)	
PSC	2799 (5.8)	101 (11.1)	1396 (9.6)	903 (5.4)	282 (2.8)	92 (2.1)	25 (1.4)	
Congenital/metabolic disease	2567 (5.3)	80 (8.8)	870 (6.0)	826 (4.9)	455 (4.6)	215 (4.8)	121 (7.0)	
PBC	2485 (5.1)	92 (10.1)	1122 (7.7)	765 (4.6)	342 (3.4)	116 (2.6)	48 (2.8)	
NASH	2247 (4.7)	13 (1.4)	226 (1.6)	585 (3.5)	660 (6.6)	522 (11.8)	241 (13.9)	
HBV	1896 (3.9)	33 (3.6)	798 (5.5)	646 (3.9)	291 (2.9)	92 (2.1)	36 (2.1)	
Other	5664 (11.7)	135 (14.7)	1824 (12.5)	1816 (10.8)	1095 (11.0)	539 (12.1)	291 (14.7)	
MELD score, median (25^{th}, 75^{th})	21 (16, 28)	22 (16, 28)	21 (16, 29)	20 (15, 28)	21 (16, 28)	21 (15, 29)	22 (16, 31)	0.000
Cold ischemia time, hours, median (25^{th}, 75^{th})	7.0 (5.4, 9.2)	7.1 (5.3, 9.0)	7.0 (5.3, 9.1)	7.0 (5.3, 9.1)	7.1 (5.4, 9.2)	7.2 (5.5, 9.3)	7.3 (5.7, 9.4)	0.288
Warm ischemia time, minutes, median (25^{th}, 75^{th})	44 (34, 55)	44 (35, 55)	43 (34, 55)	44 (35, 55)	44 (35, 56)	45 (35, 57)	41 (31, 50)	0.000
Waiting time, days (including days on hold), median (25^{th}, 75^{th})	120 (27, 335)	91 (21, 286)	106 (24, 308)	124 (28, 336)	131 (30, 358)	134 (31, 355.5)	86 (12, 314)	0.000
Hospital stay after liver transplant, days, median (25^{th}, 75^{th})	11 (8, 19)	13 (7, 23)	11 (8, 19)	11 (8, 18)	11 (8, 18)	12 (8, 19)	13 (9, 22)	0.000
Preoperative comorbidities, *n* (%)								
Diabetes type Ⅰ	536 (1.1)	9 (1.7)	108 (0.8)	181 (1.1)	155 (1.6)	60 (1.4)	23 (1.3)	0.000
Diabetes type Ⅱ	8541 (18.0)	81 (9.1)	1827 (12.9)	2896 (17.6)	2179 (22.2)	1105 (25.2)	453 (26.6)	
Dialysis	3538 (7.3)	80 (8.8)	1059 (7.3)	1160 (6.9)	693 (7.0)	366 (8.2)	180 (5.1)	0.000
Hypertension	4124 (8.5)	54 (5.9)	989 (6.8)	1424 (8.5)	990 (10.0)	481 (10.8)	186 (10.7)	0.000
Chronic obstructive pulmonary disease	447 (0.9)	13 (1.4)	133 (0.9)	153 (0.9)	86 (0.9)	45 (1.0)	17 (1.0)	0.000

HCV: Hepatitis C virus; HBV: Hepatitis B virus; PSC: Primary sclerosing cholangitis; NASH: Non-alcoholic steatohepatitis; BMI: Body mass index; WHO: World Health Organization; MELD: Model for End-Stage Liver Disease; PBC: Primary biliary cirrhosis.

Table 2 Donor demographic and clinical characteristics

Donor variable	Total number of donors (n = 48281) (100%)	WHO recipients' BMI						P value
		Underweight (n = 914) (1.9%)	Normal weight (n = 14529) (30.0%)	Overweight (n = 16724) (34.6%)	Obese recipients			
					Class Ⅰ (n = 9944) (20.5%)	Class Ⅱ (n = 4438) (9.2%)	Class Ⅲ (n = 1732) (3.5%)	
Age in years, median (25^{th}, 75^{th})	40 (24, 53)	37 (20, 52)	39 (22, 53)	40 (24, 53)	41 (24, 53)	41 (25, 53)	41 (25, 54)	0.000
BMI, median (25^{th}, 75^{th})	25.2 (22.3, 29.0)	23.6 (20.7, 27.1)	24.6 (21.7, 28.2)	25.3 (22.3, 29.0)	25.7 (22.7, 29.8)	25.9 (23.0, 29.9)	25.8 (22.8, 30.0)	0.000
Gender, n (%)								
Male	29034 (60.1)	486 (53.2)	8171 (56.2)	10219 (61.1)	6280 (63.2)	2801 (63.1)	1077 (62.2)	0.000
Female	19247 (39.8)	428 (46.8)	6358 (43.8)	6505 (38.9)	3664 (36.8)	1637 (36.9)	655 (37.8)	
Primary cause of death, n (%)								
Anoxia	6895 (14.3)	163 (17.8)	2004 (13.8)	2337 (33.9)	1465 (14.7)	675 (15.2)	251 (14.5)	0.040
Cerebrovascular	19840 (41.1)	355 (38.8)	5980 (41.2)	6869 (41.1)	4079 (41.0)	1826 (41.1)	731 (42.3)	
Head trauma	20273 (42.0)	372 (40.7)	6161 (42.4)	7098 (42.5)	4110 (41.4)	1831 (41.3)	701 (40,5)	
Central nervous system tumor	357 (0.7)	8 (0.9)	107 (0.7)	116 (0.7)	92 (0.9)	24 (0.5)	10 (0.6)	
Other	892 (1.8)	16 (1.8)	272 (1.9)	292 (1.7)	193 (1.9)	82 (1.8)	37 (2.1)	
Race, n (%)								
Non-hispanic white	34907 (72.3)	639 (69.9)	10488 (72.2)	12078 (72.2)	7196 (72.4)	3264 (73.5)	1242 (71.7)	0.000
Non-hispanic black	6758 (14.0)	127 (13.9)	1938 (13.3)	2334 (14.0)	1458 (14.7)	640 (14.4)	261 (15.1)	
Hispanic	5118 (10.6)	109 (11.9)	1623 (11.2)	1790 (10.7)	994 (10.0)	427 (9.6)	175 (10.1)	
Asian	885 (1.8)	28 (3.1)	299 (2,1)	309 (1.8)	162 (1,6)	55 (1.2)	32 (1.8)	
Other	673 (1.3)	11 (1.2)	241 (1.6)	213 (1.2)	134 (1.3)	52 (1.1)	22 (1.2)	

BMI: Body mass index; WHO: World Health Organization.

Graft survival: During the study period, 20207 grafts failed (41.9%) and median graft survival was 11.8 years (95%CI: 11.6-12.0) (Table 5).

Figure 3 represents Kaplan-Meier probability functions for graft failure stratified by recipients' BMI. Underweight (11.1 years; 95%CI: 10.1-12.1, P = 0.034) and class Ⅲ obese patients (10.7 years; 95%CI: 9.7-11.7, P = 0.001) had significant shorter median survivals when compared to normal weight recipients (12.0 years; 95%CI: 11.6-12.5).

The most frequent causes of graft failure were recipients' death (60.9%), recurrent disease (4.9%), primary graft non-function (3.5%), infections (3.5%), and unknown reasons (23.2%) (Table 6).

Cox-regression multivariate analysis showed that underweight status (AHR = 1.315; 95%CI: 1.129-1.531; P = 0.000) and class Ⅲ obesity (AHR = 1.156; 95%CI: 1.021-1.309; P = 0.022) remained significant predictors for shorter graft survival in comparison to normal weight recipients after adjusting for both recipients' and donors' characteristics (age, gender, BMI, primary cause of end-stage liver disease, comorbidities), cold and warm ischemia times and year of transplantation. On the other hand, grafts transplanted in overweight recipients had lower risk of failure with AHR of 0.931 (95%CI: 0.882-0.981; P = 0.008) in comparison to normal weight recipients.

Secondary outcomes

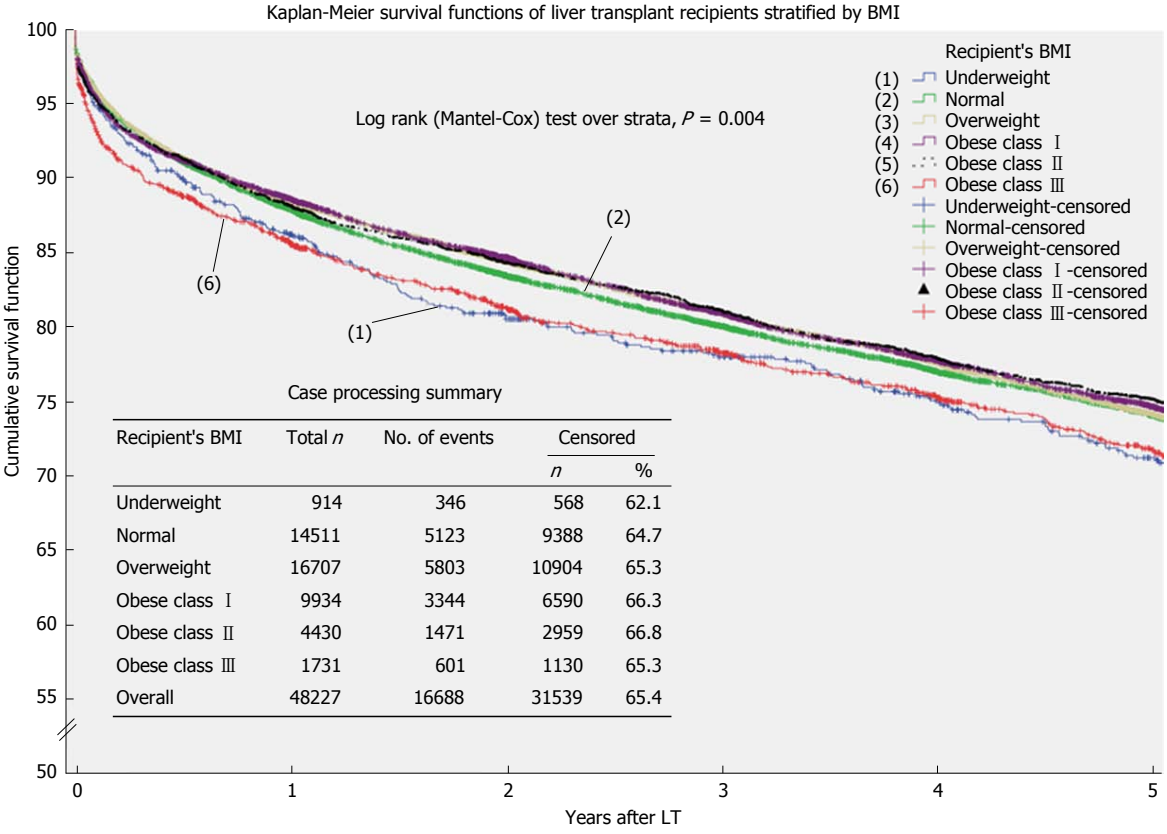
Perioperative mortality: Statistical significant differences in perioperative mortality were identified between normal weight and class Ⅱ and Ⅲ obese patients at 30, 60, 90-d and at 1-year after LT (Figure 4).

Analysis of the most common causes of perioperative deaths during the index admission is summarized in Table 7. Overall, in hospital mortality was observed in 4.6% of the entire cohort and sepsis and multiorgan failure represented 31.9% of all causes of death. Comparison across BMI categories showed that only cardiovascular ischemic or embolic events were significantly higher in class Ⅱ obese patients *vs* normal weight recipients (0.2% *vs* 0.05%).

Table 3 Mean and median overall survival by recipients' body mass index class

Recipient's BMI	Means and medians for survival time (yr)							
	Mean[1]				Median			
	Estimate	Std. error	95%CI		Estimate	Std. error	95%CI	
			Lower bound	Upper bound			Lower bound	Upper bound
Underweight	10.8	0.3	10.2	11.4	11.5	0.6	10.4	12.7
Normal	11.6	0.1	11.4	11.7	13.1	0.2	12.6	13.6
Overweight	11.5	0.1	11.4	11.7	12.8	0.2	12.5	13.2
Obese-class Ⅰ	11.3	0.1	11.2	11.5	12.4	0.2	11.9	12.8
Obese-class Ⅱ	11.1	0.1	10.8	11.4	12.2	0.3	11.6	12.9
Obese-class Ⅲ	10.7	0.2	10.2	11.1	11.3	0.5	10.3	12.3
Overall	11.5	0.0	11.4	11.5	12.7	0.1	12.5	12.9

[1]Estimation is limited to the largest survival time if it is censored. BMI: Body mass index.



Case processing summary

Recipient's BMI	Total *n*	No. of events	Censored	
			n	%
Underweight	914	346	568	62.1
Normal	14511	5123	9388	64.7
Overweight	16707	5803	10904	65.3
Obese class Ⅰ	9934	3344	6590	66.3
Obese class Ⅱ	4430	1471	2959	66.8
Obese class Ⅲ	1731	601	1130	65.3
Overall	48227	16688	31539	65.4

Figure 2 Kaplan-Meier overall survival functions of liver transplant recipients during the first 5 years stratified by their body mass index. Log rank (Mantel-Cox) test over strata showed significant survival difference (P = 0.004). In comparison to normal weight recipients, underweight recipients (P = 0.034) and class Ⅲ obese patients (P = 0.001) experienced significant lower probability of overall survival. BMI: Body mass index; LT: Liver transplantation.

At multivariate logistic regression analysis, recipients' BMI category remained a significant predictor for in-hospital, 90 d and 1 year mortality after adjusting for cold and warm ischemia time, donors' characteristics, primary indication for LT and recipients' comorbidities (Table 8). Specifically, when compared to normal weight recipients, class Ⅲ obesity was a predictor for in-hospital mortality (AOR = 1.749; 95%CI: 1.276-2.397; P = 0.001), 90 d mortality (AOR = 1.956; 95%CI: 1.473-2.597; P = 0.000) and 1 year mortality (AOR = 1.458; 95%CI: 1.154-1.842; P = 0.002). Also, being underweight was a risk factor for 90 d mortality (AOR = 1.737; 95%CI: 1.185-2.548; P = 0.005) and 1-year mortality (AOR = 1.505; 95%CI: 1.105-2.048; P = 0.009) while being overweight was protective (AOR = 0.886 at 1-year post LT; 95%CI: 0.792-0.992; P = 0.036).

All causes of death: Analysis of all primary causes of mortality after LT is reported in Figure 5. Infections were responsible for 21.1% of all deaths, cardiopulmonary complications for 14.9%, and graft failure for 10.9%. Other main causes of mortality were malignant diseases (9.4%), unknown causes (8.3%) and other less common causes that represented 27.3% of all deaths when grouped together (Figure 5A).

Table 4 Univariate and multivariate Cox analysis of predictors of overall survival

	Univariate analysis		Multivariate analysis	
Variable	**Hazard rate (95%CI)**	***P* value**	**Hazard rate (95%CI)**	***P* value**
Recipient BMI		0.003		0.000
Normal weight (reference)	1		1	
Underweight	1.125 (1.009-1.255)	0.034	1.265 (1.096-1.461)	0.001
Overweight	1.005 (0.968-1.043)	0.807	0.908 (0.864-0.954)	0.000
Obese class Ⅰ	1.024 (0.980-1.070)	0.284	0.947 (0.893-1.004)	0.067
Obese class Ⅱ	1.042 (0.983-1.104)	0.169	0.971 (0.898-1.051)	0.470
Obese class Ⅲ	1.163 (1.069-1.266)	0.000	1.157 (1.031-1.299)	0.013
Donor BMI		0.001		0.000
Normal weight (reference)	1		1	
Underweight	0.962 (0.897-1.033)	0.288	1.017 (0.928-1.114)	0.716
Overweight	1.060 (1.023-1.098)	0.001	1.009 (0.962-1.057)	0.717
Obese class Ⅰ	1.049 (0.998-1.102)	0.059	0.986 (0.921-1.057)	0.695
Obese class Ⅱ	1.129 (1.045-1.220)	0.002	1.020 (0.912-1.140)	0.729
Obese class Ⅲ	0.988 (0.999-1.112)	0.988	0.889 (0.765-1.034)	0.128
Recipient age		0.000		0.000
18-45 (reference)	1		1	
46-55	1.264 (1.212-1.319)	0.000	1.207 (1.143-1.276)	0.000
56-65	1.536 (1.471-1.603)	0.000	1.490 (1.405-1.580)	0.000
66-75	2.005 (1.887-2.130)	0.000	2.069 (1.904-2.247)	0.000
⩾ 76	3.224 (2.099-4.951)	0.000	2.476 (1.462-4.194)	0.001
Donor age		0.000		0.000
0-17 (reference)	1		1	
18-45	1.107 (1.050-1.166)	0.000	1.066 (0.996-1.141)	0.066
46-55	1.297 (1.223-1.376)	0.000	1.266 (1.170-1.370)	0.000
56-65	1.502 (1.411-1.598)	0.000	1.413 (1.300-1.537)	0.000
66-75	1.706 (1.583-1.840)	0.000	1.609 (1.453-1.782)	0.000
⩾ 76	1.661 (1.448-1.883)	0.000	1.609 (1.340-1.932)	0.000
Recipient sex (male)	1.063 (1.030-1.097)	0.000	1.025 (0.979-1.073)	0.297
Donor sex (male)	0.951 (0.922-0.980)	0.001	0.967 (0.926-1.008)	0.967
Cold ischemia time (h)	1.010 (1.006-1.013)	0.000	1.008 (1.003-1.013)	0.001
Warm ischemia time (min)	1.002 (1.001-1.003)	0.000	1.002 (1.001-1.003)	0.000
Year of transplantation	0.996 (0.992-0.999)	0.017	0.987 (0.980-0.993)	0.000
Dialysis	1.507 (1.422-1.598)	0.000	1.492 (1.367-1.629)	0.000
Diabetes	1.406 (1.355-1.460)	0.000	1.314 (1.248-1.383)	0.000
COPD	1.384 (1.218-1.573)	0.000	1.250 (1.075-1.454)	0.004
Hypertension	1.207 (1.150-1.267)	0.000	1.057 (0.998-1.120)	0.059
Primary indication		0.000		0.000
HCV	1.356 (1.313-1.400)	0.000	1.429 (1.335-1.530)	0.000
Alcohol + HCV	1.214 (1.152-1.281)	0.000	1.477 (1.351-1.616)	0.000
HCV + other viral hepatitis	1.093 (0.932-1.283)	0.274	1.342 (1.098-1.638)	0.004
Other	1.020 (0.951-1.094)	0.583	1.111 (0.993-1.244)	0.067
Alcohol	1.060 (1.018-1.103)	0.005	1.188 (1.102-1.282)	0.000
HBV	0.669 (0.613-0.729)	0.000	0.782 (0.691-0.883)	0.000
PSC	0.597 (0.554-0.644)	0.000	0.709 (0.634-0.792)	0.000
PBC	0.670 (0.623-0.721)	0.000	0.715 (0.641-0.797)	0.000
NASH	0.906 (0.821-1.001)	0.051	0.953 (0.783-1.160)	0.630
Autoimmune	0.807 (0.742-0.878)	0.000	0.916 (0.810-1.036)	0.164
Acute liver failure	0.801 (0.689-0.931)	0.004	1.049 (0.822-1.339)	0.701
Congenital or metabolic disease	0.758 (0.703-0.817)	0.000	0.825 (0.736-0.926)	0.001

At multivariate analysis, after adjusting for patients' and donors' characteristics, primary indication for liver transplantation, comorbidities, year of transplantation and warm and cold ischemia times, class Ⅲ obesity and underweight status remained independent factors associated with lower survival. HCV: Hepatitis C virus; HBV: Hepatitis B virus; PSC: Primary sclerosing cholangitis; NASH: Non-alcoholic steatohepatitis; BMI: Body mass index; COPD: Chronic obstructive pulmonary disease; PBC: Primary biliary cirrhosis.

Compared to other BMI groups, class Ⅲ obese patients died more frequently form infections and cardiopulmonary complications. On the other hand, normal weight and overweight patients experienced a higher rate of malignant diseases (Figure 5B).

Impact analysis

Analysis of the hypothetical number of lives that could have been saved within one-year post LT by allocating grafts only to low risk groups (normal weight, overweight and obese class Ⅰ recipients) was performed using observed values and ranges of this study. If no transplants had been performed for class Ⅲ obese patients, 55 deaths could have been avoided, 38 if no transplants had been done for class Ⅱ obese and 18 if no transplants had been done for underweight

Table 5 Mean and median graft survival by recipients' body mass index class

Recipient's BMI	Means and medians for graft survival time (yr)							
	Mean[1]				Median			
	Estimate	Std. error	95%CI		Estimate	Std. error	95%CI	
			Lower bound	Upper bound			Lower bound	Upper bound
Underweight	10.4	0.3	9.8	11	11.1	0.4	10.1	12.1
Normal	11.1	0.07	10.9	11.3	12	0.2	11.6	12.5
Overweight	11.1	0.07	11	11.3	12	0.1	11.6	12.4
Obese-class Ⅰ	11	0.09	10.8	11.2	11.7	0.2	11.3	12.1
Obese-class Ⅱ	10.8	0.14	10.5	11.1	11.7	0.3	11.1	12.3
Obese-class Ⅲ	10.3	0.24	9.8	10.8	10.7	0.5	9.7	11.7
Overall	11	0.04	11	11.1	11.8	0.1	11.6	12

[1]Estimation is limited to the largest survival time if it is censored. BMI: Body mass index.

Table 6 Summary of the primary causes of graft loss stratified by recipients' body mass index *n* (%)

Primary cause of graft failure	All recipients (*n* = 16715)	WHO recipients' BMI class						*P* value
		Underweight (*n* = 346) (Ⅰ)	Normal weight (*n* = 5129) (Ⅱ)	Overweight (*n* = 5813) (Ⅲ)	Obese recipients			
					Class Ⅰ (*n* = 3351) (Ⅳ)	Class Ⅱ (*n* = 1473) (Ⅴ)	Class Ⅲ (*n* = 603) (Ⅵ)	
Primary graft non-function	603 (3.6)	11 (3.2)	178 (3.5)	221 (3.8)	128 (3.8)	48 (3.3)	17 (2.8)	⩾ 0.05
Biliary complications	89 (0.5)	2 (0.6)	18 (0.4)	34 (0.6)	23 (0.7)	6 (0.4)	6 (1.0)	⩾ 0.05
Vascular thrombosis	119 (0.7)	2 (0.6)	45 (0.9)	39 (0.7)	16 (0.5)	11 (0.7)	6 (1.0)	⩾ 0.05
Recurrent disease	829 (4.9)	14 (4.0)	240 (4.6)	306 (5.2)	172 (5.1)	75 (5.0)	22 (3.6)	⩾ 0.05
Acute rejection	158 (0.9)	2 (0.6)	57 (1.1)	55 (0.9)	27 (0.8)	10 (0.7)	7 (1.2)	⩾ 0.05
Chronic rejection	270 (1.6)	7 (2.0)	104 (2.0)	82 (1.4)	50 (1.5)	17 (1.1)	10 (1.6)	⩾ 0.05
Infection	589 (3.5)	11 (3.2)	163 (3.2)	216 (3.7)	119 (3.6)	53 (3.6)	27 (4.5)	⩾ 0.05
Recipient death	10172 (60.9)	224 (64.7)	3107 (60.6)	3480 (59.9)	2037 (60.8)	945 (64.2)	379 (62.9)	⩾ 0.05
Unknown	3886 (23.2)	73 (21.1)	1217 (23.7)	1380 (23.7)	779 (23.2)	308 (20.9)	129 (21.4)	⩾ 0.05

WHO: World Health Organization; BMI: Body mass index.

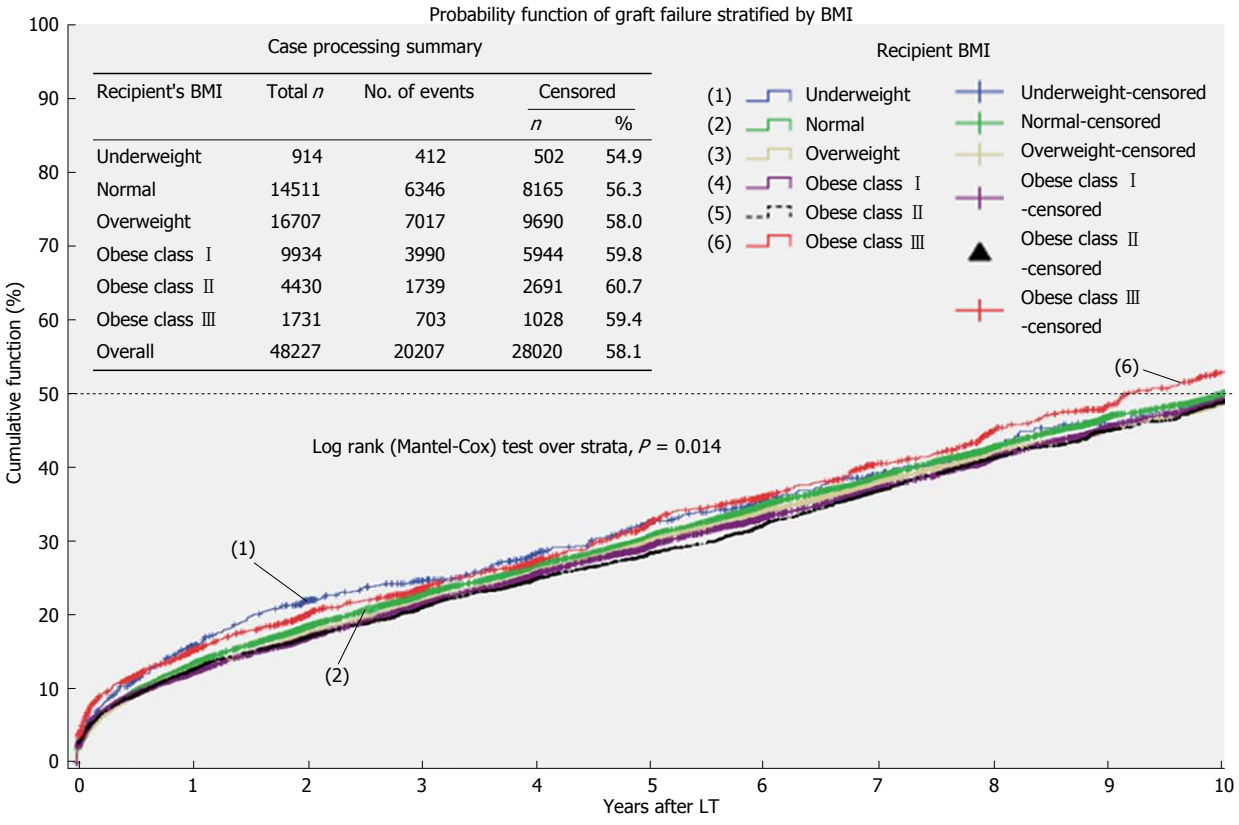


Figure 3 Kaplan-Meier functions of graft survival stratified by recipients' body mass index. BMI: Body mass index; LT: Liver transplantation.

Table 7 Summary of the primary causes mortality during the index admission for liver transplantation stratified by recipients body mass index *n* (%)

Primary cause of perioperative mortality	All recipients (*n* = 48281)	WHO recipients' MBI class						Group comparisons	*P* value
					Obese recipients				
		Underweight (*n* = 914) (Ⅰ)	Normal weight (*n* = 14529) (Ⅱ)	Overweight (*n* = 16724) (Ⅲ)	Class Ⅰ (*n* = 9944) (Ⅳ)	Class Ⅱ (*n* = 4438) (Ⅴ)	Class Ⅲ (*n* = 1732) (Ⅵ)		
Infections or multiorgan failure	718 (1.4)	22 (2.4)	202 (1.3)	229 (1.3)	155 (1.5)	67 (1.5)	43 (2.4)		⩾ 0.05
Cerebrovascular complication	155 (0.3)	4 (0.4)	47 (0.3)	60 (0.3)	25 (0.2)	14 (0.3)	5 (0.2)		⩾ 0.05
Hemmorrhagic	128 (0.2)	3 (0.3)	38 (0.2)	41 (0.2)	32 (0.3)	11 (0.2)	3 (0.1)		⩾ 0.05
Single organ failure	102 (0.2)	1 (0.1)	25 (0.1)	35 (0.2)	21 (0.2)	13 (0.2)	7 (0.4)		⩾ 0.05
Intraoperative complications	75 (0.1)	2 (0.2)	30 (0.2)	21 (0.1)	12 (0.1)	4 (0.09)	6 (0.3)		⩾ 0.05
Cardiovascular or embolic event	53 (0.1)	1 (0.1)	8 (0.05)	16 (0.09)	13 (0.13)	12 (0.2)	3 (0.17)	(Ⅱ) *vs* (Ⅴ)	⩽ 0.05
Vascular thrombosis	22 (0.04)	0	5 (0.03)	9 (0.05)	2 (0.02)	4 (0.09)	2 (0.1)		⩾ 0.05
Biliary complication	2 (0.004)	0	0	1 (0.005)	1 (0.01)	0	0		⩾ 0.05
Primary graft non-function	62 (0.12)	1 (0.1)	11 (0.07)	27 (0.16)	12 (0.12)	5 (0.1)	6 (0.3)		⩾ 0.05
Rejection	9 (0.01)	0	3 (0.02)	3 (0.01)	2 (0.02)	1 (0.02)	0		⩾ 0.05
Other causes	751 (1.5)	12 (1.3)	203 (1.3)	235 (1.4)	177 (1.7)	91 (2.0)	33 (1.9)		⩾ 0.05
Unknown	172 (0.3)	1 (0.1)	57 (0.3)	61 (0.3)	32 (0.3)	13 (0.2)	8 (0.4)		⩾ 0.05
Total	2249 (4.6)	47 (5.1)	629 (4.3)	738 (4.4)	484 (4.8)	235 (5.2)	116 (6.6)		

WHO: World Health Organization; BMI: Body mass index.

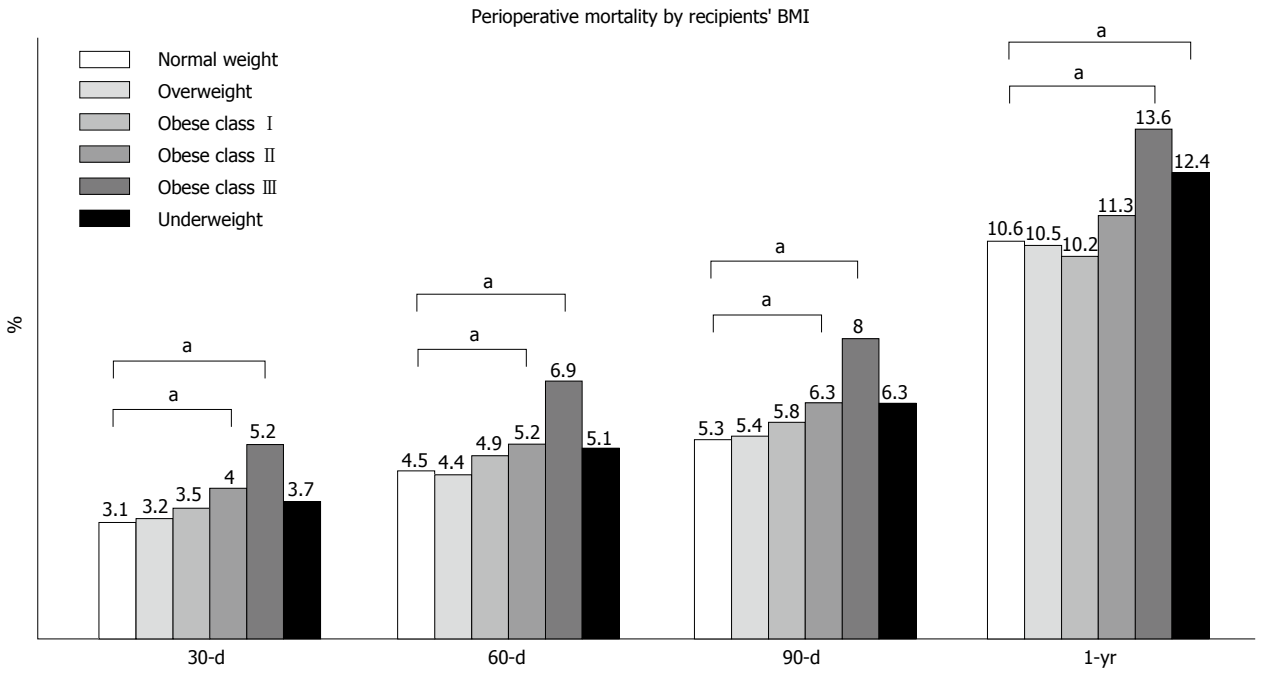


Figure 4 Graphical representation of perioperative and 1-year mortality stratified by recipients' body mass index. Statistical significant difference in perioperative mortality at 30 d, 60 d and 90 d post liver transplantation was found between normal weight recipients and class Ⅱ and class Ⅲ obese patients. At 1 year after surgery, a statistical significant difference in mortality was noted between normal weight recipient and class Ⅲ obese patients. [a]*P* ⩽ 0.05. BMI: Body mass index.

recipients. These results were equivalent to 2.38% of deaths for the entire cohort.

Analysis of the long-term impact of allocating grafts to underweight recipients showed a potential loss of 1009 life-years (95%CI: 390-1627 years), equivalent to 80 grafts (95%CI: 29-133 grafts). Allocation of grafts to obese class Ⅱ recipients resulted in a potential loss of 2311 life-years (95%CI: 1690-2932 years) or equivalent to 183 grafts (95%CI: 129-240 grafts). Allocation of grafts to obese class Ⅲ recipients resulted in a potential loss of 2056 life-years (95%CI: 1319-2793 years) or equivalent to 163 grafts (95%CI: 101-229 grafts). Overall, we estimated that avoiding LT for the two highest risk BMI groups (underweight and class Ⅲ obese recipients) would have saved 3065 life-years (95%CI: 1710-4421 years) that were equivalent to 243 extra grafts (95%CI: 131-363 grafts).

In the best hypothetical scenario where all the extra-grafts were allocated to patients with the longest median survival (normal weight recipients), the net gain for the entire cohort was 15921 life-years (95%CI: 15375-22754 life-years) that corresponded to a 2.7%

Table 8 Multivariate logistic analysis of in hospital, 90-d and 1-year mortality stratified by recipients' body mass index at the time of liver transplantation

	Multivariate analysis: In hospital mortality		Multivariate analysis: 90-d mortality		Multivariate analysis: 1-yr mortality	
Variable	Odds ratio (95%CI)	*P* value	Odds ratio (95%CI)	*P* value	Odds ratio (95%CI)	*P* value
Recipient BMI						
Normal weight	1		1		1	
Underweight	1.359 (0.865-2.135)	0.184	1.737 (1.185-2.548)	0.005	1.505 (1.105-2.048)	0.009
Overweight	0.942 (0.798-1.112)	0.481	0.995 (0.856-1.157)	0.950	0.886 (0.792-0.992)	0.036
Obese class Ⅰ	1.171 (0.974-1.408)	0.094	1.185 (1.000-1.430)	0.050	0.900 (0.792-1.028)	0.120
Obese class Ⅱ	1.135 (0.889-1.450)	0.309	1.197 (0.959-1.495)	0.112	1.004 (0.846-1.193)	0.960
Obese class Ⅲ	1.749 (1.276-2.397)	0.001	1.956 (1.473-2.597)	0.000	1.458 (1.154-1.842)	0.002
Donor BMI						
Normal weight	1		1		1	
Underweight	1.581 (1.214-2.060)	0.001	1.449 (1.131-1.857)	0.003	1.200 (0.978-1.472)	0.080
Overweight	1.025 (0.881-1.194)	0.746	1.008 (0.878-1.158)	0.907	1.025 (0.922-1.140)	0.647
Obese class Ⅰ	0.983 (0.788-1.226)	0.878	1.016 (0.832-1.239)	0.879	1.061 (0.913-1.233)	0.437
Obese class Ⅱ	1.217 (0.878-1.686)	0.239	1.189 (0.880-1.606)	0.259	1.223 (0.973-1.536)	0.996
Obese class Ⅲ	1.013 (0.647-1.585)	0.955	0.952 (0.626-1.448)	0.817	0.999 (0.731-1.365)	0.996
Recipient age						
18-45	1		1		1	
46-55	1.305 (1.089-1.563)	0.004	1.324 (1.122-1.583)	0.001	1.302 (1.146-1.479)	0.000
56-65	1.563 (1.292-1.891)	0.000	1.719 (1.447-2.041)	0.000	1.650 (1.443-1.888)	0.000
66-75	2.251 (1.737-2.917)	0.000	2.451 (1.941-3.094)	0.000	2.570 (2.146-3.078)	0.000
⩾ 76	5.081 (1.410-18.316)	0.013	6.345 (2.060-20.105)	0.001	2.694 (0.871-8.328)	0.344
Donor age						
0-17	1		1		1	
18-45	1.207 (0.961-1.516)	0.106	1.216 (0.988-1.496)	0.065	1.138 (0.968-1.337)	0.118
46-55	1.492 (1.154-1.929)	0.002	1.411 (1.117-1.784)	0.004	1.464 (1.221-1.755)	0.000
56-65	1.357 (1.027-1.793)	0.032	1.388 (1.079-1.785)	0.011	1.365 (1.123-1.660)	0.002
66-75	1.308 (0.929-1.843)	0.124	1.379 (1.014-1.875)	0.041	1.550 (1.230-1.954)	0.000
⩾ 76	0.639 (0.291-1.403)	0.265	0.864 (0.456-1.638)	0.654	1.527 (1.034-2.253)	0.033
Recipient sex (male)	0.909 (0.787-1.050)	0.194	0.966 (0.847-1.102)	0.608	1.051 (0.948-1.164)	0.344
Donor sex (male)	1.017 (0.888-1.164)	0.807	1.009 (0.892-1.142)	0.888	0.993 (0.904-1.092)	0.888
Cold ischemia time (h)	1.033 (1.019-1.047)	0.000	1.021 (1.007-1.035)	0.003	1.024 (1.013-1.035)	0.000
Warm ischemia time (min)	1.008 (1.005-1.011)	0.000	1.007 (1.005-1.010)	0.000	1.006 (1.004-1.008)	0.000
Year of transplantation	0.987 (0.968-1.006)	0.182	0.970 (0.953-0.987)	0.000	1.003 (0.989-1.016)	0.686
Dialysis	2.922 (2.378-3.590)	0.000	2.824 (2.326-3.429)	0.000	2.436 (2.071-2.865)	0.000
Diabetes	1.165 (0.990-1.371)	0.065	1.149 (0.992-1.331)	0.063	1.226 (1.095-1.374)	0.000
COPD	1.276 (0.803-2.027)	0.303	1.155 (0.996-1.341)	0.057	1.233 (0.883-1.720)	0.219
Hypertension	0.957 (0.796-1.151)	0.642	0.959 (0.810-1.135)	0.624	1.027 (0.905-1.166)	0.680
Primary indication for transplant						
HCV	1.369 (1.114-1.682)	0.003	1.215 (1.049-1.408)	0.009	0.980 (0.847-1.135)	0.791
Alcohol + HCV	1.307 (0.976-1.752)	0.073	1.038 (0.808-1.332)	0.772	0.961 (0.788-1.173)	0.698
HCV + Other viral hepatitis	1.307 (0.976-1.752)	0.073	0.895 (0.509-1.575)	0.701	0.876 (0.564-1.362)	0.558
Other	0.730 (0.549-0.970)	0.030	0.693 (0.544-0.883)	0.003	0.843 (0.677-1.050)	0.128
Alcohol	1.380 (1.095-1.740)	0.006	1.172 (0.988-1.391)	0.068	1.366 (1.153-1.618)	0.000
HBV	1.230 (0.871-1.737)	0.239	1.263 (0.912-1.747)	0.159	1.326 (1.023-1.719)	0.033
PSC	1.749 (1.229-2.489)	0.002	1.608 (1.190-2.172)	0.002	1.777 (1.377-2.293)	0.000
PBC	1.989 (1.394-2.837)	0.000	1.834 (1.352-2.487)	0.000	1.754 (1.365-2.252)	0.000
NASH	1.564 (0.899-2.722)	0.113	1.043 (0.660-1.649)	0.857	1.298 (0.902-1.869)	0.160
Autoimmune	1.179 (0.825-1.686)	0.365	0.879 (0.648-1.191)	0.405	1.027 (0.794-1.329)	0.840
Acute liver failure	0.839 (0.462-1.522)	0.839	0.797 (0.450-1.410)	0.435	1.183 (0.712-1.964)	0.517
Congenital or metabolic disease	1.139 (0.832-1.522)	0.563	1.094 (0.821-1.458)	0.540	1.162 (0.919-1.469)	0.211

HCV: Hepatitis C virus; HBV: Hepatitis B virus; PSC: Primary sclerosing cholangitis; NASH: Non-alcoholic steatohepatitis; COPD: Chronic obstructive pulmonary disease; PBC: Primary biliary cirrhosis.

(95%CI: 2.5%-3.6%) improvement in overall survival for the entire cohort.

DISCUSSION

The main findings of this study were that class Ⅲ obesity and underweight status were associated with higher perioperative mortality and inferior patient and graft survival in comparison to normal weight recipients.

To our knowledge, this is the largest multicentric retrospective observational study on the impact of BMI in LT recipients. One of its strengths is the fact that its sample size allowed us to adjust the analysis of primary and secondary outcomes for several confounders. Our study corroborated the results of several other investigators[4,15-19] but it went against the findings of other groups[8,9,18,20,21] including a recent meta-analysis[9] of 13 studies involving 76620 LT recipients that found

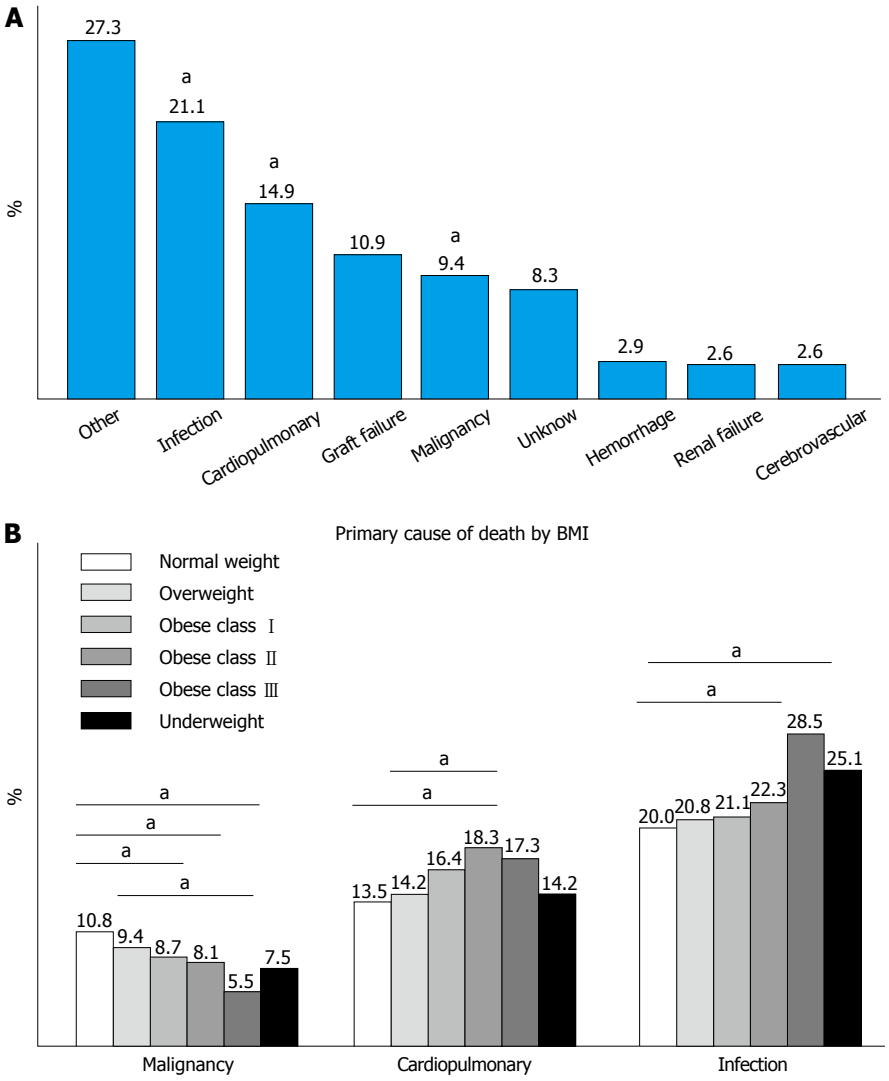


Figure 5 All causes of deaths after liver transplantation. A "a" represent statistical significant differences among recipients' BMI categories (A). Infections were more frequently observed in class Ⅱ and class Ⅲ obese recipients in comparison to normal weight patients. Similarly, deaths caused by cardiopulmonary complications were more frequent in class Ⅱ obese patients *vs* normal weight or overweight recipients. On the other hand, deaths caused by malignancies were more frequent in normal weight and overweight recipients in comparison to other BMI categories (B). $^{a}P \leq 0.05$. BMI: Body mass index.

that obesity did not impact survival of patients undergoing LT.

In 2008, Segev *et al*[22] found that in the United States, obese and morbidly obese patients were more likely turned down for a LT in comparison to normal weight candidates. A possible explanation for this is finding that LTs for obese patients can be challenging and require more resources in comparison to recipients with lower BMI indices[6,7,23]. Yet, transplant centers are dealing with obese patients with increasing frequency because obesity is prevalent in many countries[24] and in the contest of insufficient number of grafts, this creates a unique ethical dilemma[25,26]. One of the possible strategies is to deny LT to certain groups of high risk patients based on the utilitarian principle of maximizing results by transplanting only patients who have the best potential outcomes, and to accept the fact that patients who do not receive a LT would have significant shorter lives. In our study, 5-year survival for class Ⅲ obese recipients was 71.5% *vs* 73.9% for normal weight patients. Although statistically significant, the absolute difference was clinically irrelevant. Therefore, the exclusion of patients based only on their BMI might be unethical in vision of the fact that 5-year survival of obese and underweight LT recipients was higher than 50% conventionally considered the minimum survival benefit to justify allocation of liver grafts to patients with ESLD[27,28].

One of the most pressing questions we wanted to address in this study was to quantify the impact of abnormal BMIs on the overall survival of the entire cohort of patients waiting for a LT. Therefore, we simulated clinical scenarios where different graft allocation policies were implemented. By excluding underweight and morbidly obese recipients (the two highest-risk categories for perioperative mortality but representing only 5.4% of the entire cohort), an extra 243 grafts (95%CI: 131-363) could have been used to transplant low risk patients. This strategy would result in an overall 5-year survival improvement of 0.5% (95%CI:

0.27%-0.75%) for the entire cohort. The main reasons for this marginal increase were the fact that underweight and class Ⅲ obese patients represented only a very small percentage of the cohort, and the fact that the absolute difference in median survival between normal weight recipients and class Ⅲ obese and underweight patients was only 1.8 and 1.6 years respectively. These relatively small differences are most likely due to the fact that LT recipients undergo rigorous cardiopulmonary testing prior to listing, and only the healthiest of the morbidly obese patients are cleared for transplantation with overall acceptable results.

Our study has several limitations. One of the most important is its retrospective design. Therefore, confounders like immunosuppression protocols, surgical skills and pre and postoperative care provided by so many transplant centers could not be controlled in the final analysis. Another main finding of this study was that the proportion of patients who died from malignant diseases was inversely correlated with their BMI. This phenomenon was observed also by Valentijn *et al*[29] in patients undergoing non-transplant related surgeries where 52% of underweight patients died of cancer-related deaths in comparison to 24% for class Ⅲ obese. This might be due to different factors (*e.g.,* smoking habits), or to the fact that obese patients might have lower risk of developing cancer[30]. Further investigations are needed to test if obesity is indeed a protective condition against malignancies after transplantation as one of the most important limitations of this study is its retrospective design.

Another limitation was our inability to adjust for the amount of ascites that often affects patients with ESLD. Therefore, the true incidence of obesity might have been overestimated. In addition, we intentionally included recipients transplanted over a long period of time to increase the study population. The advantage of having a large number of patients had to be weighed against the fact that over the study period, there have been significant changes such as immunosuppresssion protocols, perioperative care and patient selection with significant decrease in morbidity and mortality for obese patients undergoing LT during the last ten years. These improvements might have decreased our ability to detect any clinically significant differences in overall survivals across different BMI categories.

Despite these limitations, our study has the strength of including a very large number of patients that allowed us to perform multivariate analyses to test if selected obese patients have significant worse outcomes than normal weight patients after LT. The results suggested that even for very selected class Ⅲ obese and underweight recipients, perioperative morbidity and mortality are higher than normal-weight recipients. However, these differences are clinically inconsequential as these patients have good long-term outcomes and their exclusion has a minimal survival benefit for the entire cohort of patients waiting for LT. These finding might be of some help to clinicians and policy makers who deal with the ethical dilemma of allocating liver grafts to recipients with abnormal BMI. The biggest challenge ahead of transplant programs remains the selection of those recipients who, despite their abnormal BMI, will have good outcomes and long-term benefit from LT.

COMMENTS

Background

Liver transplantation (LT) is the only treatment that can save patients' lives in the presence of irreversible liver failure. There has been a persistent discrepancy between the number of patients who are waiting for a liver transplant and the number of available livers. Several strategies have been used to increase the number of donors, but despite all the best efforts, a significant proportion of patients affected by end-stage liver disease still die while waiting for a suitable organ. Since organs are limited, the transplant community has used some criteria to prioritize the allocation of livers grafts to patients who are in urgent need of a transplant. The main reason for these criteria is to maximize the benefits and minimize the potential risks associated with such extensive surgeries. One of the emerging controversies in the field of transplantation is the allocation of livers to patients who are obese as they are considered at high risk of developing serious complications that can lead to death after LT. Therefore, there is evidence that obesity might be a negative factor that disadvantages some groups of patients who have lower probabilities of being selected for LT.

Research frontiers

The authors' group analyzed a very large database containing data prospectively collected from patients who underwent LT in the United States to assess if abnormal body mass index (BMI) was a negative predictor for survival after LT. Previous studies, using different databases, had conflicting results and controversy regarding LT, especially for obese patients, still persists.

Innovations and breakthroughs

This paper found that, although underweight and morbid obese patients had increased risks for perioperative complications and lower long term survival in comparison to normal weight recipients of liver transplants, the absolute differences were clinically negligible. In addition, impact analysis revealed that exclusion of high risk patients from undergoing LT did not improve the overall results for the entire group of patients who needed a LT.

Applications

Selected obese and underweight patients affected by end-stage liver disease should not be excluded from LT as their overall outcomes are clinically comparable to normal weight patients.

Terminology

BMI is the ratio between a person's stature and respective weight. In most cases, the higher is the BMI, the higher is the concentration of fat in the body. Persons with BMI higher than 30 are considered obese and individuals with BMI higher than 40 are considered morbidly obese. Obesity is associated with increased risks for metabolic derangements such as diabetes, hypertension, hypercholesterolemia and atherovascular diseases. Because of this association, obese patients are considered at higher risk of developing cardiopulmonary complications after LT and they absorb more resources when undergoing complex surgical interventions like LT.

Peer-review

This is a large retrospective study to attempt to answer if BMI affect outcomes of liver transplant patients. The study is well designed, performed, and written.

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P- Reviewer: Chiu KW, Marino IR, Qin JM, Xia V
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Liu SQ

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DOI: 10.5500/wjt.v6.i2.370

World J Transplant 2016 June 24; 6(2): 370-379
ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Risk factors for fracture in adult kidney transplant recipients

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Author contributions: All authors contributed to revising the manuscript.

Institutional review board statement: This study was approved by the institutional review board at Sunnybrook Health Sciences Centre, Toronto, Canada.

Informed consent statement: Data was obtained from data holdings at the Institute for Clinical Evaluative Sciences (ICES). ICES is named as a prescribed entity in Ontario's privacy law Personal Health Information Protection Act. Prescribed entity status means that health information custodians of all types can legally disclose personal health information to ICES without informed consent for purposes of analysis, evaluation and compiling statistical information about our health care system.

Conflict-of-interest statement: William Leslie: Speaker bureau: Amgen, Eli Lilly, Novartis. Research grants: Amgen, Genzyme. Jonathan Adachi: Speaker/Consultant: Amgen, Eli Lilly, Merck, Novartis, Warner Chilcott. Clinical Trials: Amgen, Eli Lilly, Merck, Novartis. Greg Knoll has received investigator-initiated research grants from Astellas, Pfizer, Roche and Novartis. Amit Garg received an investigator-initiated grant from Astellas and Roche for a Canadian Institutes of Health Research study in living kidney donors. The other authors declare that they have no competing interests.

Data sharing statement: No additional data are available.

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Received: January 19, 2016
Peer-review started: January 20, 2016
First decision: March 24, 2016
Revised: April 7, 2016
Accepted: June 1, 2016
Article in press: June 3, 2016
Published online: June 24, 2016

Abstract

AIM: To determine the general and transplant-specific risk factors for fractures in kidney transplant recipients.

METHODS: We conducted a cohort study of all adults who received a kidney-only transplant (n = 2723) in Ontario, Canada between 2002 and 2009. We used multivariable Cox proportional hazards regression to determine general and transplant-specific risk factors for major fractures (proximal humerus, forearm, hip, and clinical vertebral). The final model was established using the backward elimination strategy, selecting risk factors with a P-value $\leqslant$ 0.2 and forcing recipient age and sex into the model. We also assessed risk factors for other fracture locations (excluding major fractures, and fractures involving the skull, hands or feet).

RESULTS: There were 132 major fractures in the follow-up (8.1 fractures per 1000 person-years). General risk factors associated with a greater risk of major fracture were older recipient age [adjusted hazard ratio (aHR) per 5-year increase 1.11, 95%CI: 1.03-1.19] and female sex (aHR = 1.81, 95%CI: 1.28-2.57). Transplant-specific risk factors associated with a greater risk of fracture included older donor age (5-year increase) (aHR = 1.09, 95%CI: 1.02-1.17) and end-stage renal disease (ESRD) caused by diabetes (aHR = 1.72, 95%CI: 1.09-2.72) or cystic kidney disease (aHR = 1.73, 95%CI: 1.08-2.78) (compared to glomerulonephritis as the reference cause). Risk factors across the two fracture locations were not consistent (major fracture locations *vs* other). Specifically, general risk factors associated with an increased risk of other fractures were diabetes and a fall with hospitalization prior to transplantation, while length of time on dialysis, and renal vascular disease and other causes of ESRD were the transplant-specific risk factors associated with a greater risk of other fractures.

CONCLUSION: Both general and transplant-specific risk factors were associated with a higher risk of fractures in kidney transplant recipients. Results can be used for clinical prognostication.

Key words: Fracture; Risk factors; Kidney transplant recipient; Prognostication; Cohort study

Core tip: We examined risk factors for major and other fractures in adult kidney transplant recipients. Increasing age and female sex were associated with an increased major fracture risk, while diabetes or cystic kidney disease as the cause of end-stage renal disease and increasing age of the kidney donor were the transplant-specific risk factors associated with an increased major fracture risk. Risk factors were variable across fracture locations (major *vs* other fractures). General and transplant-specific risk factors for fracture should be considered when assessing fracture risk in kidney transplant recipients. Different risk factors may need to be considered depending on the fracture location.

Naylor KL, Zou G, Leslie WD, Hodsman AB, Lam NN, McArthur E, Fraser LA, Knoll GA, Adachi JD, Kim SJ, Garg AX. Risk factors for fracture in adult kidney transplant recipients. *World J Transplant* 2016; 6(2): 370-379 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/370.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.370

INTRODUCTION

Kidney transplant recipients are at a higher risk of fracture compared to the general population[1-4]. Reasons for the increased fracture risk are multifactorial, and may include perturbations in bone and mineral metabolism that occur in renal bone disease, and the administration of glucocorticoids after transplantation[5]. However, the risk factors for fracture after transplant remain uncertain. In a recent systematic review many classical risk factors for fracture in the general population (*e.g.,* older age, female sex) were inconsistently associated with fractures in kidney transplant recipients[6]. Unlike the transplant population, risk factors for fracture in the general population are well-established and are included in the World Health Organization's (WHO) Fracture Risk Assessment tool (FRAX). FRAX is used to guide treatment decisions in the general population by incorporating age, sex, clinical risk factors (body mass index, parental hip fracture, glucocorticoid use, rheumatoid arthritis, smoking, alcohol intake $\geqslant$ 3 units per day), and hip bone mineral density (optional) to predict the 10-year probability of hip fracture or major osteoporotic fracture (proximal humerus, forearm, hip, or clinical vertebral)[7-9]. However, kidney transplant recipients may have different risk factors for fracture given the unique pathophysiology that underlies their bone disease[10]. For example, in a recent cohort study the only classical risk factor for fracture that reached statistical significance in kidney transplant recipients was high alcohol use[11]; however, this study had only 21 fracture events and may have had inadequate statistical power to identify other risk factors[11]. The same study also found that FRAX may be a useful tool to predict fracture in kidney transplant recipients (area under the receiver operating curve 0.62); however, the authors hypothesized that incorporating transplant-specific risk factors for fracture may further improve the performance of FRAX[11].

The WHO has called for a global strategy on fracture prevention and management[12]. Such strategies require an understanding of well-validated fracture risk factors and prediction tools so populations at high risk can be targeted for diagnosis, treatment, and therapeutic trials.

Given that risk factors for fracture in kidney transplant recipients have not been well-established, we conducted this study to determine general risk factors (*e.g.,* age, sex, previous fracture, previous fall) and transplant-specific risk factors (*e.g.,* length of time on dialysis prior to transplant) associated with major fractures (proximal humerus, forearm, hip, and clinical vertebral) in kidney transplant recipients. In an additional analysis we assessed risk factors for other fracture locations (excluding major fractures, and fractures involving the skull, hands or feet).

MATERIALS AND METHODS

Design and setting

We performed a population-based cohort study using healthcare databases held at the Institute for Clinical Evaluative Sciences (ICES) in Ontario, Canada. Ontario residents have universal access to hospital and physician services. These datasets were linked using unique encoded identifiers and analyzed at ICES. This study was approved by the institutional review board at Sunnybrook Health Sciences Centre, Toronto, Canada.

Data sources

We used several databases to obtain our study cohort, characteristics, risk factors, and outcome data. Information on all kidney transplant recipients who received their transplant in Ontario was provided by the Canadian Organ Replacement Register. Information on provincial physicians' billing claims was provided by the Ontario Health Insurance Plan database. The Canadian Institute for Health Information Discharge Abstract Database provided information on diagnostic and procedural codes during Ontario hospitalizations and information on emergency room visits was provided by the National Ambulatory Care Reporting System. The Ontario Registered Persons Database provided information on vital status and demographics.

Cohort

We identified all first-time kidney-only transplants in Ontario from April 1st, 2002 to December 31st, 2009, restricting to individuals $\geqslant$ 18 years of age at the transplant date. We selected April 1st, 2002 as our cohort entry date as this was when Canada changed the International Classification of Disease (ICD) system from version 9 to 10. The cohort entry date (index date) was the date an individual received their kidney transplant.

Risk factors

We assessed several general risk factors for fracture (*e.g.,* age, sex, and prior major fracture) which are incorporated in FRAX. We also assessed other general risk factors found to increase fracture risk in the non-transplant population, including: A fall with hospitalization in the year prior to transplantation, race/ethnicity, and diabetes (only type 1 diabetes is included in FRAX)[13-15]. We assessed several transplant-specific risk factors including: Length of time on dialysis prior to transplant (years), type of donor (living *vs* deceased), cause of end-stage renal disease [ESRD, *e.g.,* diabetes mellitus, glomerulonephritis, renal vascular disease, cystic kidney disease, or other (*i.e.,* any cause of ESRD not included in the aforementioned categories such as pyelonephritis)], pre-transplant dialysis modality (peritoneal, hemodialysis, or pre-emptive), and donor characteristics (age and sex).

Outcomes

We followed kidney transplant recipients from the date of transplant until first fracture, death, or end of follow-up (March 31st, 2013). We did not censor kidney transplant recipients if they returned to chronic dialysis or if they had another transplant during follow-up. Our primary outcome was major fractures which were defined as a composite of hip, forearm, proximal humerus, and clinical vertebral fractures. We chose to assess risk factors for major fractures with hospital presentation (emergency room visit or hospital admission) as these fracture locations are associated with excess morbidity and mortality in the general population[16-18]. We also assessed other fracture locations, defined as: Lower leg (ankle, tibia, fibula, patella), femoral shaft, rib/sternum/trunk, scapula, clavicle, and pelvis fractures. These fractures as a whole were considered the secondary outcome as they may be more common in kidney transplant recipients[10]. For example, in prior studies ankle fractures were common in kidney transplant recipients[1,19]. We included both high and low trauma fractures because, similar to low-trauma fractures, high-trauma fractures occur more commonly when an individual has compromised bone strength[20]. We identified fracture events using the 10th version of the ICD system. To increase accuracy, diagnosis codes for hip, forearm, and femoral shaft fractures had to be accompanied by procedural codes identified from hospital encounters and physician billing codes[21].

Statistical analysis

We compared differences in baseline characteristics of recipients with and without a fracture using the Mann Whitney *U* test for continuous variables and the chi-square test for categorical variables. We calculated the incidence rate of fracture (per 1000 person-years) censoring the observation period on the date of death, first fracture, or end of follow-up (March 31, 2013). We used the Cox proportional hazards model to assess effects of risk factors on the hazard of the first fracture. Prior to obtaining the adjusted hazard ratio (aHR) to quantify the effect of each risk factor, model assumptions such as the proportional hazards assumption and linearity of continuous factors (Martingale residuals) were assessed with a *P*-value < 0.05 used as criteria for a violation[22-24]. We used the backward elimination

Table 1 Characteristics of kidney transplant recipients classified by major fracture status[1] *n* (%)

	No fracture (*n* = 2591)	Major Fracture (*n* = 132)	*P*-value
General risk factors			
Age (yr)	50.5 (41-61)	56.5 (45-63)	0.01
Women	928 (35.8)	66 (48.5)	0.004
Race/ethnicity			0.40
White	1845 (71.2)	103 (78)	
Asian	208 (8.0)	8 (6.1)	
Black	198 (7.6)	7 (5.3)	
Other[2]	340 (13.1)	14 (10.6)	
Diabetes	673 (25.6)	40 (30.3)	0.27
Fall with hospitalization in the year prior to the transplant date	92 (3.6)	8 (6.1)	0.15
Major fracture prior to the transplant date[3]			
Transplant specific risk factors			
Length of time on dialysis prior to transplant (measured in years)[4]	2.8 (1.2-5.4)	2.7 (0.92-5.1)	0.56
Type of donor			0.47
Deceased (*vs* living)	1458 (56.3)	70 (53.0)	
Cause of end-stage renal disease[5]			0.004
Glomerulonephritis	951 (36.7)	39 (29.6)	
Cystic kidney disease	385 (14.9)	31 (23.5)	
Diabetes	560 (21.6)	37 (28.0)	
Other	695 (26.8)	25 (18.9)	
Pre-transplant dialysis modality[6]			0.99
Peritoneal dialysis	701 (27.1)	35 (26.5)	
Hemodialysis	1622 (62.6)	83 (62.9)	
Pre-emptive	268 (10.3)	14 (11.6)	
Donor characteristics			
Type of donor			0.47
Deceased (*vs* living)	1458 (56.3)	70 (53.0)	
Donor age (yr)	46 (36-54)	48 (41-55)	0.16
Donor sex			0.73
Women	1295 (50.0)	68 (51.5)	

Data are median (interquartile range) or *n* (%). [1]Major fracture events were comprised of forearm (*n* = 81), hip (*n* = 22), proximal humerus (*n* = 18), and clinical vertebral fractures (*n* = 13); [2]Other was defined as a composite of: Indian Sub-Continent, Pacific Islander, Aboriginal, Mid East/Arabian, Latin American, Other/Multiracial; [3]Due to the small number of recipients with a prior major fracture this risk factor was not able to be assessed; [4]Includes individuals who received a pre-emptive transplant where the time spent on dialysis was defined as 0 years; [5]Due to the small number of recipients with a major fracture who had renal vascular disease as the cause of their ESRD this category was combined into the other category; [6]We defined hemodialysis and peritoneal dialysis based on the modality the recipient first received. We defined pre-emptive transplant as no evidence of hemodialysis or peritoneal dialysis prior to transplant. ESRD: End-stage renal disease.

strategy to select risk factors that would be entered into the final model, with recipient age and sex forced into the model. To decrease the possibility of missing important risk factors for fracture post-transplant, a priori we chose a *P*-value of $\leqslant$ 0.2 to select variables for inclusion in the final model[25]. We assessed for multicollinearity among variables prior to entering variables into the backward elimination model. We found limited concern for multicollinearity, since all variance inflation factors were less than 2[26]. There were missing data for the following variables: Donor age (2.2%), donor sex (< 1%), cause of ESRD (11.6%), race (10.7%), and donor type (< 1%). We handled missing data by assigning values randomly selected from observed values with the exception of donor age for which we supplemented missing values with the median age. In the final model we interpreted two-sided *P*-values < 0.05 as statistically significant. We performed all analyses using Statistical Analysis Software, version 9.4 (www.sas.com). The statistical methods of this study were reviewed by a biostatistician, Guangyong Zou, PhD.

RESULTS

Incidence of fracture

Of the 2723 kidney transplant recipients the total follow-up was 16274 person-years (average 6 years), during which 402 (14.8%) died and 132 (4.8%) sustained a major fracture (8.1 fractures per 1000 person-years, 95%CI: 6.8-9.6).

Baseline characteristics

Recipients who sustained a major fracture in follow-up compared to recipients with no major fracture had a significantly higher median age (56.5 years *vs* 50.5 years), were more likely to be women (48.5% *vs* 35.8%), and were less likely to have glomerulonephritis as their cause of ESRD (29.6% *vs* 36.7%) (Table 1).

Univariable analysis

We found older recipient age and female recipient sex were the general risk factors associated with an increased risk of major fracture (Table 2). For example,

Table 2 Univariable and multivariable analysis of risk factors for major fracture in kidney transplant recipients

Risk factors	Univariable analysis HR (95%CI)	Multivariable analysis HR (95%CI)
Age (per 5 yr increase)	1.13 (1.06-1.21)	1.11 (1.03-1.19)
Sex		
Men	Reference	
Women	1.65 (1.18-2.33)	1.81 (1.28-2.57)
Race/ethnicity		
White	Reference	
Asian	0.72 (0.35-1.47)	
Black	0.65 (0.30-1.39)	
Other[1]	0.78 (0.44-1.36)	
Diabetes (*vs* none)	1.40 (0.96-2.02)	
Fall with hospitalization in the year prior to the transplant date (*vs* none)	2.00 (0.98-4.09)	1.72 (0.84-3.50)
Major fracture prior to the transplant date[2] (*vs* none)		
Length of time on dialysis prior to transplant (measured in years)[3]	1.06 (0.61-1.84)	
Type of donor		
Living	0.99 (0.70-1.39)	
Deceased	Reference	
Cause of end-stage renal disease[4]		
Glomerulonephritis	Reference	Reference
Cystic kidney disease	1.93 (1.20-3.08)	1.73 (1.08-2.78)
Diabetes	1.80 (1.15-2.82)	1.72 (1.09-2.72)
Other	0.92 (0.56-1.53)	0.88 (0.53-1.46)
Pre-transplant dialysis modality[5]		
Hemodialysis	Reference	
Peritoneal dialysis	0.99 (0.67-1.47)	
Pre-emptive	0.96 (0.54-1.68)	
Type of donor		
Living	0.99 (0.70-1.39)	
Deceased	Reference	
Donor age (per 5 yr increase)	1.11 (1.04-1.18)	1.09 (1.02-1.17)
Donor sex		
Men	Reference	
Women	1.03 (0.73-1.44)	

[1]Other was defined as a composite of: Indian Sub-Continent, Pacific Islander, Aboriginal, Mid East/Arabian, Latin American, Other/Multiracial; [2]Due to the small number of recipients with a prior major fracture this risk factor was not able to be assessed; [3]Includes individuals who received a pre-emptive transplant where the time spent on dialysis was defined as 0 years; [4]Due to the small number of recipients with a major fracture who had renal vascular disease as the cause of their ESRD this category was combined into the other category; [5]We defined hemodialysis and peritoneal dialysis based on the modality the recipient first received. We defined pre-emptive transplant as no evidence of hemodialysis or peritoneal dialysis prior to transplant. ESRD: End-stage renal disease; HR: Hazard ratio.

female recipients had almost a two-fold greater risk of major fracture (HR = 1.65, 95%CI: 1.18-2.33). Due to the small number of recipients with a prior major fracture this risk factor was not able to be assessed. Regarding transplant-specific risk factors, cystic kidney disease (HR = 1.93, 95%CI: 1.20-3.08) and diabetes (HR = 1.80, 95%CI: 1.15-2.82) as the cause of ESRD (compared to glomerulonephritis as the reference cause) were both associated with a higher risk of major fracture. Each 5-year increase in donor age was also associated with a greater risk of major fracture (HR = 1.11, 95%CI: 1.04-1.18).

Multivariable analysis

In the multivariable model, older recipient age (5-year increase) (aHR = 1.11, 95%CI: 1.03-1.19) and female recipient sex (aHR = 1.81, 95%CI: 1.28-2.57) were the general risk factors associated with a greater risk of major fracture (Table 2). Regarding transplant-specific risk factors diabetes (aHR = 1.72, 95%CI: 1.09-2.72) and cystic kidney disease (aHR = 1.73, 95%CI: 1.08-2.78) as the cause of ESRD (compared to glomerulonephritis as the reference cause), and older donor age (5-year increase) (aHR = 1.09, 95%CI: 1.02-1.17) were associated with a greater risk of major fracture.

Other fractures

When we assessed other fracture events (excluding the major fractures, and skull, hands, or feet) kidney transplant recipients had 141 fractures (8.7 fractures per 1000 person-years, 95%CI: 7.3-10.2). Recipients with *vs* without such fractures were significantly more likely to have diabetes (40.4% *vs* 25.4%) and were more likely to have had a fall with hospitalization in the year prior to transplant (7.1% *vs* 3.5%) (Table 3). In the multivariable model we found diabetes and a fall with hospitalization prior to transplantation were the general risk factors associated with an increased risk of fracture, while length of time on dialysis, and renal

Table 3 Characteristics of kidney transplant recipients classified by other fractures status[3] *n* (%)

	No fracture (*n* = 2582)	Other fracture (*n* = 141)	*P*-value
General risk factors			
Age (yr)	52 (42-61)	54 (44-61)	0.18
Women	944 (36.6)	48 (34.0)	0.55
Race/ethnicity			0.33
White	1838 (71.2)	110 (78.0)	
Asian	208 (8.1)	8 (5.7)	
Black	198 (7.8)	7 (5.0)	
Other[1]	338 (13.1)	16 (11.4)	
Diabetes	656 (25.4)	57 (40.4)	< 0.001
Fall with hospitalization in the year prior to the transplant index	90 (3.5)	10 (7.1)	0.03
Major fracture prior to the transplant date[5]	69 (2.7)	13 (9.2)	< 0.001
Transplant specific risk factors			
Length of time on dialysis prior to transplant (measured in years)[2]	2.7 (1.1-5.4)	3.0 (1.7-5.3)	0.068
Type of donor			
Deceased	1439 (55.7)	89 (63.1)	0.09
Cause of end-stage renal disease			0.003
Glomerulonephritis	958 (37.1)	32 (22.7)	
Cystic kidney disease	397 (15.4)	19 (13.5)	
Diabetes	555 (21.5)	42 (29.8)	
Renal vascular disease	294 (11.4)	23 (16.3)	
Other	378 (14.6)	25 (17.7)	
Pre-transplant dialysis modality[4]			0.09
Peritoneal dialysis	694 (26.7)	42 (29.8)	
Hemodialysis	1613 (62.5)	92 (65.3)	
Pre-emptive	275 (10.7)	7 (5.0)	
Donor characteristics			
Type of donor			
Deceased	1439 (55.7)	89 (63.1)	0.09
Donor age (yr)	46 (36-54)	48 (40-54)	0.13
Donor sex			
Women	1298 (50.3)	65 (46.1)	0.33

Data are median (interquartile range) or *n* (%). [1]Other was defined as a composite of: Indian Sub-Continent, Pacific Islander, Aboriginal, Mid East/Arabian, Latin American, Other/Multiracial; [2]Includes individuals who received a pre-emptive transplant where the time spent on dialysis was defined as 0 years; [3]Other fracture events were comprised of pelvis (*n* = 15), ankle (*n* = 37), patella (*n* = 8), tibia/fibula (*n* = 37), rib/sternum (*n* = 34), and other (femoral shaft, scapula, clavicle; *n* = 16); [4]We defined hemodialysis and peritoneal dialysis based on the modality the recipient first received. We defined pre-emptive transplant as no evidence of hemodialysis or peritoneal dialysis prior to transplant; [5]Prior major fracture had to occur from 1991 to cohort entry (date of transplant).

vascular disease and other causes of ESRD were the transplant-specific risk factors associated with a greater risk of other fractures (Table 4).

DISCUSSION

Similar to the general population, we found increasing recipient age and female sex were associated with an increased major fracture risk in kidney transplant recipients. Unique to the kidney transplant population, we also found diabetes or cystic kidney disease as the cause of ESRD and increasing age of the kidney donor were associated with a significantly increased major fracture risk. However, risk factors were not consistent across fracture locations with increasing age and female sex not associated with an increased other fracture risk. Our findings suggest that both general and transplant-specific risk factors for fracture should be considered by clinicians when assessing fracture risk in kidney transplant recipients. However, different risk factors may need to be taken into account when considering different fracture locations.

We previously published a study of 321 kidney transplant recipients from Manitoba, Canada and found that FRAX was able to modestly predict fracture and may be a useful tool for clinicians to use to help guide treatment decisions; the area under the receiver operating curve value was 0.62 and there was concordance in the observed *vs* predicted 10-year major osteoporotic fracture probability (6.3% *vs* 5.6%, respectively)[11]. However, the number of major osteoporotic fracture events was small (*n* = 21), with correspondingly wide 95%CIs[11]. We hypothesized that a fracture prediction tool incorporating both general and transplant-specific risk factors may improve fracture prediction[11]. However, model updating may not be needed as the absolute fracture rate found in the current study was lower than previously reported, similar to other recently conducted studies[27,28]. Moreover, the strength of the transplant-specific risk factors was only moderate. Additionally, the large sample size needed to update a model and the reasonable performance of the original FRAX model in kidney transplant recipients further suggests model updating may not be needed. However,

Table 4 Univariable and multivariable analysis of risk factors for other fracture in kidney transplant recipients

Risk factor	Univariable analysis HR (95%CI)	Multivariable analysis HR (95%CI)
Age (per 5 yr increase)	1.09 (1.02-1.17)	1.03 (0.96-1.10)
Sex		
Men	Reference	
Women	0.99 (0.63-1.26)	0.97 (0.68-1.39)
Race/ethnicity		
White	Reference	
Asian	0.67 (0.33-1.37)	0.67 (0.32-1.39)
Black	0.59 (0.27-1.26)	0.47 (0.21-1.02)
Other[1]	0.82 (0.49-1.39)	0.73 (0.43-1.26)
Diabetes (*vs* none)	2.2 (1.57-3.08)	2.19 (1.38-3.49)
Fall with hospitalization in the year prior to the transplant date (*vs* none)	2.37 (1.25-4.52)	2.05 (1.07-3.93)
Length of time on dialysis prior to transplant (measured in years)[2]	1.06 (1.00-1.12)	1.07 (1.01-1.14)
Type of donor		
Living	Reference	
Deceased	0.67 (0.47-0.92)	
Cause of end-stage renal disease		
Glomerulonephritis	Reference	Reference
Cystic kidney disease	1.4 (0.8-2.47)	1.35 (0.76-2.39)
Diabetes	2.47 (1.56-3.91)	1.40 (0.78-2.49)
Renal vascular disease	2.40 (1.41-4.10)	2.11 (1.22-3.65)
Other	2.04 (1.21-3.44)	2.03 (1.20-3.45)
Pre-transplant dialysis modality[3]		
Hemodialysis	Reference	
Peritoneal dialysis	1.06 (0.74-1.53)	
Pre-emptive	0.43 (0.2-0.92)	
Donor age (per 5 yr increase)	1.07 (1.01-1.14)	1.06 (0.99-1.12)
Donor sex		
Men	Reference	
Women	0.83 (0.6-1.16)	

[1]Other was defined as a composite of: Indian Sub-Continent, Pacific Islander, Aboriginal, Mid East/Arabian, Latin American, Other/Multiracial; [2]Includes individuals who received a pre-emptive transplant where the time spent on dialysis was defined as 0 years; [3]We defined hemodialysis and peritoneal dialysis based on the modality they first received. We defined pre-emptive transplant as no evidence of hemodialysis or peritoneal dialysis prior to transplant.

to gain a more complete understanding of fracture risk, it is likely important for clinicians to consider some transplant-specific risk factors (*e.g.,* cause of ESRD) in isolation, when assessing fracture risk. Future research should assess other potential transplant-specific risk factors (unavailable in our current analyses), including: Change in body mass index after transplantation (weight changes found to increase fracture risk in the general population) and fibroblast growth factor 23 (suppresses mineralization of the bone matrix)[29,30].

We found that risk factors for fracture may vary across fracture locations. For example, there were different risk factors for fracture between our two fracture classifications (major fracture locations *vs* other fracture locations). A possible explanation for this finding is that in the kidney transplant population risk factors for fractures are site specific. For example, similar to what some studies have found in the general population, in our study increasing recipient age and female recipient sex were both associated with an increased major fracture risk[31-33]. However, increasing recipient age and female sex were not associated with an increased risk of other fractures. This provides a potential explanation for the results of a previous systematic review which found risk factors for fracture in kidney transplant recipients were inconsistent; studies in the review included different fracture locations[6]. However, we cannot discount the possibility that the differences in risk factors across fracture locations found in this study were the result of a type Ⅱ error. Future studies with larger sample sizes should assess site-specific risk factors for fractures (*e.g.,* ankle) in kidney transplant recipients.

Of concern, several of the risk factors for fracture identified in this study are becoming more common in recent eras of kidney transplant recipients. For example, we found diabetes as the cause of ESRD and older recipient age were significant risk factors for major fractures. The number of recipients with diabetes and the average recipient age has been increasing[34,35]. Similar to results found in a previous study[36], increasing donor age was also associated with an increased risk of major fracture. This is concerning as there has been an increase in the number of recipients receiving a kidney from older donors[37,38]. It is important to note that donor age may only be a surrogate measure for recipient age, with kidneys from older donors often being allocated to older recipients. Nevertheless, the increase in the aforementioned risk factors may have important implications for fracture risk in future recipients.

Unfortunately, none of the risk factors for major

fractures found in this study are easily modifiable. However, a hospitalized fall in the year prior to transplant was a significant risk factor for other fractures; falls are potentially modifiable through the use of fall prevention programs[39-41]. This is an important finding given the commonality of falls in kidney transplant recipients with over 10% of women recipients aged ⩾ 50 years sustaining a fall with hospitalization in the first 3-years after transplant[4]. The paucity of modifiable risk factors is concerning as one of the best ways to prevent fractures in the general population is to provide therapy (*e.g.,* bisphosphonates); the efficacy of these therapies in kidney transplant recipients is unclear[42]. However, given that not many recipients sustained a fracture the lack of modifiable risk factors may be less of a concern.

Limitations of the study are noted. First, we were unable to assess drug use (*e.g.,* glucocorticoids) as a potential risk factor for fracture; drug information in our databases was only available for a minority of kidney transplant recipients; therefore, our sample size would have been decreased, limiting statistical power. It is important to note that a previous study found that kidney transplant recipients who received early corticosteroid withdrawal had a 1.6% reduction in fracture compared to recipients who received standard corticosteroid based immunosuppression[43]. Future studies should explore this further, including measuring glucocorticoid use as a continuous variable and assessing the impact of reduced dose on fracture risk, with a consideration given to the impact this may have on long-term immunological outcomes (*e.g.*, graft loss)[44]. Second, we were unable to assess several risk factors, such as body mass index and estimated glomerular filtration rate, due to a high proportion of missingness (> 50%). Third, the small number of fracture events may have limited statistical power and increased concerns about the validity of the model. However, we selected a liberal *P*-value in our backward elimination analysis to ensure we were not excluding potentially important variables. Additionally, it is unlikely there were type Ⅰ errors given there were at least 10 events per variable[45]. Finally, due to the small number of fracture events we were also not able to assess several of the other risk factors included in the FRAX algorithm (*e.g.*, rheumatoid arthritis). Last, the generalizability of these results to other races/ethnic groups may be limited as the majority (72%) of our sample was White.

Both general and transplant-specific risk factors for fracture should be considered by clinicians when assessing fracture risk in this unique patient population; however, risk factors may be variable across fracture locations. Future studies with larger sample sizes should assess the ability of other transplant-specific risk factors to predict fracture.

ACKNOWLEDGMENTS

Dr. Naylor was supported by the Canadian Institute of Health Research Allied Health Professional Fellowship the Canadian National Transplant Research Program Astellas Training Award. Dr. Garg was supported by the Dr. Adam Linton Chair in Kidney Health Analytics. Dr. Lam was supported by a KRESCENT New Investigator award. This study was supported by the Institute for Clinical Evaluative Sciences (ICES) Western site. ICES is funded by an annual grant from the Ontario Ministry of Health and Long-Term Care (MOHLTC). Core funding for ICES Western is provided by the Academic Medical Organization of Southwestern Ontario (AMOSO), the Schulich School of Medicine and Dentistry (SSMD), Western University, and the Lawson Health Research Institute (LHRI). The research was conducted by members of the ICES Kidney, Dialysis and Transplantation team, at the ICES Western facility, who are supported by a grant from the Canadian Institutes of Health Research (CIHR). The opinions, results and conclusions are those of the authors and are independent from the funding sources. No endorsement by ICES, AMOSO, SSMD, LHRI, CIHR, or the MOHLTC is intended or should be inferred. Parts of this material are based on data and information compiled and provided by Canadian Institute for Health Information (CIHI). However, the analyses, conclusions, opinions and statements expressed herein are those of the author, and not necessarily those of CIHI. Aspects of this project were conducted in the Lilibeth Caberto Kidney Clinical Research Unit.

COMMENTS

Background

Compared to the general population kidney transplant recipients are at an increased risk of fracture due to a multitude of factors, including: Chronic kidney disease-mineral and bone disorder and glucocorticoid administration post-transplant. However, risk factors for fracture are not well-established in the kidney transplant population. An understanding of risk factors for fracture is important to target high risk recipients for treatment and therapeutic trials.

Research frontiers

To determine the general and transplant-specific risk factors for fractures in kidney transplant recipients.

Innovations and breakthroughs

General and transplant-specific risk factors for fracture should be considered by clinicians when assessing fracture risk in kidney transplant recipients. However, different risk factors may need to be taken into account when considering different fracture locations. Unfortunately, none of the risk factors for major fractures found in this study are easily modifiable. However, a hospitalized fall in the year prior to transplant was a significant risk factor for other fractures; falls are potentially modifiable through the use of fall prevention programs. This is an important finding given the commonality of falls in kidney transplant recipients.

Applications

The risk factors for fracture identified in this study are useful for clinical prognostication.

Terminology

Major fractures were defined as a composite of hip, forearm, proximal humerus, and clinical vertebral fractures. Other fracture locations were defined as a composite of lower leg (ankle, tibia, fibula, patella), femoral shaft, rib/sternum/trunk, scapula, clavicle, and pelvis fractures.

Peer-review

This is a well written study on an important topic in transplantation as the risk of

bone fractures after transplantation. The statistical analysis is well conducted.

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P- Reviewer: Cantarovich F, Fulop T, Kin T, Markic D, Salvadori M
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Transplantation

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5500/wjt.v6.i2.380

World J Transplant 2016 June 24; 6(2): 380-388
ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Study

Long term outcomes of cardiac transplant for immunoglobulin light chain amyloidosis: The Mayo Clinic experience

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Institutional review board statement: The Mayo Foundation Institutional Review Board approved this study.

Informed consent statement: Patients were not required to give informed consent because of observational, retrospective nature of the study.

Conflict-of-interest statement: The authors of this manuscript have no conflicts of interest to disclose pertinent to this research.

Data sharing statement: No additional data available.

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Received: January 25, 2016
Peer-review started: January 27, 2016
First decision: February 29, 2016
Revised: March 20, 2016
Accepted: April 7, 2016
Article in press: April 11, 2016
Published online: June 24, 2016

Abstract

AIM: To determine the outcome of orthotopic heart transplantation (OHT) in immunoglobulin light chain (AL) amyloidosis.

METHODS: The medical records of patients with AL who underwent orthotopic heart transplantation at the Mayo Clinic in Rochester Minnesota from 1992 to 2011 were reviewed. Patients met at least one of the following at: New York Heart Association class Ⅳ heart failure, ventricular thickness > 15 mm, ejection fraction < 40%. Selection guidelines for heart transplant included age < 60 years, absence of multiple myeloma and significant extra-cardiac organ involvement. Baseline characteristics including age, gender, organ

involvement, and New York Heart Association functional class were recorded. Laboratory data, waiting time until heart transplant, and type of treatment of the underlying plasma cell disorder were recorded. Survival from the time of OHT was calculated using Kaplan-Meier survival curves. Survival of patients undergoing OHT for AL was compared to that of non-amyloid patients undergoing OHT during the same time period.

RESULTS: Twenty-three patients (median age 53 years) with AL received OHT. There were no deaths in the immediate perioperative period. Twenty patients have died post OHT. For the entire cohort, the median overall survival was 3.5 years (95%CI: 1.2, 8.2 years). The 1-year survival post OHT was 77%, the 2-year survival 65%, and the 5-year survival 43%. The 5-year survival for non-amyloid patients undergoing OHT during the same era was 85%. Progressive amyloidosis contributed to death in twelve patients. Of those without evidence of progressive amyloidosis, the cause of death included complications of autologous hematopoietic stem cell transplantation for 3 patients, post-transplant lymphoproliferative disorder for 2 patients; and for the remaining one death was related to each of the following causes: acute rejection; cardiac vasculopathy; metastatic melanoma; myelodysplastic syndrome; and unknown. Eight patients had rejection at a median of 1.8 mo post OHT (range 0.4 to 4.9 mo); only one patient died of rejection. Median survival of seven patients who achieved a complete hematologic response to either chemotherapy or autologous hematopoietic stem cell transplantation was 10.8 years.

CONCLUSION: Our data demonstrate that long term survival after heart transplant is feasible in AL patients with limited extra-cardiac involvement who achieve complete hematologic response.

Key words: Heart transplantation; Autologous stem cell transplantation; Amyloidosis; Chemotherapy; Heart failure

Core tip: Heart failure due to immunoglobulin light chain (AL) amyloidosis is a devastating disease with poor prognosis. Orthotopic heart transplantation (OHT) is controversial. Twenty-three patients with AL amyloid underwent OHT at our institution over a twenty-year period. Median survival was 3.5 years following OHT. Median survival of seven patients who achieved a complete hematologic response to treatment for AL was almost 11 years. This study demonstrates that long term survival after heart transplant is feasible in AL patients with limited extra-cardiac involvement who achieve complete hematologic response.

Grogan M, Gertz M, McCurdy A, Roeker L, Kyle R, Kushwaha S, Daly R, Dearani J, Rodeheffer R, Frantz R, Lacy M, Hayman S, McGregor C, Edwards B, Dispenzieri A. Long term outcomes of cardiac transplant for immunoglobulin light chain amyloidosis: The Mayo Clinic experience. *World J Transplant* 2016; 6(2): 380-388 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/380.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.380

INTRODUCTION

Immunoglobulin light chain (AL) amyloidosis is a systemic plasma cell disorder, characterized by the production of a kappa or lambda monoclonal light chain by a clonal population of bone marrow plasma cells[1]. The monoclonal light chain misfolds into an insoluble beta-pleated sheet conformation. The aberrant protein subsequently accumulates in tissues, leading to organ dysfunction.

Cardiac involvement occurs in 50% of patients with systemic AL amyloidosis and is the most important risk factor for adverse prognosis and death[2,3]. Amyloid fibrils infiltrate the extracellular space of the valves, atria and ventricles, as well as the perivascular spaces, resulting in biventricular wall thickening without ventricular dilation[4]. As a result, atrial pressure increases, and atrial dilation occurs despite amyloid infiltration. Clinical features of cardiac amyloidosis include restrictive physiology, heart failure, dysrhythmias, and sudden cardiac death[1,4,5]. In addition, there is evidence suggesting that the immunoglobulin free light chains are toxic to the myocardium[6].

Patients with advanced heart failure due to AL have an extremely poor prognosis and often do not survive long enough to benefit from therapy for amyloidosis. Orthotopic heart transplantation (OHT) in AL has been limited due to the risk of disease progression in other organs and recurrence of amyloid deposition in the transplanted heart[7,8]. Although earlier studies suggested inferior outcomes of OHT for compared with non-amyloid indications[8,9], more recent reports have found survival similar to other forms of heart failure[10]. Controversy regarding the role of OHT in AL remains[11,12] and many centers consider amyloidosis to be a contraindication to OHT. The purpose of the current study was to determine the long term outcome and predictors of survival in a large single-center cohort of patients undergoing OHT for AL.

MATERIALS AND METHODS

Study population

Patients were identified from our institutional database of cardiac transplantation recipients. The diagnosis of amyloidosis was confirmed by demonstrating Congo red positivity in tissue samples. AL amyloid was confirmed by laser dissection mass spectrometry in all but two patients, who had typing performed by immunohistochemistry. During the period from May 31, 1992 to December 31, 2011, 3117 patients with

AL amyloidosis were seen at the Mayo Clinic. Twenty-one percent (668 patients) had overt congestive heart failure. One hundred and thirty-seven were referred for OHT evaluation, and 77 patients completed their evaluation for OHT. Of those completing the evaluation, 33 were deemed ineligible for OHT. Reasons for ineligibility included extensive amyloid in 29 and one instance of each of the following: Coexisting myeloma; coexisting lymphoma; improving cardiac status due to chemotherapy; and lack of financial approval. Forty-four patients (7% of patients with overt heart failure) completed evaluation and were listed for OHT, but only 23 were transplanted. Twenty-one were removed from the listing for the following reasons: Death (n = 12); further medical decline (n = 5); patient refusal (n = 2); myeloma (n = 1); and transplant elsewhere (n = 1). The median time to de-listing was 48 d (interquartile range 14, 111 d; range 0-341 d).

Throughout the 20-year period, all patients met at least one of the following at time of listing: New York Heart Association class Ⅳ heart failure, ventricular thickness > 15 mm, ejection fraction < 40%. In 1998, additional selection guidelines were added: Age < 60 years; combination of the urine light chain, serum monoclonal protein and bone marrow plasmacytosis that does not infer the presence of multiple myeloma or related disorders including low bone marrow plasma cell labeling index; absence of renal involvement as defined by a 24-h urine total protein excretion of < 500 mg and creatinine clearance > 50 mL/min per square meter unless combined renal transplant planned; absence of liver involvement - if elevation of alkaline phosphatase was thought to be due to heart failure, liver biopsy was to be done to exclude interstitial amyloid deposits. The presence of vascular deposits in a biopsy of the rectum, fat or viscera was not an exclusionary criterion. Assignment of organ involvement was according to the consensus criteria from the 10th International Symposium on Amyloid and Amyloidosis[13]. The modified body mass index (mBMI) was calculated as BMI multiplied by serum albumin level in gram per litre. For most patients the values used for listing and pre-operative BMI (and mBMI) were the same given the proximity of listing to OHT.

The autologous hematopoietic stem cell transplantation (AHSCT) protocol is as previously described, and 11 of the patients have been previously reported[14]. Demographic, clinical and laboratory data were collected from the Mayo Clinic Transplant Center database, the Robert A Kyle Dysproteinemia database, and all medical records were reviewed. Because most of these patients were treated before era of the serum immunoglobulin free light chain assay, the ability to assign a hematologic response was limited. The determination of hematologic response was a hybrid of the two consensus guidelines. If patients had serum immunoglobulin free light chains measured (n = 9), then the 2012 consensus response criteria were applied[15]; otherwise, the 10th consensus response criteria from the International Symposium on Amyloid and Amyloidosis were applied[13]. Two patients had measurable M-spikes, 8 had positive immunofixation of the serum or urine that could be followed, and 4 either had none of the aforementioned detected (or testing not performed prior to starting chemotherapy).

Immunosuppression

Post OHT, all patients received standard therapy for immunosuppression, according to our institutional protocol at the time of transplant. The first twenty-one patients received OKT3, cyclosporine, prednisone, and azathioprine or mycophenate mofetil. A gradual taper of cyclosporine was done over the first year to baseline immunosuppression. Surveillance endomyocardial biopsies to monitor for rejection were used to help guide prednisone taper. The last two patients received prednisone, mycophenate mofetil, and tacrolimus.

Statistical analysis

Medical records for the patients undergoing OHT for AL amyloidosis were reviewed. Survival from the time of OHT was calculated using Kaplan-Meier survival curves. Comparison of survival curves was done with the log-rank test. Baseline variables were tested for their impact on overall survival using Cox proportional modeling. The database was closed to follow up as of March 18, 2015. All statistics were calculated using JMP 10.0.0 (SAS, Carey, North Carolina).

RESULTS

Twenty-three patients with AL amyloidosis underwent OHT (Table 1). Fifty-two percent were female (n = 12), and all but two were Caucasian. Twenty-one patients had isolated cardiac involvement at baseline clinical evaluation; one patient (OHT#14) had mild peripheral nerve and gastrointestinal involvement, and one (OHT #15) had peripheral nerve involvement. Twenty-two patients had had a clonal lambda plasma cell disorder; one had a kappa clone. Three patients had renal transplantation, one simultaneous with the OHT and the others at 23 and 53 mo post OHT.

Twenty patients have died post OHT (Table 1 and Figure 1A). The baseline disease burden is outlined in Table 2. For the entire cohort, the median overall survival was 3.5 years (95%CI: 1.2, 8.2 years). The 1-year survival post OHT was 77%, the 2-year survival 65%, and the 5-year survival 43% (Figure 1A). Progressive amyloidosis contributed to death in twelve patients. Of those without evidence of progressive amyloidosis, the cause of death included post-AHSCT complications for 3 patients, post-transplant lymphoproliferative disorder for 2 patients; and for the remaining there was one death related to each of the following causes: Acute rejection; cardiac vasculopathy; metastatic melanoma; myelodysplastic syndrome; and unknown. Eight patients had rejection at a median of 1.8 mo post OHT (range 0.4 to 4.9 mo); only one patient died of rejection).

Table 1 Demographics and orthotopic heart transplantation outcomes

AL-OHT	M/F	Age at OHT	List to OHT (d)	Year OHT	PO FU (yr)	Major outcomes
8	F	52	62	1997	16.5	Alive, doing well
22	F	53	1160	2011	3.9	Alive, PTLD in remission, VGPR on bortezomib
23	M	58	13	2011	3.1	Alive, doing well
1	M	45	86	1992	14.1	Died, progressive amyloid; renal transplantation 53 mo post OHT
17	F	51	94	2003	10.8	Died, renal failure, debility; hematologic relapse and renal amyloid
9	M	56	16	1998	8.6	Died, metastatic melanoma
2	M	44	126	1993	8.4	Died, PTLD, sepsis, progressive amyloidosis
12	F	57	44	1999	8.2	Died, cardiogenic shock secondary to cardiac amyloid, required dialysis for renal amyloid post ASHCT#2
3	M	56	14	1994	7.5	Died, progressive amyloid
14	M	33	30	1999	6.3	Died, cardiac allograft vasculopathy
16	F	53	33	2000	5.4	Died, progressive GI amyloid and stroke; renal transplant 23 mo post-op
7	M	61	415	1997	3.5	Died, progressive amyloid autonomic and peripheral neuropathy
18	M	56	33	2004	3.1	Died, complications of myelodysplastic syndrome
5	F	47	68	1995	2.6	Died, PTLD, progressive multifocal leukoencephalopathy
10	F	54	5	1998	2.2	Died, progressive amyloid
19	M	62	18	2005	2.1	Died, progressive amyloid peripheral neuropathy and GI involvement, recurrent pneumonia
4	M	49	86	1994	1.2	Died, progressive amyloid
21	F	51	29	2007	1.2	Died, progressive GI amyloid, right heart failure, renal failure, steroid myopathy *vs* amyloid neuropathy
13	F	51	103	1999	0.9	Died, sepsis, multiorgan failure after AHSCT
11	F	48	31	1999	0.7	Died, disseminated fungal infection after AHSCT
15	F	60	33	1999	0.6	Died, progressive amyloid peripheral and autonomic neuropathy
20	F	52	33	2006	0.6	Died, progressive amyloid and overwhelming infection
6	M	56	99	1996	0.04	Died, refractory rejection; had combined renal and cardiac transplant
Median (IQR)		53 (33, 62)	33 (29, 94)			

The first three rows are alive. M: Male; F: Female; PO FU: Post-operative follow-up; PTLD: Post-transplant lymphoproliferative disorder; GI: Gastrointestinal; VGPR: Very good partial response; IQR: Interquartile range.

Table 2 Baseline disease burden

AL-OHT	IFE positive	g/dL	dFLC (mg/L)	Tx mBMI, kg g/L m^2	Creatinine (mg/dL)	Alkaline phosphatase (U/L)	IVS (mm)	EF (%)	BM PC (%)
8	Yes			688	0.9	115	15	33	12
22	Yes	1.6	298	977	0.8	60	14	55	18
23	NA	0	1059	951	1.5	150	15	50	10
1	Yes			1120	1.4	112	16	40	8
17	Yes	0	70	913	0.9	302	16	64	12
9	Yes	0	271	822	1.7	334	12	78	8
2	Yes			1191	1.1	130	15	60	5
12	Yes	0		742	1.2	312	17	30	13
3	NA			729	1.2		18	56	20
14	Yes			620	0.9	207	16	20	12
16	No	0		402	1.1	145	12	53	15
7	No				1.2	132	15	57	6
18	Yes	0	245	935	1.2	90	18	56	17
5	No			599	0.9	428	22	40	4
10	Yes	0		629	0.7	110	16	44	5
19	Yes	0	304	744	1.1	90	16	24	12
4	NA			693	1.1		14	20	NA
21	Yes	1.9	138	632	0.9	70	13	43	13
13	Yes	0.14		779	1.0	312	16	40	5
11	Yes	0	279	513	0.7	156	21	30	12
15	Yes			788	1.2	118	17	50	9
20	Yes	0	87	865	1.5	112	14	40	7
6	Yes			894	1.1	229	13	35	19
Median (IQR)			27 (11-30)	761 (631, 919)	1.1 (0.9, 1.2)	132 (111, 267)	15 (14, 16)	40 (34, 56)	12 (7, 13)

The first three rows are alive. IFE: Immunofixation of serum and/or urine; dFLC: Difference between involved and uninvolved immunoglobulin free light chains; Tx: Transplant; mBMI: Modified body mass index [albumin × weight/(height)^2]; IVS: Interventricular septum; EF: Ejection fraction; BM PC: Bone marrow plasma cells; NA: Not available; IQR: Interquartile range.

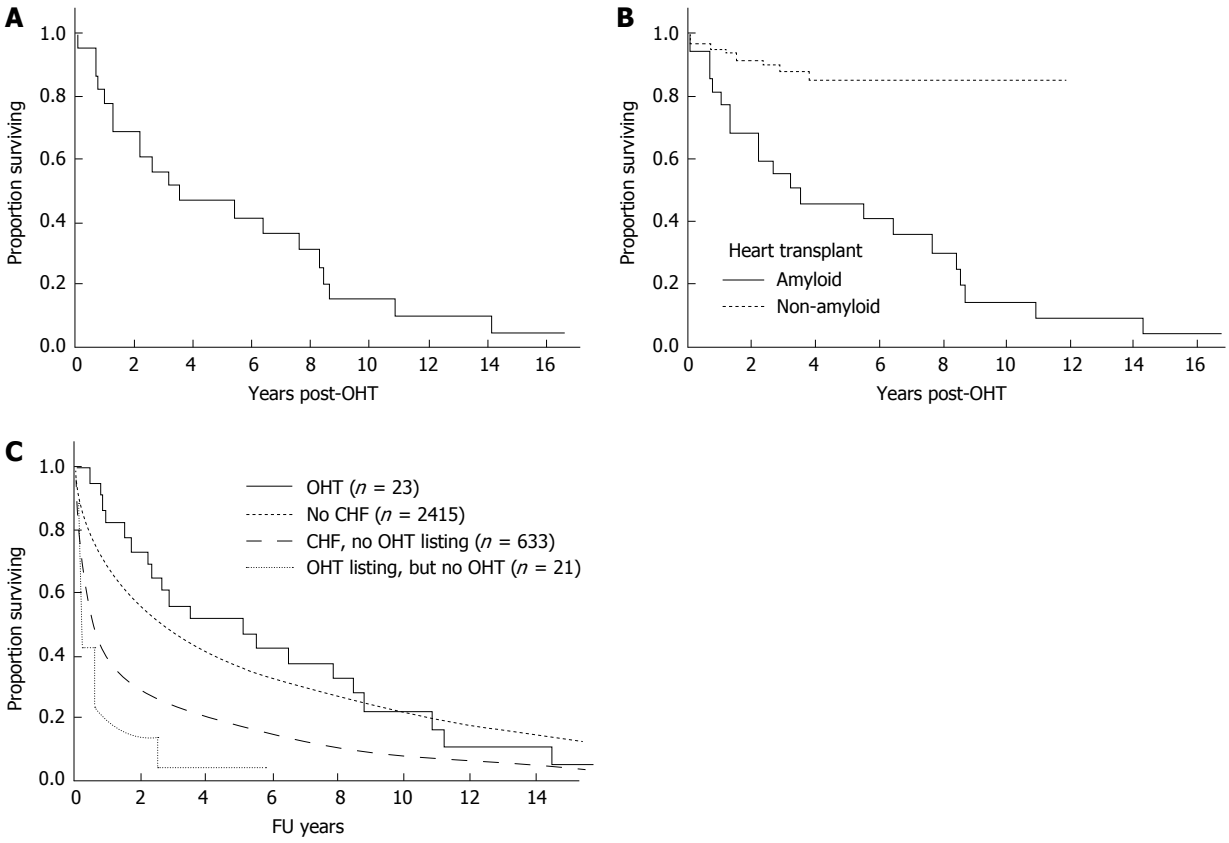

Figure 1 Overall survival. A: Overall survival from orthotopic heart transplant; B: Overall survival comparing OHT for AL amyloidosis to OHT from 1992 to 2011 for non-amyloid indications; C: Comparison of survival with non OHT subgroups. OHT: Orthotopic heart transplantation; CHF: Congestive heart failure; AL: Immunoglobulin light chain.

Figure 1B shows a comparison of this cohort to patients undergoing isolated OHT at our center for non- amyloid indications, where 1 year overall survival is 94.8% ± 2.1% and 5-year survival 85.2% ± 4.4%. Given the small sample size, it is difficult to assess baseline factors that might predict for early death. Notably, no patient died within the immediate OHT peri-operative period. On univariate analysis none of the following factors were significant risk factors for poor overall survival: Age, gender, BMI or mBMI (at listing or at transplant), time from listing to OHT, serum creatinine, or bone marrow plasmacytosis.

Figure 1C shows the comparison of patients with AL who underwent OHT with AL patients without and with overt heart failure who did not undergo OHT, and with those who were listed but did undergo OHT.

Three patients had no therapy (chemotherapy or AHSCT) for amyloidosis and only four received treatment prior to OHT (Table 3). Reasons for no chemotherapy/AHSCT were: Inability to harvest stem cells for planned AHSCT; rejection two weeks after OHT; and death 7 mo after OHT. One patient received chemotherapy only prior to OHT, 4 received chemotherapy pre- and post-OHT, and 15 only post-OHT. The non-AHSCT first line therapies are shown in Table 3. Therapies beyond first line therapies included bortezomib, AHSCT, melphalan with corticosteroids, or an IMiD with dexamethasone; one patient received doxorubicin.

After their first line therapy, seven patients achieved a complete hematologic response (CR), 3 a very good partial response, 2 a partial response, and 11 remained immunofixation positive or were not assessed before death (Table 3). As shown in Figure 2A, patients achieving a CR fared much better than those who did not, achieving a median survival of 10.8 years.

Thirteen patients underwent AHSCT, performed at a median of 8 mo (range 3-24 mo) post OHT with one patient having a second AHSCT 82 mo post OHT. In two patients AHSCT was planned but could not be performed due to inability to harvest stem cells. The median survival of those undergoing AHSCT was 6.3 years (95%CI: 1.2, 8.6 years). Figure 2B demonstrates survival outcomes of those who received AHSCT *vs* those who did not. Among the patients who underwent AHSCT, 8 received full dose melphalan conditioning (200 mg/m^2), and 5 received attenuated doses. Two of the eight patients receiving full dose melphalan conditioning died within three years post AHSCT, and one is alive 33 mo at last follow-up. In contrast 3 of the 5 receiving attenuated melphalan conditioning died within 3-year post AHSCT. For the 2 who died within 100 d of AHSCT, the cause of death was disseminated fungal infection in one and sepsis leading to multi-organ failure in the other. Four of the AHSCT patients achieved a CR, 5 a very good partial response (PR) or PR, and 4 no significant response or not assessable.

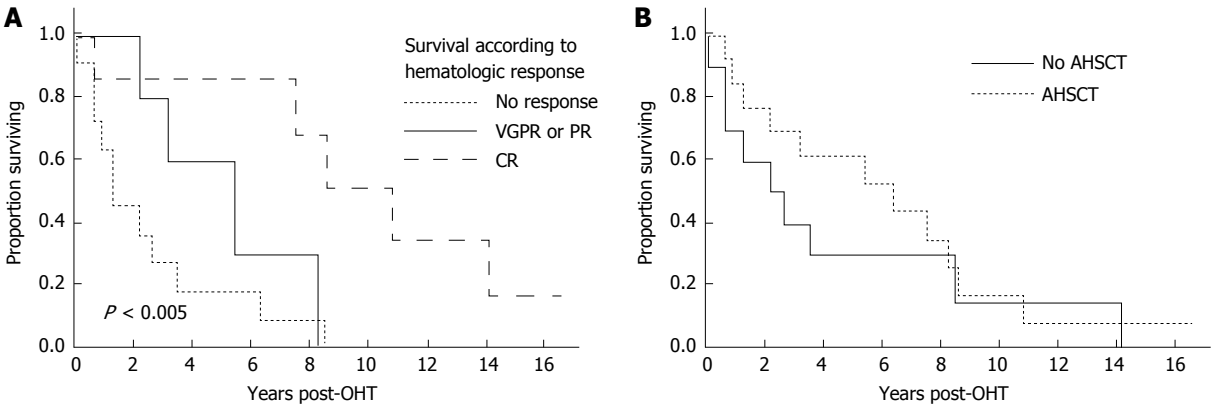

Figure 2 Influence of chemotherapy on overall survival. A: Overall survival (OS) based on hematologic response. The median OS for the 11 non-responders/non-evaluable patients was 1.2 years. The median OS for the 5 patients achieved VGPR or PR was 5.4 years. The median OS for the 7 patients achieving CR was 10.8 years; B: OS based on whether or not patient received AHSCT. The median OS for the 10 patients not undergoing AHSCT was 2.4 years; the median OS For the 13 patients undergoing AHSCT was 6.3 years. VGPR: Very good partial response; CR: Complete hematologic response; AHSCT: Autologous hematopoietic stem cell transplantation.

Table 3 Chemotherapy and response

AL-OHT	Rx relative to OHT	First amyloid directed therapy (Rx)	Response to1st Rx	Lines of Rx
8	Only Rx post-OHT	AHSCT[1]	CR	1
22	Rx pre- and post-OHT	Mel-Dex	CR	⩾ 2
23	Rx pre- and post-OHT	Bortezomib-Dex[3]	VGPR	⩾ 2
1	Only Rx post-OHT	Mel-Pred	CR	⩾ 2
17	Only Rx post-OHT	AHSCT[1]	CR	⩾ 2
9	Only Rx post-OHT	AHSCT[1]	CR	⩾ 2
2	Only Rx pre-OHT	Mel-Pred	IFE positive	1
12	Only Rx post-OHT	AHSCT[2]	PR	⩾ 2
3	Only Rx post-OHT	AHSCT[1]	CR	1
14	Only Rx post-OHT	AHSCT[2]	IFE positive	1
16	Only Rx post-OHT	AHSCT[1]	VGPR	⩾ 2
7	Rx pre- and post-OHT	VBMCP	IFE positive	⩾ 2
18	Only Rx post-OHT	AHSCT[1]	VGPR	1
5	Only Rx post-OHT	Mel-Pred	NA	1
10	No treatment	None	NA	0
19	Only Rx post-OHT	AHSCT[2]	PR	1
4	Rx pre- and post-OHT	Mel-Pred	IFE positive	⩾ 2
21	Only Rx post-OHT	AHSCT[2]	No response	⩾ 2
13	Only Rx post-OHT	AHSCT[1]	IFE positive	1
11	Only Rx post-OHT	AHSCT[2]	IFE positive	1
15	No treatment	None	NA	0
20	Only Rx post-OHT	Dex	CR	1
6	No treatment	None	NA	0

[1]Melphalan conditioning 200 mg/m^2; [2]Melphalan conditioning 140 mg/m^2 in all but OHT #12 who got 150 mg/m^2; [3]Patient had AHSCT as second line and received Melphalan conditioning 200 mg/m^2. Amyloid directed therapy: AHSCT: Autologous hematopoietic stem cell transplant; Mel: Oral melphalan; Pred: Prednisone; Bortez: Bortezomib; Dex: Dexamethasone; VBMCP: Vincristine, BCNU, melphalan, cytoxan, prednisone; NA: Not available; CR: Complete hematologic response; PR: Partial response; VGPR: Very good partial response; IFE: Immunofixation.

DISCUSSION

Given the limited supply of donor hearts, OHT in AL amyloidosis remains controversial due to the risk of recurrent amyloidosis in the graft or progression of other organ involvement. This long term follow-up study reports the largest single center experience of OHT in AL. Our results support the use of OHT in AL amyloidosis patients with predominant cardiac involvement and no evidence of myeloma, especially if they have achieved (or are able to achieve) a complete hematologic response. Although the median overall survival of our cohort was only 3.5 years, those patients who achieved a complete hematologic response had a remarkably good overall median survival of more than 10 years.

Superior survival is reported in patients with AL amyloidosis and cardiac involvement who undergo OHT compared to patients who do not[14,16]. In a report of 14 patients from the United Kingdom, median overall survival was 7.5 years from OHT; in 8 patients who underwent AHSCT and OHT, survival was increased to 9.7 years[17]. These data are confounded by selection

Table 4 Orthotopic heart transplantation in patients with amyloidosis

Ref.	*n*	AHSCT	Outcomes
Current series	23	13	1-yr OS 77% 5-yr OS 43%
MGH[10]	18	14	5-yr OS 60%
United Kingdom 2004[19]	17[1]	3	1-yr 59% 5-yr approximately 37%
United Kingdom 2010[17]	14[1]	8	1-yr OS 86% 5-yr OS 45%
Spanish registry[28]	13	3	1-yr OS 43% 5-yr OS 36%
German group[29]	12	5	1-yr OS 83% 3-yr OS 83%
ISHLT Registry[8,30]	10[2]	None	1-yr 88% 4-yr 38%
Maurer[16]	10	8	1-yr 90%
Stanford[20]	9	5	1-yr 100%
French registry[31]	8	3	1-yr 89%

[1]Unclear how much overlap between these two groups. Intervals for Dubrey series was 1982-2002 and for Sattianayagam series, interval was 1984-2004, but there was no reference of which patients had been previously reported; [2]At least 8 were AL; unclear what other 2 were. ISHT: International Society for Heart Transplant; OS: Overall survival; AHSCT: Autologous hematopoietic stem cell transplantation.

biases, but the fact remains that 30%-40% of patients with AL amyloidosis die within the first 6 mo of their diagnosis due to cardiac causes[18] making consideration of aggressive strategies imperative.

Most series of heart transplantation in AL have reasonable 1-year survival rates (Table 4). In our study there was no perioperative mortality. The major causes of death in our and other series are infection and progressive amyloidosis. If performed without chemotherapeutic support, 5-year survival is just 20%[19], Improved survival rates have been seen in patients who undergo AHSCT, with 1- and 5-year survival of 82% and 65% respectively in our earlier report[14]. Recent reports suggest improved short term outcomes with advances in chemotherapy and AHSCT, with the Stanford series reporting 1 year survival of 100%[20].

In the MGH series of 18 AL patients undergoing OHT approximately 60% of patients were alive at 6 years[10], and, in contrast to earlier studies[8,9,21], was similar to that of non-amyloid patients. Although overall survival in our study was reduced compared with patients transplanted for non-amyloid indications, our series includes many early era patients who did not receive the benefit of current therapy for amyloidosis. Nevertheless, the long term survival of the patients in our series who achieved complete hematologic response was remarkably good.

Selecting patients with primarily cardiac involvement in AL is challenging. Subclinical extra-cardiac organ involvement may progress post heart transplant to clinically important disease. Perivascular intestinal amyloid is common and not viewed as a barrier to cardiac transplantation. However, in our experience, patients with significant mucosal intestinal involvement do poorly and are often not able to tolerate aggressive treatment for AL. Clearly not all patients with cardiac involvement will require heart transplant; there are patients with significant cardiac involvement who can have cardiac improvement with effective chemotherapy alone[22-25]. Perhaps cardiac biomarkers like ST-2 may lend insight to those with irreparable damage despite effective chemotherapy[26].

"Better selection" also means choosing those patients in whom the underlying plasma cell clone can be controlled, since in our study and others effective chemotherapy has resulted in the best outcomes post-OHT[10,16,20]. Most of the patients in our series did not receive chemotherapy prior to OHT because the only chemotherapies available at the time of their diagnosis were oral melphalan and prednisone and high dose melphalan with AHSCT. Newer treatment options[27], especially bortezomib containing regimens, are less myelosuppressive, making pre-OHT therapy a possibility. Furthermore, the improvement in chemotherapeutic regimens makes hematologic response more likely in the current era.

Achieving a hematologic response pre-OHT is not a simple matter. Time is of the essence in these patients. In our experience and others, approximately 40% of AL patients listed do not undergo OHT either due to death or deterioration[10]. This seems to be related to both delayed diagnosis, as well as inability to support these patients with traditional heart failure therapy and devices. In the MGH series, patients with amyloidosis had a mortality hazard ratio of 4.7 (95%CI: 2.8, 11.8) as compared to non-amyloidosis patients while on the waiting list[10]. The only predictive factor of survival to OHT in that study was BMI - patients with lower BMI fared better than those with higher BMI, although this was not confirmed in our study.

The number of AL amyloid patients transplanted at our institution in recent years has declined. This reduction is multifactorial, and reflects patients receiving earlier and more effective bone marrow directed treatment, more rigorous selection, the availability of OHT for AL at other medical centers, and our own reluctance to offer OHT after some discouraging outcomes. However, the excellent long term survival in this study of patients achieving CR , coupled with markedly improved short term survival recently reported[20] have prompted renewed enthusiasm for OHT in AL in highly selected patients.

We recognize that our study is limited by being a small series of highly selected patients, lacking currently available cardiac biomarkers and modern markers of clonal burden and access to current treatment regimens. Despite these limitations, in carefully selected patients, long term survival can be achieved. Moving forward the challenge will continue to be the selection of the appropriate patients. The patients likely to derive the most benefit are those who: (1) have plasma cells that are responsive to chemotherapy; (2) have clinically significant involvement of the heart only; and (3) are

not demonstrating significant cardiac response despite effective chemotherapy. The current lack of effective short term cardiac support and the rapidly progressive nature of AL cardiac amyloidosis warrant consideration of revised guidelines for organ allocation in these patients.

ACKNOWLEDGMENTS

Robert A Kyle Hematologic Malignancies Program, the Predolin Foundation, and the Multiorgan Transplant Database from the William J Von Liebig Center for Transplantation and Clinical Regeneration, Mayo Clinic Rochester, MN, United States.

COMMENTS

Background

Cardiac involvement is present in approximately 50% of patients with immunoglobulin light chain (AL) amyloidosis amyloidosis and is associated with a dismal prognosis. Heart transplant for AL amyloid is controversial, due to concerns about amyloid deposition in the transplanted heart and the potential for increased morbidity and mortality from the underlying plasma cell disorder.

Research frontiers

The research goal was to review a single center experience with cardiac transplantation for AL amyloid and determine outcome.

Innovations and breakthroughs

This study demonstrates that long term survival is possible in highly selected patients with AL amyloid who undergo cardiac transplantation if the underlying plasma cell disorder can be controlled.

Applications

Patients with cardiac AL amyloid and limited extra cardiac involvement may be considered for cardiac transplantation. Long term survival is possible in those who achieve a complete hematologic response to chemotherapy or autologous stem cell transplantation.

Terminology

Immunoglobulin light chain AL is a plasma cell disorder which results in deposition of amyloid fibrils in the organs and tissues of the body. Autologous hematopoietic stem cell transplantation is a strategy to treat the underlying plasma cell disorder that causes AL amyloidosis.

Peer-review

The authors presented a good overview of patients with AL amyloidosis + advanced heart failure who received cardiac transplantation.

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DOI: 10.5500/wjt.v6.i2.389

World J Transplant 2016 June 24; 6(2): 389-395
ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Study

Ventilator associated pneumonia following liver transplantation: Etiology, risk factors and outcome

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Author contributions: Siniscalchi A and Faenza S performed the research; Aurini L and Beatrice B wrote the paper; Gamberini L analyzed and collected the data; Nava S and Viale P revised the manuscript.

Institutional review board statement: The study was reviewed and approved by the Sant'Orsola Hospital Institutional Review Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors have no conflicts of interest to disclose.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

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Received: January 16, 2016
Peer-review started: January 18, 2016
First decision: February 29, 2016
Revised: April 4, 2016
Accepted: May 7, 2016
Article in press: May 9, 2016
Published online: June 24, 2016

Abstract

AIM: To determine the incidence, etiology, risk factors and outcome of ventilator-associated pneumonia (VAP) in patients undergoing orthotopic liver transplantation (OLT).

METHODS: This retrospective study considered 242 patients undergoing deceased donor OLT. VAP was diagnosed according to clinical and microbiological criteria.

RESULTS: VAP occurred in 18 (7.4%) patients, with an incidence of 10 per 1000 d of mechanical ventilation (MV). Isolated bacterial etiologic agents were mainly *Enterobacteriaceae* (79%). Univariate logistic analysis showed that model for end-stage liver disease (MELD) score, pre-operative hospitalization, treatment with terlipressin, Child-Turcotte-Pugh score, days of MV and red cell transfusion were risk factors for VAP. Multivariate

analysis, considering significant risk factors in univariate analysis, demonstrated that pneumonia was strongly associated with terlipressin usage, pre-operative hospitalization, days of MV and red cell transfusion. Mortality rate was 22% in the VAP group *vs* 4% in the group without VAP.

CONCLUSION: Our data suggest that VAP is an important cause of nosocomial infection during post-operative period in OLT patients. MELD score was a significant risk factor in univariate analysis. Multiple transfusions, treatment with terlipressin, preoperative hospitalization rather than called to the hospital while at home and days of MV constitute important risk factors for VAP development.

Key words: Liver transplantation; Ventilator associated pneumonia; Perioperative period; Infection

Core tip: Ventilator associated pneumonia (VAP) is a serious perioperative complication in liver transplant recipients, and its etiology and risk factors are still poorly understood. Therefore, we conducted this retrospective study in a big sample of patients to evaluate the incidence, risk factors, etiological agents and outcome of VAP considering 242 consecutive liver transplant recipients. VAP occurred with an incidence of 10 per 1000 d of mechanical ventilation (MV). Multivariate analysis demonstrated that VAP was strongly associated with terlipressin usage, pre-operative hospitalization, days of MV and red cell transfusion. Mortality rate was 22% in the VAP group *vs* 4% in the group without VAP.

Siniscalchi A, Aurini L, Benini B, Gamberini L, Nava S, Viale P, Faenza S. Ventilator associated pneumonia following liver transplantation: Etiology, risk factors and outcome. *World J Transplant* 2016; 6(2): 389-395 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/389.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.389

INTRODUCTION

Ventilator-associated pneumonia (VAP) is the main hospital acquired infection in intensive care unit (ICU) and correlates with increased duration of mechanical ventilation (MV), length of ICU and hospital stay, and healthcare costs[1]. The reported rates vary significantly depending on the population, the specific ICU, the preventive strategies and the definition[2].

Liver recipients have high risk for prolonged post-operative MV due to multiple causes: Slow resolution of hepatic encephalopathy, muscle atrophy caused by pre-transplant poor nutrition and postoperative diaphragmatic dysfunction related to upper abdominal surgery.

The risk of pneumonia may be increased because of the presence of alveolar oedema and pleural effusion, as a consequence of low serum protein concentration, large amount of blood product transfusions, immuno-suppression and pre-existing risk factors like cardiac or renal failure.

The reperfusion damage has an important role in delaying extubation, which seems to be caused by the increased tumor necrosis factor (TNF) release from Kupffer cells. TNF leads to a histological damage in liver and lung tissue and could be a cause of alveolar oedema, haemorrhage and leukocyte invasion of the parenchyma.

Our study aimed to determine the incidence, etiology, risk factors and outcome of VAP in patients receiving orthotopic liver transplantation (OLT) from a deceased donor.

MATERIALS AND METHODS

Study design

After institutional review board approval, this retrospective study involved the patients who were admitted to our liver transplantation center from December 2006 to December 2010 and survived for at least 48 h. All patients had a diagnosis of end stage liver disease (ESLD) and underwent deceased donor OLT at the Transplantation Center of St. Orsola-Malpighi Policlinic in Bologna.

ESLD referred to the 4th stage or cirrhosis and was defined as the development of either a first major clinical complication of cirrhosis (variceal bleeding, ascites, jaundice, encephalopathy or spontaneous bacterial peritonitis) or hepatocellular carcinoma (HCC)[3]. Clinical evaluation of those patients used the model for end-stage liver disease (MELD) score reporting the value of the day of the transplantation.

The exclusion criteria were acute liver failure, simultaneous kidney/liver or liver/heart transplantation.

We analyzed the incidence, etiology, risk factors and impact of VAP on clinical outcome. All patients were evaluated, at the moment of the admission, to confirm the absence of pneumonia. Patients were followed until hospital discharge or death.

Definitions

The suspicion of VAP was based on clinical criteria (new or progressive radiological pulmonary infiltrates plus two or more of the following: Temperature > 38.3 ℃ or < 36 ℃, leukocyte count > 10 × 10^9/L or < 4 × 10^9/L and purulent respiratory secretions)[4] appearing 48-72 h post intubation and initiation of MV.

A microbiologic strategy was then followed for diagnosis: Microbiologic lower respiratory tract samples were obtained with bronchoalveolar lavage (BAL) or endotracheal aspirate.

VAP diagnosis was defined in case of positive results

Table 1 Etiologic agents of ventilator-associated pneumonia

Microorganism	Total (n = 18)
Klebsiella pneumonia	5/18 (28%)
Escherichia coli	5/18 (28%)
Klebsiella oxytoca	2/18 (11%)
Enterobacter spp.	1/18 (6%)
Citrobacter spp.	1/18 (6%)
Pseudomonas aeruginosa	8/18 (44%)
Staphylococcus aureus	4/18 (22%)
Corynebacterium striatum	4/18 (22%)
Xantomonas spp.	2/18 (11%)
Acinetobacter spp.	2/18 (11%)

The *Enterobacteriaceae* are written in bold. Note that in some patients more than one microorganism was found.

of quantitative culture of specimens from BAL or tracheoaspirate with protected brush (considering a threshold of 1×10^5 cfu/mL in a BAL fluid specimen, and 1×10^6 cfu/mL in an endotracheal aspirate specimen[5].

Postoperative management

Immunosuppressive induction was achieved by administrating 1 g of methylprednisolone at the time of reperfusion; the immunosuppressive regimen consisted of a combination of calcineurin-inhibitor and prednisone.

Postoperative interventions according to the European guidelines since 2002[6] for VAP prevention consisted of semi-recumbent patient positioning, sedation resolution and use of a weaning protocol, strict hand hygiene, non-invasive ventilation, oral care with chlorhexidine, no ventilatory circuit tube changes unless specifically indicated, appropriately educated and trained staff, cuff pressure control every 24 h, enteral feeding, use of heat moisture exchangers and unit-specific microbiological surveillance.

Data collection

Pre-operative, intra-operative and post-operative data were recorded.

Preoperative data included age, weight, height, body mass index (kg/m^2), body surface (m^2), etiology of cirrhosis, presence of HCC at pre-operative investigation, MELD score at the transplantation day, Child-Turcotte-Pugh (CTP) score, serum bilirubin (mg/dL), serum creatinine (mg/dL), international normalized ratio, glycated haemoglobin (%), serum urea (mg/dL), serum glucose (mg/dL), serum albumin (g/dL), transjugular intrahepatic portosystemic shunt presence, ongoing therapy with diuretics, and terlipressin (instead of its indications as the clinical and laboratoristic parameters are included in other scores) at the time of transplantation, patient preoperative hospital stay rather than called to the hospital while at home.

Intra-operative data included length of surgery, anhepatic phase duration, number of packed red blood cells (RBC), fresh frozen plasma and platelets transfusions (units), duration of cold ischemia (h), vasopressors usage in pre and post-reperfusion phase, donor age and gender. Quality of liver allograft was classified on the basis of Donor risk index (DRI)[7] as low risk (DRI < 1.8) or high risk graft (DRI > 1.8)[8].

Postoperative data considered: VAP incidence and etiology, duration of MV, time between intubation and VAP clinical manifestation, length of ICU stay and hospital mortality.

Statistical analysis

Statistical analyses were performed with SPSS 16.00. Continuous data are expressed as medians (25-75 interquartile range) while discrete data are represented by numerosity and relative frequencies. Patients were divided into two subgroups on the basis of presence or absence of VAP. Incidence of VAP is reported as episodes per 1000 d of MV. Differences between groups were assessed using χ^2 test or Fisher exact test for categorical variables and student's t-test or Mann-Whitney test for continuous variables. Variables which were significantly different between the two groups were individually analyzed with a univariate logistic regression model, considering VAP insurgence the dependent variable. Predictor variables found in univariate analysis were included into a multivariate logistic regression model using the Enter method, considering VAP insurgence the dependent variable. Results are expressed as hazard ratios, and P values with 95%CIs.

RESULTS

During the study period from 2006 to 2010, 284 patients underwent OLT at the Transplant Center of St. Orsola-Malpighi Hospital. Forty-two patients were not included in the analysis because they had: Combined liver/kidney or liver/heart transplantation (29 cases), transplantation for acute liver failure (6 cases) and other causes without concomitant cirrhosis (7 cases). The final analysis considered 242 patients with ESLD related to histologically proven liver cirrhosis.

Microbiologically confirmed VAP occurred in 18 (7.4%) patients, with an incidence of 10 episodes per 1000 d of MV, and none of these patients presented any criteria of pneumonia, from the in-hospital admission to the time of transplantation. The 18 patients received a diagnosis of VAP after positive BAL culture, and all of them were extubated within 48 h since pneumonia detection.

Isolated microbes belonged mainly to the group of *Enterobacteriaceae* (79%, 14 patients), including *Klebsiella pneumoniae*, *Escherichia coli*, *Klebsiella oxytoca*, *Enterobacter* spp. and *Citrobacter* spp. The remaining bacterial etiologic agents were represented by *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) (Table 1).

We observed that 25% of VAP episodes involved more than one microorganism.

Demographic data of the study population and

Table 2 Preoperative, intraoperative and postoperative variables

Variable	Patients in study (*n* = 242)	VAP-yes (*n* = 18)	VAP-no (*n* = 224)	*P*-value
Age (yr)	56 (19-69)	55 (37-66)	56 (19-69)	0.624
Weight (kg)	72 (39-106)	73 (47-93)	72 (39-106)	0.515
Height (cm)	170 (148-193)	169 (155-182)	170 (148-193)	0.495
BMI (kg/m^2)	25 (16-38)	24 (19-34)	25 (16-38)	0.452
BSA (m^2)	1.9 (1.3-2.3)	1.9 (1.4-2.1)	1.9 (1.3-2.3)	0.505
HCV$^+$	128 (53%)	8 (44%)	120 (54%)	0.455
HBV$^+$	49 (20%)	3 (17%)	46 (21%)	0.486
Alcohol abuse	37 (15%)	3 (17%)	34 (15%)	0.540
HCC	118 (49%)	4 (22%)	114 (51%)	0.019
MELD score	21 (6-48)	23 (14-48)	20 (6-45)	0.032
CTP score	11 (5-15)	11 (9-14)	11 (5-15)	0.060
Bilirubin (mg/dL)	5.9 (0.4-71.1)	8.0 (2.1-71.1)	5.6 (0.4-68.6)	0.054
Creatinine (mg/dL)	1.0 (0.0-5.2)	1.1 (0.5-5.2)	1.0 (0.0-4.9)	0.708
INR	1.6 (0.8-7.6)	1.9 (1.3-3.8)	1.6 (0.8-7.6)	0.020
HbA1c (%)	10.8 (4.5-17)	9.9 (8.1-14.9)	10.9 (4.5-17)	0.085
Urea (mg/dL)	0.3 (0.1-3.1)	0.3 (0.1-2.6)	0.3 (0.1-3.1)	0.554
Serum glucose (mg/dL)	105 (60-358)	102 (63-284)	105 (60-358)	0.369
Albumin (g/dL)	3.5 (2.0-5.3)	3.3 (2.6-4.5)	3.5 (2.0-5.3)	0.189
TIPS presence	15 (6%)	1 (6%)	14 (6%)	0.691
Furosemide therapy	144 (60%)	11 (61%)	133 (59%)	0.885
Canrenoate therapy	112 (46%)	5 (28%)	107 (48%)	0.102
Terlipressin therapy	20 (8.3%)	7 (39%)	13 (5.8%)	< 0.001
Preoperative hospital stay	82 (34%)	11 (61%)	71 (32%)	0.018
Intraoperative and postoperative variables				
Length of surgery (min)	560 (512-650)	570 (490-630)	580 (460-660)	0.067
Anhepatic phase duration (min)	120 (88-138)	118 (85-138)	140 (116-145)	0.067
RBC transfusions (units)	8 (0-65)	16 (6-48)	7 (0-65)	< 0.05
FFP transfusions (units)	9 (0-75)	10 (0-31)	9 (0-35)	0.122
Platelet transfusions (units)	2 (0-4)	2 (1-3)	2 (1-3)	0.587
CIT (h)	7 (6-9)	7 (7-9)	8 (7-9)	0.354
Pre-reperfusion VP infusion	68 (28%)	8 (22%)	60 (26%)	0.530
Post-reperfusion VP bolus	110 (45%)	8 (44%)	102 (45%)	0.520
Post-reperfusion VP infusion	118 (48%)	10 (55%)	108 (48%)	0.510
Duration of ICU stay (d)	5 (3-10)	16 (20-59)	5 (3-8)	< 0.05
Hospital mortality	14 (6%)	4 (22%)	10 (4%)	< 0.05
Median duration of MV (d)	0.42 (0.208-0.417)	1.125 (0.375-11.75)	0.38 (0.208-0.864)	< 0.05
Time between intubation and VAP insurgence (h)	-	72 (48-336)	-	-

Statistical analyses were performed using parametric tests and nonparametric tests (Wilcoxon's rank sum) when normality or variance assumptions were not met. Proportions were compared by Fisher's test. Statistical significance was defined as $P < 0.05$. HCV: Hepatitis C virus; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; BMI: Body mass index; BSA: Body surface; MELD: Model for end-stage liver disease; CTP: Child-Turcotte-Pugh; INR: International normalized ratio; HbA1c: Glycated hemoglobin; TIPS: Trans-jugular intrahepatic porto-sistemicity shunt; RBC: Packed red blood cells; FFP: Fresh frozen plasma; CIT: Cold ischemia time; VP: Vasopressor; ICU: Intensive care unit; MV: Mechanical ventilation; VAP: Ventilator-associated pneumonia.

Table 3 Donor variables *n* (%)

Variable	Patients in study (*n* = 242)	VAP-yes (*n* = 18)	VAP-no (*n* = 224)	*P*-value
Donor age (yr)	56 (14-89)	58 (20-86)	56 (19-80)	0.624
Donor gender (male)	182 (75)	14 (77)	168 (69)	0.624
Donor risk index > 1.8	52 (21)	4 (22)	48 (21)	0.345

VAP: Ventilator-associated pneumonia.

general preoperative, intraoperative and postoperative characteristics are reported in Table 2 and the donor variables in Table 3.

Significant differences in MELD score were observed between the two groups; VAP patients had a mean MELD score of 23 *vs* 20 of control patients. Treatment with terlipressin was associated with a higher risk of pneumonia (39% of VAP episodes receiving terlipressin *vs* 5.8% in the control group).

Intraoperative data (Table 2) showed statistically significant differences between the two groups in red cell transfusion (red cell transfusion refers to the large amount of red cell transfusion): A median of 16 units per patient in the VAP group *vs* 7 in controls. Postoperative data (Table 2) showed that ICU stay of VAP patients was significantly longer (16 d *vs* 5 d) and was associated with a higher hospital mortality (22% of VAP patients died *vs* 4% of controls). VAP was

Table 4 Variables associated with ventilator-associated pneumonia in multivariate analysis

Variable	OR	95%CI	*P*-value
MELD score	0.98	0.8-10	0.670
CTP score	0.79	0.5-1.1	0.27
RBC transfusions (units)	1.1	1.04-1.1	< 0.001
MV (d)	1.10	1.03-1.15	< 0.001
Terlipressin therapy	31.49	4.7-49.2	< 0.001
Preoperative hospital stay (d)	1.8	1-1.9	< 0.05

Statistical significance was defined as $P < 0.05$. MELD: Model for end-stage liver disease; CTP: Child-Turcotte-Pugh; RBC: Packed red blood cells; MV: Mechanical ventilation.

documented after a median of 72 h post intubation. Median intubation duration among all studied patients was 0.42 d, patients without VAP required a median of 0.38 (0.208-0.864) d of MV, while VAP patients required a median MV duration of 1.13 (0.375-11.75) d. This interval ran from the first intubation to the extubation or need for reintubation. The time from the second intubation to the extubation/exitus was not considered in the study.

Univariate logistic regression analysis found that MELD score, treatment with terlipressin, CTP score, days of MV, preoperative hospitalization and red cell transfusion were significantly associated with VAP (data not shown).

The multivariate logistic regression model constructed considering the variables which resulted significantly associated with VAP in univariate analysis resulted in a significantly increased risk of VAP for terlipressin use, red cell transfusion, duration of MV and preoperative hospitalization (Table 4).

DISCUSSION

It has been reported that the rate of VAP is usually 1 to 3% per day of intubation and MV and the rates of pneumonia are increased 6- to 21-fold for intubated patients and show a further rise with the duration of MV. It has been estimated that the overall rates are most commonly 10 to 15 cases per 1000 ventilator days for ICU patients, depending on the population studied. The National Nosocomial Infections Surveillance System reports a median occurrence of VAP of 4.6 -5.1 for 1000 ventilator days either in medical or surgical ICUs. Also, rates are generally higher in surgical ICU patients than in medical ICU patients[9,10].

Data about the incidence of VAP in OLT patients are poor and highly variable, the incidence rates range from 5% to 48% and the rates of the VAP-related mortality from 36% to 53%[11]. A recent monocentric Italian study was not able to detect increased frequency of VAP in a small population of OLT patients compared to a control group of non-OLT patients admitted to the same surgical ICU[12]. Another study[13] on the infections after OLT reported the occurrence of VAP in 17.5% of their samples.

Our results show a higher incidence of VAP than previous results from similar patients. We have to underline that our patients presented a higher MELD score (mean values 20-23) than those considered in other studies (mean values 14-15), which could reflect worse general preoperative conditions predisposing to infections, although the mortality rate was comparable (22%).

As stated before, MELD score has already been associated with postoperative complications, and this association is concordant with the correlation between MELD score and the seriousness of the post-operative complications[9].

The early identification of clinical predictors of severe prognosis, *i.e.*, the MELD score, could help to identify patients at major risk and to take appropriate measures, earlier intensive treatment and several strategies including the use of non-invasive ventilation when possible to reduce the rate of VAP[14,15].

The quality of the liver graft, which has an important role in determining prognosis of transplanted organs, does not seem to play a role in early infectious complications like VAP. In fact, high risk grafts were equally distributed in the two groups, and this result has been corroborated in the literature[11].

The microorganisms associated with VAP vary widely depending on the characteristics of the patients, the different ICUs and the length of in-hospital stay. Common pathogens include *Enterobacteriaceae*, *P. aeruginosa* and *S. aureus*[16]. In our series, the microorganisms associated with VAP, after liver transplantation, are not different from those in non-OLT patients in ICU[17]. The *Enterobacteriaceae* predominated over *P. aeruginosa* and *S. aureus.*

Our study confirmed previous finding that multiple blood transfusions were associated with VAP insurgence. This is because longer duration of significant bleeding during OLT may lead to more alveolo-capillary membrane damage and prolonged postoperative intubation.

Our study shows that patients receiving terlipressin for hepatorenal syndrome had an odds ratio of 31.49 times higher for VAP, in the multivariate analysis. Further studies may investigate if hepatorenal syndrome (HRS) or its treatment with terlipressin is the effective risk factor for VAP. That is a limitation of the current study. Terlipressin therapy for HRS requires hospital admission and this could influence the outcome, but it has a notorious detrimental effect on splancnic microcirculation. We suppose that the vasoconstricting action could damage intestinal barrier and foster bacterial migration through haematic and lymphatic circulation to pulmonary parenchyma, and this mechanism could also explain the high incidence of *Enterobacteriaceae* among etiologic agents in our case series. Westphal *et al*[17] showed in an animal study that terlipressin treatment induced important alterations in pulmonary circulation, decreased cardiac index, and

diminished systemic oxygen delivery and consumption.

Despite the mentioned results, this study presents some limitations. We reported a low number of pneumonia cases due to its globally low incidence and the limited sample size, since our data came from a single center.

In conclusion, this study was designed to investigate the incidence, the risk factors and the outcome of VAP after OLT. Incidence has been estimated to be 10 per 1000 d of MV. Our study confirms some of the risk factors for VAP found in other studies: RBC transfusion, duration of MV and preoperative hospitalization rather than direct admission from home. MELD score is higher in the VAP group and it represents a significant risk factor in univariate analysis, reflects worse general conditions and prospects higher postoperative complications. The adoption of MELD score could rationalize VAP prevention practice in patients at major risk, earlier intensive treatment to increase the ventilator-free days and several stategies including the use of non-invasive ventilation. Among the risk factors, we found the therapy with terlipressin, used for the treatment of hepatorenal syndrome. This drug exhibited, in animal models, some effects on pulmonary circulation and has a detrimental effect on splancnic blood flow that could contribute to bacterial migration. Also hepatorenal syndrome could have contributed to this effect. Further studies are needed to clarify this correlation.

COMMENTS

Background

Patients undergoing orthotopic liver transplant (OLT) represent a special subpopulation at risk for nosocomial infections, in particular ventilator-associated pneumonia (VAP) is the main hospital acquired infection in intensive care unit and it is a serious perioperative complication in liver transplant recipients.

Research frontiers

VAP's etiology and risk factors are still poorly understood.

Innovations and breakthroughs

The authors conducted this retrospective study in a big (considering the peculiar population) sample of patients, 242 consecutive liver transplant recipients. Of course, none of the patients who developed VAP presented signs or symptoms of infection before liver transplantation. Model for end-stage liver disease score was a significant risk factor in univariate analysis, and probably it reflects worse general conditions. In multivariate analysis the authors found a statistically significant association with terlipressin therapy. Patients who were taking terlipressin received the last dose until the OLT to treat the hepatorenal syndrome that could be a risk factor by itself. The authors did not refer to clinical and laboratory parameters as they are included in other scores. Some patients received other vasopressors during the OLT, but there were no statistically significant differences between the two groups. As the authors remarked in the discussion, further studies are needed to clarify this finding.

Applications

The application of the authors' results aims to individualize patients at major risk, to apply earlier intensive treatment and several strategies to prevent VAP.

Peer-review

The authors performed a study on a very important infectious complication in post-operative OLT setting. The paper is well written and the aim is clear.

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P- Reviewer: Boin IFSF, Giannella M, Mouloudi E
S- Editor: Kong JX **L- Editor**: Wang TQ **E- Editor**: Liu SQ

World Journal of Transplantation

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5500/wjt.v6.i2.396

World J Transplant 2016 June 24; 6(2): 396-402
ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Study

Liver transplantation for hepatocellular carcinoma in Ireland: Pre-operative alpha-fetoprotein predicts tumour recurrence in a 14-year single-centre national experience

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Institutional review board statement: The study was approved by the St Vincent's University Hospital ethics review board.

Informed consent statement: Informed consent was not required for this study.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Data sharing statement: No additional data.

Manuscript source: Unsolicited manuscript

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Received: November 11, 2015
Peer-review started: November 15, 2015
First decision: January 4, 2016
Revised: April 12, 2016
Accepted: May 7, 2016
Article in press: May 9, 2016
Published online: June 24, 2016

Abstract

AIM: To examine the results of orthotopic liver transplantation (OLT) for hepatocellular carcinoma (HCC) in Ireland over a 14-year period.

METHODS: Cases of HCC receiving OLT between January 1995 and September 2009 in the Irish Liver Transplant Unit were reviewed from a prospectively maintained database. Outcome measures included overall and recurrence free survival, alpha-fetoprotein (AFP) and tumour pathological features.

RESULTS: On explant pathology, 57 patients had HCC. The median follow-up time was 42.7 mo. The overall 1, 3 and 5 years survival was 87.7%, 72.1% and 72.4%. There was no difference in survival when compared

to patients undergoing OLT without malignancy. The tumour recurrence rate was 14%. The Milan criteria were exceeded in 32% of cases but this did not predict overall survival or recurrence. On multivariate analysis pre-operative AFP > 100 ng/mL was an independent risk factor for recurrence (RR = 5.2, CI: 1.1-24.3, *P* = 0.036).

CONCLUSION: Patients undergoing OLT for HCC had excellent survival even when conventional listing criteria were exceeded. Pre-operative AFP predicts recurrence independent of tumour size and its role in selection criteria should be investigated in larger studies.

Key words: Liver transplantation; Alpha-fetoprotein; Hepatocellular carcinoma; Transplantation selection criteria; Liver cirrhosis

Core tip: We have shown good survival from a medium volume transplant centre in a small cohort of patients exceeding Milan criteria. We show an association between a pre-operative alpha-fetoprotein (AFP) > 100 and hepatocellular carcinoma (HCC) recurrence, independent of tumour size. Our study supports other single centre experience on survival after transplant for HCC with low AFP and indicates that AFP needs to be interrogated in large, multi-centre studies to see if it can be included in transplant listing criteria to augment the current radiology based dimensional criteria.

O'Connor DB, Burke JP, Hegarty J, McCormick AP, Nolan N, Hoti E, Maguire D, Geoghegan J, Traynor O. Liver transplantation for hepatocellular carcinoma in Ireland: Pre-operative alpha-fetoprotein predicts tumour recurrence in a 14-year single-centre national experience. *World J Transplant* 2016; 6(2): 396-402 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/396.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.396

INTRODUCTION

Hepatocellular carcinoma (HCC) is the 5th most common cancer and 3rd leading cause of cancer-related death worldwide[1]. The incidence and related mortality are increasing, particularly in Western countries[2]. It is now well accepted that the optimal treatment for small HCC in the setting of cirrhosis is orthotopic liver transplantation (OLT)[3]. Since the publication and adaptation of the Milan criteria[4] the outcomes have improved dramatically compared to results from the era prior to established selection criteria[5]. Patients undergoing OLT for HCC within the Milan criteria achieve outcomes comparable to non-malignant transplant cohorts. However, recurrence is the most important cause of post-transplant death[6]. Appropriate patient selection is crucial as patients with large or biologically unfavourable tumours have unacceptable recurrence and overall survival rates[7]. It is essential that centres which provide OLT for HCC audit their results to ensure outcomes compare to international survival rates thus enabling appropriate patient prioritisation and organ allocation.

The aim of the current study was to determine the outcomes of OLT for HCC in a single, national institution over a 14-year period. Overall and recurrence free survival rates were compared to clinical and pathological factors using multivariate analysis to identify independent predictors of recurrence.

MATERIALS AND METHODS

All patients undergoing OLT with HCC proven on explant pathology between January 1995 and September 2009 were included in the study. All OLT in the Republic of Ireland are carried out in the Liver Unit of St Vincent's University Hospital. The Liver Unit maintains a prospective database containing patients' clinical details. Tumour characteristics are recorded on a computerised pathology database. A retrospective review of this data was performed. Patient characteristics recorded included age at OLT, sex, aetiology of underlying liver disease, pre-operative alpha-fetoprotein (AFP), survival, and recurrence status. Tumour data recorded included size and number of tumours, compliance with the Milan and University of California San Francisco (UCSF) criteria and microvascular invasion. The study was approved by the St Vincent's University Hospital ethics review board.

Patient selection and transplant protocol

All patients listed were classified clinically as cirrhotic. HCC diagnosis was based on a combination of ultrasound, computed tomography (CT) and double-contrast magnetic resonance imaging (MRI). After 1996 patients were listed for OLT if they met Milan criteria on pre-operative imaging. Patients in Ireland listed for OLT for HCC receive an adjusted Model for End-Stage Liver Disease (MELD) score[8]. All OLT were from deceased donor transplants and organs were retrieved before cardiac death. Patients were followed up at a dedicated transplant clinic every three months. In general, post-transplant immunosuppression consisted of a reducing dose of corticosteroids and a calcineurin inhibitor (tacrolimus) or azothioprine. Follow up included annual abdominal ultrasound and CT where appropriate.

Survival and recurrence

Overall patient survival was determined from date of OLT until the most recently attended clinic. HCC recurrence free survival was determined by the date of the most recently available radiological imaging. Deaths from recurrence were prospectively recorded in the database. Patients without recurrence that died were documented free of recurrence only if the most recent available imaging or post-mortem report excluded recurrence. Two investigators (O'Connor DB

Table 1 Patient demographics

Male:female	44:13
Age, median (IQR)	59.1 (53.5-63.6)
Aetiology of HCC	
Alcoholic liver disease	17 (29.8%)
Hepatitis C	17 (29.8%)
Haemochromatosis	13 (22.8%)
α-1-antitrypsin deficiency	3 (5.3%)
Primary sclerosing cholangitis	2 (3.5%)
Primary biliary cirrhosis	2 (3.5%)
Autoimmune hepatitis	2 (3.5%)
Hepatitis B	2 (3.5%)
Cryptogenic	2 (3.5%)
Cystic fibrosis	1 (1.8%)
Sarcoidosis	1 (1.8%)
Nash	1 (1.8%)
Pre-operative α-fetoprotein, median (IQR)	8.8 (3.3-29.2)
Compliant with Milan criteria	41 (71.9%)
Compliant with UCSF criteria	49 (86.0%)
Largest lesion, median (IQR)	3 (2.5-4.5)
Cirrhosis	53 (93.0%)
Steatosis	2 (3.5%)
Multifocal lesions	24 (42.1%)
Micro-vascular invasion	24 (42.1%)
Tumour differentiation	
Well	24 (42.1%)
Moderate	28 (49.1%)
Poor	5 (8.8%)
Incidental lesions	5 (8.8%)

HCC: Hepatocellular carcinoma; UCSF: University of California San Francisco; IQR: Interquartile range.

and Cooney A) independently reviewed the database to ensure accuracy of the survival and recurrence data. Patients were censored in September 2009 to ensure a minimum of 5-year follow-up.

Tumour characteristics

All explants were examined by a histopathologist experienced in HCC pathology (Nolan N). Tumour size, number of lesions, presence of macro or microvascular invasion, and condition of the non-tumour bearing liver were recorded. Tumours were graded as well, moderate or poorly differentiated. Compliance with Milan or UCSF criteria was based on size and number of lesions and was determined by explant pathology rather than pre-operative imaging.

Statistical analysis

Patients were divided into groups based on meeting or exceeding listing criteria, presence or absence of vascular invasion, tumour grade, and pre-operative AFP levels to determine impact on overall overall and recurrence free survival. Data is presented as median (interquartile range). Factors affecting survival were determined by a Cox Proportional Hazard Model and significant factors were incorporated into a multivariate analysis. Kaplan-Meier analysis and the log-rank test were used to illustrate differences between recurrence free and overall survival according to clinical factors. Comparisons between the HCC and control cohort were made using Fisher's Exact test. All calculations were done using SPSS version 12.0 (SPSS, Inc., Chicago, IL). $P < 0.050$ was set as the threshold for statistical significance.

RESULTS

During the 14-year study period 57 patients underwent OLT for HCC confirmed on explant pathology. One patient received OLT in 1995 and 56 patients were transplanted between 1998 and 2009. This represented 11.3% of the 504 patients undergoing OLT in the Liver Unit during that time. HCC was diagnosed radiologically in 52 cases pre-operatively and 5 cases were incidental findings in cirrhotic patients. HCC was absent on explant pathology in 4 additional patients transplanted for presumed HCC, representing false positives who were excluded from the analysis. Pre-operative AFP, tumour histopathology and clinical follow up data were available for all 57 patients. Median follow up was 42.7 (14.6-67.6) mo.

The median age at OLT was 59 years. The most common underlying causes of cirrhosis were alcoholic liver disease (30%), hepatitis C (30%) and Haemochromotosis (23%). The Milan criteria were exceeded in 16 (28%) and 8 patients (14%) exceeded UCSF criteria. Median largest tumour size was 3 (2.5-4.5) cm. Micro-vascular invasion was present in 24 (42%) tumours. The mean time to OLT following diagnosis was 3 mo. Bridging therapy was not routinely used. Only 4 patients underwent trans-arterial chemo-embolization and this was not included in statistical analysis. Patient and tumour characteristics are outlined in Table 1.

Survival

Overall survival at 1, 3 and 5 years was 87.7% (50/57), 72.1% (31/43) and 72.4% (21/29) respectively. The HCC transplant group were compared to a cohort of 313 patients undergoing OLT between 1998 and 2008 who underwent their primary, non-emergent, transplant during that period. There was no statistical difference between the HCC and control cohort in 1 (87.7% *vs* 89.1%, $P = 0.450$), 3 (72.1% *vs* 84.2%, $P = 0.050$) and 5 years (72.4% *vs* 80.9%, $P = 0.211$) overall survival rates. No clinical or pathological variable significantly affected overall survival in those undergoing OLT for HCC (Table 2). Overall survival was not affected by patients exceeding the Milan (Figure 1A) or UCSF (Figure 1B).

Recurrence

Recurrence free survival was 86%, 69.7% and 69.5% at 1, 3 and 5 years respectively. There were 8 recurrences in total (14%) and 5 patients died from recurrence. Recurrence occurred within 1 year in 3 patients, within 2 years in 3 and beyond 3 and 5 years in one patient each. The location of recurrent disease was hepatic in 3 (including 2 patients with additional

Table 2 Univariate analysis of factors affecting overall survival

	HR	CI	*P*-value
Male sex	0.786	0.301-2.055	0.623
Age	1.001	0.954-1.051	0.952
Aetiology of HCC			
Alcoholic liver disease	0.523	0.175-1.567	0.247
Hepatitis C	2.098	0.849-5.183	0.108
Haemochromatosis	0.715	0.239-2.143	0.549
Other	1.198	0.459-3.126	0.712
Pre-operative α-fetoprotein > 100 ng/mL	1.502	0.437-5.165	0.519
Compliant with Milan criteria	0.994	0.381-2.590	0.989
Compliant with UCSF criteria	0.871	0.290-2.618	0.805
Largest lesion	1.207	0.963-1.513	0.102
Cirrhosis	23.309	0.024-224.813	0.369
Steatosis	0.044	0.000-187.285	0.465
Multi-focal lesions	1.201	0.499-2.890	0.683
Micro-vascular invasion	1.489	0.619-3.578	0.374
Tumour differentiation			
Well	0.862	0.349-2.131	0.748
Moderate	1.100	0.448-2.698	0.835
Poor	1.159	0.268-5.022	0.843
Incidental lesions	0.450	0.060-3.391	0.438

HCC: Hepatocellular carcinoma; UCSF: University of California San Francisco.

Table 3 Univariate analysis of factors affecting recurrence free survival

	HR	CI	*P*-value
Male sex	0.681	0.156-2.971	0.609
Age	1.004	0.932-1.081	0.922
Aetiology of HCC			
Alcoholic liver disease	0.775	0.155-3.887	0.757
Hepatitis C	3.272	0.798-13.417	0.100
Haemochromatosis	1.210	0.243-6.024	0.816
Other	0.027	0.000-16.500	0.271
Pre-operative α-fetoprotein > 100 ng/mL	6.668	1.661-26.768	0.007
Compliant with Milan criteria	1.354	0.271-6.761	0.712
Compliant with UCSF criteria	0.739	0.148-3.692	0.712
Largest lesion	1.326	0.976-1.801	0.071
Cirrhosis	23.025	0.000-327.873	0.604
Steatosis	0.045	0.000-546.731	0.664
Multifocal lesions	2.100	0.494-8.930	0.315
Micro-vascular invasion	1.560	0.376-6.463	0.540
Tumour differentiation			
Well	0.249	0.046-1.340	0.105
Moderate	1.553	0.374-6.443	0.544
Poor	5.631	1.074-29.510	0.041
Incidental lesions	0.041	0.000-720.752	0.523

HCC: Hepatocellular carcinoma; UCSF: University of California San Francisco.

extra-hepatic metastases), porta-hepatis lymph nodes in 2, and in one patient multiple recurrence occurred in lung, omentum and sacrum. Hepatic recurrences were diagnosed on CT and extra hepatic disease was confirmed by biopsy. Recurrence free survival was similar between patients meeting or exceeding the Milan (Figure 1C) and the UCSF criteria (Figure 1D). Underlying liver disease, tumour size or vascular invasion did not affect recurrence free survival. On univariate analysis only poorly differentiated tumours and AFP levels > 100 ng/mL were associated with reduced disease free survival (Table 3) and a shorter time to recurrence (Figure 2). On multivariate analysis, pre-operative AFP > 100 ng/mL remained an independent predictor of recurrence free survival (HR = 5.2, $P = 0.036$).

Patients exceeding Milan and UCSF criteria

Eight patients exceeded both Milan and UCSF criteria. Five were alive at 5 years and one patient with recurrence was alive after 3 years follow-up. Recurrence only occurred in 2 cases. One patient died from recurrence after 14 mo and one died from a separate malignancy at 2 years. Micro-vascular invasion was present in 4 cases. AFP exceeded 100 ng/mL in the patient who died from recurrence.

DISCUSSION

The current study confirms that OLT for HCC is an effective treatment modality and that survival rates are comparable to those undergoing OLT for non-malignant disease. Patients exceeding the Milan or UCSF criteria were not at increased risk of reduced overall survival or increased recurrence. Pre-operative serum AFP is an independent risk factor for recurrence.

The landmark Milan publication in 1996 established listing criteria based on a single HCC of less than 5 cm or up to 3 tumours, each less than 3 cm[4]. This was validated in other single-centre studies and together demonstrated a 5-year survival of 70% and recurrence rates of less than 15% which became the gold standard outcome in OLT for HCC[9-11]. These criteria continue to be used in Ireland and many centres worldwide. Our institution is a medium volume centre performing approximately 60-70 OLT per year. The outcomes of our patient cohort compare favourably to recently published series' from higher volume centres[12,13]. Our patient demographic is different to most centres as in over 50% of patients the underlying liver pathology was alcoholic liver disease or haemochromatosis. Worldwide, the main causes of HCC are hepatitis B and C virus but in this study they only accounted for 33% of HCC[14]. However aetiology of HCC did not significantly impact on overall or disease free survival.

The majority of patients with HCC present with disease beyond the Milan criteria[15]. Acceptable 5-year survival and recurrence rates observed in a subgroup of patients with larger tumours led to the publication of the UCSF criteria which proposes listing patients with a single tumour up to 6.5 cm or up to 3 lesions, none larger than 4.5 cm and total tumour burden not exceeding 8 cm[16]. This has been reproduced in single-centre studies with short follow up but never in multi-centre or nationwide population studies but in recent years several units have called for an extension of the criteria. Patients beyond the Milan criteria did not experience inferior survival in our centre but our numbers are too small to support calls for extension of the criteria based simply

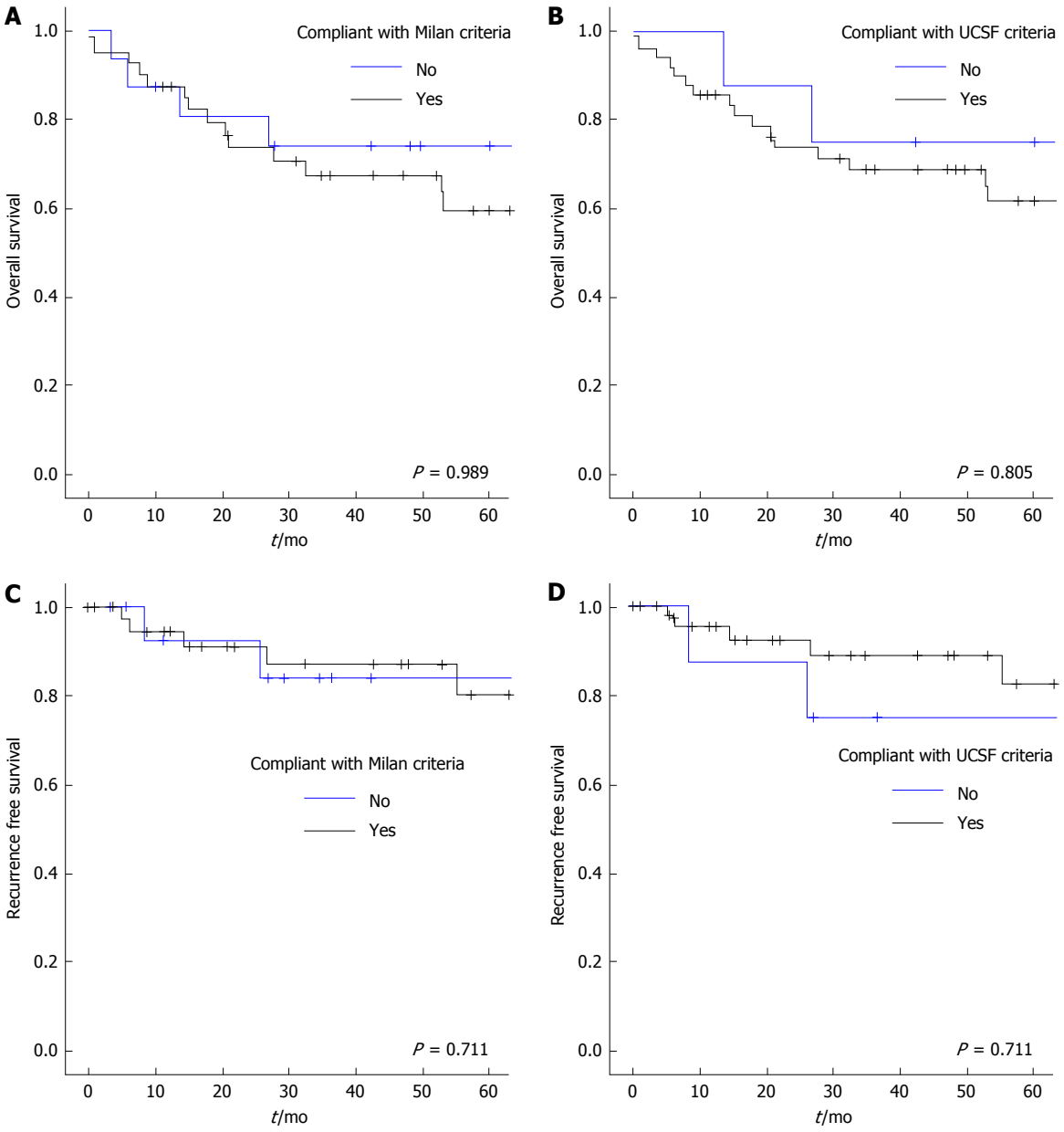

Figure 1 Kaplan-Meier estimates of overall survival (A and B) and recurrence free survival (C and D) in relation to compliance with the Milan and University of California San Francisco criteria. UCSF: University of California San Francisco.

on size and number of tumours. The limitations of pre-operative imaging for staging in the setting of cirrhosis also impede raising the threshold. One large study showed pre-operative imaging to under stage over 40% of patients[17]. In the current study almost 30% were not compliant with Milan criteria on explant pathology. Interval tumour growth is a possible explanation for patients who meet criteria on imaging and then exceed them on pathology. However our cohort experienced a short waiting period of 3 mo and relatively small tumours (median 3 cm) which makes tumour doubling unlikely. Even with advances such as double-contrast MRI, extending the criteria based solely on size risks transplanting patients with tumours too large to benefit.

The limitation of established criteria is that they are based on tumour dimensions. While results from single centre studies have justified its use for organ allocation, in a North American population study, a subgroup of patients with larger tumours within the Milan criteria had significantly poorer survival outcomes than those without HCC[18]. It is imperative that any selection criteria be accurate in predicting prognosis to justify the large proportion of transplants undertaken for HCC in the setting of a shortage of organs. For example 25% of all United States OLT have been for HCC since the introduction of priority MELD scores for HCC in 2002[18] and 11% of OLT in Ireland are for patients with HCC.

There is growing evidence that the biological behaviour of the tumour rather than size dictates recurrence. Patients with larger tumours beyond the Milan criteria but without micro-vascular invasion can have excellent survival, such as outlined in the "up-to-seven-criteria",

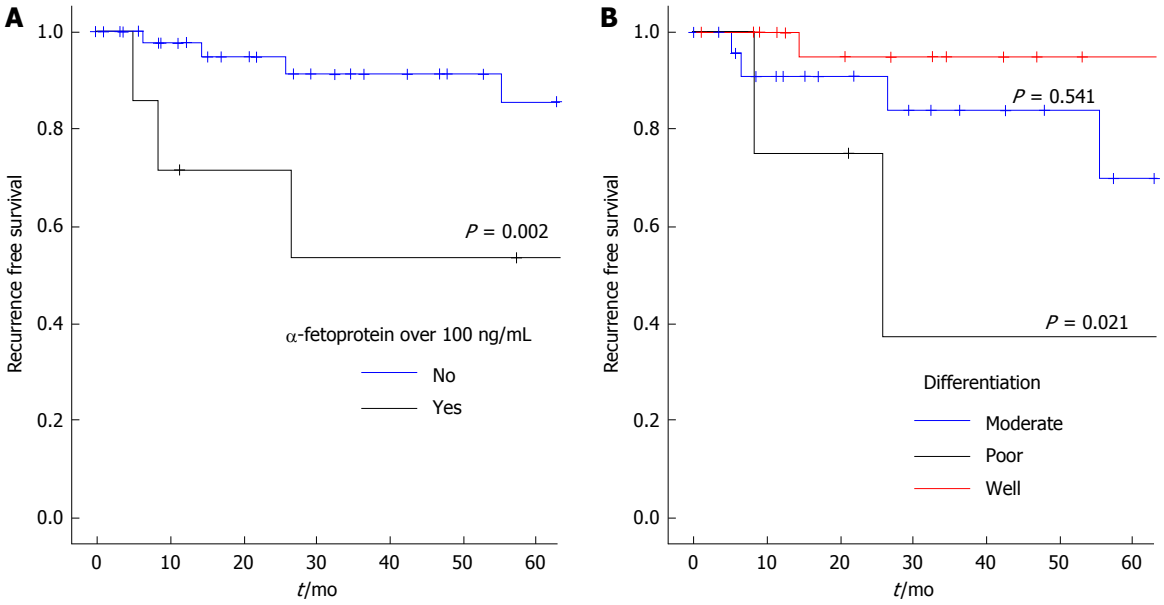

Figure 2 Kaplan-Meier estimates of recurrence free survival in relation to pre-operative α-fetoprotein (A) and tumour differentiation (B).

but this cannot be diagnosed pre-operatively[19]. The impact of micro-vascular invasion was not found to be statistically significant in our cohort but in large studies it has been shown to double the risk of death[7]. Pre-operative AFP may be the best available surrogate marker for micro-vascular invasion and the biological aggressiveness of the tumour. Several studies have identified a high pre-operative AFP as a risk factor for recurrence and reduced survival[18,20-22]. We have shown AFP predicted reduced disease free survival, independent of both tumour size and micro-vascular invasion. Furthermore, patients exceeding Milan or even UCSF criteria experienced excellent overall and recurrence free survival with a pre-operative AFP < 100 ng/mL. This supports the finding of another group where an AFP level < 30 ng/mL predicted disease free survival in patients beyond Milan criteria[23]. Both studies are limited by the small number of patients exceeding Milan criteria. Recent large studies have not examined the impact of AFP in the context of tumours beyond the Milan criteria. The largest study reporting survival in patients with tumours exceeding the Milan criteria (1112 patients) unfortunately did not examine the impact of AFP level[7]. Analysis from the United Network for Organ Sharing on 2253 patients demonstrated a significant survival advantage in patients with low pre-transplant AFP (< 20 ng/mL) but this effect wasn't explored in patients with tumours outside Milan criteria[24]. It would therefore be intriguing if AFP could be examined in a large population database or multicentre study to determine if patients with large tumours but low pre-operative AFP had higher survival rates. Only then can AFP be used to augment existing eligibility criteria to safely expand the pool of patients suitable for OLT.

In conclusion, in appropriately selected patients with HCC undergoing OLT, survival was comparable to non-HCC patients. A subgroup of patients with larger tumours and low AFP may benefit from OLT but this association should be examined in larger, multicentre studies.

ACKNOWLEDGMENTS

Anne Cooney, database manager, the Liver Unit, for assistance in database retrieval.

COMMENTS

Background

Orthotopic liver transplantation (OLT) is the most effective treatment for hepatocellular carcinoma (HCC) in the setting of cirrhosis. Survival in well selected patients with a small burden of tumour is similar to patients undergoing OLT for non-cancer related indications.

Research frontiers

Existing selection criteria are based on the size and number of the tumour. Several datasets have demonstrated good survival outcomes in patients exceeding these criteria. The biological characteristics, for example micro-vascular invasion may just as important as the tumour dimensions. However these cannot be reliably detected pre-operatively.

Innovations and breakthroughs

This study also demonstrates that patients with larger tumours can still have good survival outcomes. Pre-operative alpha-fetoprotein (AFP) predicted tumour recurrence. AFP may be a useful surrogate marker for less favourable biological characteristics of the tumour.

Applications

The prognostic value of AFP could be evaluated in large, multi-centre datasets to determine its potential as an adjunct to existing selection criteria.

Terminology

OLT: Orthotropic liver transplant involves fully explanting the diseased liver immediately prior to the transplant; HCC: Hepatocellular carcinoma is the most common primary liver tumour. Because most cases occur in the setting of cirrhosis, it is often not amenable to resection; AFP: Alpha-fetoprotein has no known function in adults but it has clinical significance as a tumour marker in

the diagnosis of HCC.

Peer-review

This is an interesting attempt to evaluate the results of liver transplantation for HCC with regard to potential relation with pre-operative values of AFP. The paper is well written and results are clarified.

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P- Reviewer: Boucek CD, Balaban YH, Panda CK, Silva R, Smyrniotis V
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Transplantation

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5500/wjt.v6.i2.403

World J Transplant 2016 June 24; 6(2): 403-410
ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Study

Higher plasma bilirubin predicts veno-occlusive disease in early childhood undergoing hematopoietic stem cell transplantation with cyclosporine

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Supported by The Education and Research Encouragement Fund of Seoul National University Hospital.

Institutional review board statement: This study was approved by the Institutional Review Board of Seoul National University Hospital (SNUH; H-1112-087-390, 2012.3.17), a 1961-bed medical center, on March 17, 2012.

Informed consent statement: The authors petition BPG for waiver of informed consent because this study was not a clinical trial, but was retrospectively done using anonymized electronic medical records of the study subjects. In addition, authors declared that the study subjects were at no risk due to this study.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

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Received: January 2, 2016
Peer-review started: January 3, 2016
First decision: February 2, 2016
Revised: February 22, 2016
Accepted: March 17, 2016
Article in press: March 18, 2016
Published online: June 24, 2016

Abstract

AIM: To analyze the association between plasma bilirubin levels and veno-occlusive disease (VOD) in non-adult patients undergoing hematopoietic stem cell transplantation (HSCT) during cyclosporine therapy.

METHODS: A total of 123 patients taking cyclosporine

were evaluated using an electronic medical system at the Seoul National University Children's Hospital from the years 2004 through 2011. Patients were grouped by age and analyzed for incidence and type of adverse drug reactions (ADRs) including VOD.

RESULTS: The HSCT patients were divided into three age groups: G#1 $\geqslant$ 18; 9 $\leqslant$ G#2 $\leqslant$ 17; and G#3 $\leqslant$ 8 years of age). The majority of transplant donor types were cord blood transplantations. Most prevalent ADRs represented acute graft-*vs*-host disease (aGVHD) and VOD. Although the incidences of aGVHD did not vary among the groups, the higher frequency ratios of VOD in G#3 suggested that an age of 8 or younger is a risk factor for developing VOD in HSCT patients. After cyclosporine therapy, the trough plasma concentrations of cyclosporine were lower in G#3 than in G#1, indicative of its increased clearance. Moreover, in G#3 only, a maximal total bilirubin level (BILmax) of $\geqslant$ 1.4 mg/dL correlated with VOD incidence after cyclosporine therapy.

CONCLUSION: HSCT patients 8 years of age or younger are more at risk for developing VOD, diagnosed as hyperbilirubinemia, tender hepatomegaly, and ascites/weight gain after cyclosporine therapy, which may be represented by a criterion of plasma BILmax being $\geqslant$ 1.4 mg/dL, suggestive of more sensitive VOD indication in this age group.

Key words: Hematopoietic stem cell transplantation; Veno-occlusive disease; Cyclosporine; Adverse drug reaction; Total bilirubin

Core tip: This study analyzed the association between plasma bilirubin and veno-occlusive disease (VOD) in childhood undergoing hematopoietic stem cell transplantation (HSCT) during cyclosporine therapy. Here, we report that age of 8 or under may be a risk factor for VOD in CsA-treated patients who underwent HSCT with differential clearance of CsA. Another finding is that a criterion of 1.4 mg/dL of plasma maximal total bilirubin level or higher content alone closely represents the incidence of VOD in early childhood patients with HSCT in CsA therapy. Information shown in this study would be of great help to understand VOD occurring during CsA medication and to find optimal pharmacotherapy in HSCT patients.

Kim KS, Moon A, Kang HJ, Shin HY, Choi YH, Kim HS, Kim SG. Higher plasma bilirubin predicts veno-occlusive disease in early childhood undergoing hematopoietic stem cell transplantation with cyclosporine. *World J Transplant* 2016; 6(2): 403-410 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/403.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.403

INTRODUCTION

Cyclosporine is a major immunosuppressant for organ transplantation, and is widely used for the prophylactic treatment of acute graft-*vs*-host disease (aGVHD) after hematopoietic stem cell transplantation (HSCT)[1]. However, the morbidity and mortality resulting from acute, or subsequently following chronic GVHD, and veno-occlusive disease (VOD), as indicated by hyperbilirubinemia, tender hepatomegaly, and ascites, are obstacles to the use of cyclosporine alone or in combination with other agents[2]. Clinical studies on cyclosporine therapy demonstrated differences between neonate, child and adult populations in the incidence of adverse drug reactions (ADRs)[3]. Since these events are closely linked to the metabolic burden and/or clearance of the drug, ADRs should be monitored and avoided depending on the types of transplantation, age groups and pharmacokinetic profiles. In particular, the dose regimens and therapeutic concentrations need to be appropriately adjusted for optimal efficacy and/or minimized ADRs.

Cyclosporine therapy should be carefully monitored as a therapeutic drug monitoring system[4]. Monitoring of pharmacokinetic profiles, including oral bioavailability, has been claimed in the context of successful pharmacotherapy because intestinal absorption of cyclosporine varies depending on the type of transplantation, age, and other parameters of patients[5-9]. In general, patients of a young age seem to be more at risk for ADRs to cyclosporine, and exhibit different ADR profiles[10]. Therefore, the oral dose of cyclosporine required for the maintenance of therapeutic blood levels is significantly augmented in childhood patients[5]. In addition to the narrow therapeutic range of cyclosporine, the types and incidences of cyclosporine-induced ADRs vary depending on the types and severities of diseases, as well as patient age[11].

It has been recognized that wide variations exist in the plasma concentrations of cyclosporine among HSCT patients[12]. A limited number of studies have been performed in cyclosporine-treated neonates and children who underwent HSCT in the context of ADR monitoring[13]. In Seoul National University Hospital, the administered dose of cyclosporine was equally determined by the post-surgical day of HSCT, which frequently resulted in cyclosporine plasma concentrations being out of therapeutic range (150-250 ng/mL). Although the normalized doses of cyclosporine for transplant patients of childhood age were usually higher than those for adults, the plasma concentrations were significantly lower[3]. This raised the contention that biotransformation and/or excretion of cyclosporine is accelerated in childhood patients, which may be linked to ADRs, such as GVHD, nephrotoxicity, and neurotoxicity[13].

Age-different effects of cyclosporine therapy on the types and incidences of ADRs in HSCT patients are

Table 1 The characteristics of hematopoietic stem cell transplantation patients treated with cyclosporine (*n* = 123)

Characteristics	G#1 (*n* = 25)	G#2 (*n* = 70)	G#3 (*n* = 28)
Age (mean, SD)	20.3, 1.7	13.0, 2.5	5.8, 2.2
Initial body weight (mean, SD)	51.8, 11.1	37.8, 12.7	14.0, 4.0
Gender (M, %)	15 (60.0)	35 (50.0)	17 (60.7)
Liver function (mean, SD)			
ALT (mg/dL)	67.6, 95.8	67.1, 67.3	104, 133
AST (mg/dL)	70.2, 73.1	72.9, 61.5	161, 356
Doner types, *n* (%)			
Cord blood	15 (60.0)	41 (58.6)	21 (75.0)
Related donor	10 (40.0)	29 (41.4)	7 (25.0)
Types of disease, *n* (%)			
AA	5 (20.0)	6 (8.6)	1 (3.6)
ABL	4 (16.0)	5 (7.1)	2 (7.1)
ALL	10 (40.0)	28 (40.0)	7 (25.0)
AML	5 (20.0)	20 (28.6)	10 (35.7)
CML	0 (0.0)	2 (2.9)	0 (0.0)
JMML	0 (0.0)	0 (0.0)	4 (14.3)
MDS	1 (4.0)	2 (2.9)	1 (3.6)
Others	0 (0.0)	7 (10.0)	3 (10.7)
Observed events, *n* (%)			
aGVHD	13 (61.9)	26 (46.4)	13 (54.2)
cGVHD	1 (4.8)	4 (7.1)	0 (0.0)
VOD	2 (9.5)	13 (23.2)	7 (29.2)
DIC	4 (19.0)	4 (7.2)	2 (8.3)
Relapse	1 (4.8)	2 (3.6)	1 (4.2)
EF	0 (0.0)	7 (12.5)	1 (4.2)

Age groups: G#1, 18 years older; G#2, 9 to 17 years old; G#3, 8 years old or under. AA: Aplastic anemia; ABL: Acute biphenotypic leukemia; ALL: Acute lymphocytic leukemia; AML: Acute myelocytic leukemia; CML: Chronic myelocytic leukemia; JMML: Juvenile myelomonocytic leukemia; MDS: Myelo dysplastic syndromes; aGVHD: Acute graft versus host disease; cGVHD: Chronic graft versus host disease; VOD: Veno-occlusive disease; DIC: Disseminated intravascular coagulation; EF: Engraft failure; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

known[14-16]. Nevertheless, more sensitive indicator(s) would be of great help to avoid or minimize serious ADRs and to accomplish successful pharmacotherapy, especially in patients of the childhood population who would be more prone to drug-induced harmful effects. This study analyzed the association between plasma bilirubin levels and VOD in non-adult patients undergoing HSCT during cyclosporine therapy. Here, we report that marginally high levels of total plasma bilirubin reliably indicate VOD during cyclosporine therapy in the HSCT patient of early childhood.

MATERIALS AND METHODS

Datasets

This study was approved by the Institutional Review Board of Seoul National University Hospital (SNUH; H-1112-087-390, 2012.3.17), a 1961-bed medical center, on March 17, 2012. The data collected had anonymous codes representing patient files comprising the following medical information: Age, gender, medical diagnosis codes, date of HSCT, absolute neutrophil count, post-transplantation day, donor types (cord blood and related donor), body weight, body surface area, body temperature, types of ADRs, peak and trough concentrations of cyclosporine, serum hematocrit, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum total bilirubin levels, dates of labs drawn, medications (generic and brand name, prescription date), and duration of chemotherapeutic agent along with co-prescribed drugs.

Patients

The database contained records of 123 patients (ages ranging from 2 to 24 with 68 males and 55 females) who had been hospitalized in SNUH from September 15, 2004 to December 31, 2012 and had undergone measurements of plasma cyclosporine levels using a radioimmunoassay kit. Cyclosporine concentrations were monitored on day 1, 4, 11 18, 24 and 28 after HSCT and at intervals of three or seven days after day 28 of HSCT in SNUH. Laboratory data were obtained from 123 patients, with three of them having HSCTs twice in this period. The total number of cyclosporine measurements was 2149, with an average of 17.5 measurements per patient.

HSCT patients who had been administered cyclosporine were divided into three groups based on age: G#1, 18 years of age or older; G#2, between 9 and 17 years of age; and G#3, 8 years of age or under. The median ages in G#1, G#2, and G#3 were 20, 13 and 6, respectively. Each group was additionally split into four subgroups by the levels of a maximal total bilirubin level (BILmax) [*i.e.*, BILmax (-), lower than 1.4 mg/dL of total plasma bilirubin; and BILmax (+), 1.4 mg/dL of total plasma bilirubin or higher] and VOD incidence [*i.e.*, VOD (-), no existing VOD; and VOD (+), existing VOD].

Statistical analysis

The Fisher exact test was chosen to determine differences in the frequency of BILmax $\geqslant$ 1.4 mg/dL between VOD (-) and VOD (+) groups. Multivariate analysis was performed to find risk factors for drug therapy. Data represent the median (0.5-24.0 mg/dL). Results were considered statistically significant if the *P*-value was less than 0.05. Statistical analyses were conducted using the Duncan and Fisher's tests in SPSS Version 12.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

The characteristics of HSCT patients treated with cyclosporine (*n* = 123) are summarized in Table 1. Of the patients examined, cord blood transplantation constituted the majority of the transplant donor type (G#1, 60.0%; G#2, 58.6%; and G#3, 75.0%). The most prevalent ADR event observed was aGVHD (G#1, 61.9%; G#2, 46.4%; and G#3, 54.2%), whereas the second most frequent ADR event was VOD (9.5%-29.2%). Although the incidences of aGVHD, diagnosed as cytopenia and delayed immune reconstitution, did not vary much between the groups, the frequency ratios of VOD were significantly higher in G#3. Thus, being 8 years of age or under at the time of transplantation

Table 2 Median trough plasma concentrations and doses of cyclosporine in hematopoietic stem cell transplantation patients

Contents		G#1 (n = 25)	G#2 (n = 70)	G#3 (n = 28)
iv	Trough plasma concentration[b] (ng/mL)	535.6 (264.0-1214.0)[a]	448.9 (184.5-1070.0)	333.1 (152.5-819.0)
	Dose (mg/kg)	5.8 (3.9-7.7)	6.1 (3.5-14.2)	6.0 (3.8-9.3)
PO	Trough plasma concentration[b] (ng/mL)	345.9 (166.0-686.5)	247.7 (40.0-496.5)	204.4 (33.0-302.5)
	Dose (mg/kg)[c]	8.2 (3.4-11.4)	8.2 (1.6-17.5)	10.6 (6.0-24.6)

[a]The values in parenthesis represent the minimum and maximum trough plasma concentrations of cyclosporine; [b]G#1 was significantly different from G#2 or G#3 using Duncan test; [c]G#3 was significantly different from G#1 or G#2 using Duncan test. *iv*: Intravenous administration; PO: Per Os, which means oral administration.

Table 3 Two by two analyses between maximal plasma bilirubin contents and veno-occlusive disease in hematopoietic stem cell transplantation patients

	G#1 (n = 25)		G#2 (n = 70)		G#3 (n = 28)	
	BILmax (-)	BILmax (+)	BILmax (-)	BILmax (+)	BILmax (-)	BILmax (+)
VOD (+)	0	2	4	9	0	7
VOD (-)	5	18	28	29	17	4
P-value	1.00		0.356		0.0001	

BILmax (+): 1.4 mg/dL or higher; BILmax (-): Lower than 1.4 mg/dL. Data were analyzed using Fisher's test program. BILmax: A maximal total bilirubin level; VOD: Veno-occlusive disease.

would be a possible risk factor for VOD in patients who underwent HSCT from cord blood donors. In types of diseases, acute lymphoblastic leukemia and acute myeloid leukemia highly occurs in all three groups of patients, but there was no significant difference of the disease incidence rate depending on the age. Also the liver functions (*i.e.*, ALT and AST activities) were comparable in all groups of patients.

After intravenous administration, the trough plasma concentrations of cyclosporine were significantly lower (83.8% and 62.2% in G#2 and G#3, respectively, *vs* G#1) in G#2 or G#3 than in G#1, although the injected dose of cyclosporine was normalized to the patient body weight (Table 2). The trough plasma concentrations of cyclosporine were approximately 40% lower in G#3 than in G#1, indicative of its accelerated clearance in G#3. The trough plasma cyclosporine levels were similarly changed in the groups examined after oral administration; in this case, the oral dose was approximately 30% greater in G#3 than in G#1 (or G#2), suggesting the possibility that the bioavailability of cyclosporine was significantly lower in G#3 (Table 2). These results indicate that the clearance and/or turnover rate of cyclosporine in plasma might be augmented in G#3, whereas the oral bioavailability was lower in this group, implying the potential of increased detoxifying burden in the patients presumably due to accelerated biotransformation and excretion of cyclosporine.

Given the distinct difference in plasma cyclosporine concentrations and the potential of increased cyclosporine clearance in G#3, we next asked whether the incidences of VOD statistically correlated with total bilirubin levels in plasma among the patients examined. Setting the BILmax cutoff level at 2.0 mg/dL demonstrated an obvious increment in VOD incidences in high BILmax groups when G#2, G#3 or the total population was analyzed, although it failed in demonstrating increased VOD incidences when G#1 was solely analyzed (data not shown). More importantly, setting the BILmax cutoff level at 1.4 mg/dL (a minimal significant value obtained empirically) revealed an augmented incidence of VOD in the high BILmax group in G#3 ($P < 0.0001$), but not in G#1 or G#2, as determined by two-by-two analyses (Table 3).

DISCUSSION

ADR-related admissions are a problem with a high prevalence[17,18]. Pérez Menéndez-Conde *et al*[18] reported that 19.4% of admissions were direct consequences of ADRs, 65% of which were preventable[19]. In particular, cyclosporine therapy causes various ADRs (*e.g.*, 20% of infectious complications during the therapy and 5% of severe GVHD)[20,21], with approximately 6% of admissions eliciting permanent damage, including seizures or death[22]. In general, the dose of cyclosporine is calculated for transplant patients primarily on the basis of body weight[23]. However, this approach has limitations, such as the development of aGVHD, cGVHD, hepatotoxicity, gastrointestinal disorders, infections and hemorrhagic cystitis[24]. Large variations in plasma cyclosporine concentrations exist in individuals (*i.e.*, 5-8 fold differences)[25]. Since the biotransformation capacities of endogenous and exogenous substances vary depending on the stage of development and maturation, attention should be directed to cyclosporine clearance. The results of this study demonstrated the impact of age differences on the incidence and type of ADRs during cyclosporine therapy in HSCT patients of early childhood as compared to adolescent patients.

A major advantage of HSCT is the potential for therapeutic benefits from graft-*vs*-leukemia effects, which are mediated by donor T and natural killer cells[26]. Unfortunately, graft-*vs*-leukemia effects are closely linked to aGVHD as the major limiting toxicity of allogeneic transplantation, which causes damage to the skin, gastrointestinal tract, and liver[27]. Studies have shown that aGVHD frequently occurred when plasma concentrations of cyclosporine decreased to 125-200 ng/mL 12 h after treatment[25,28]. Depletion of T cells from the graft effectively prevented aGVHD, but it also limited graft-*vs*-leukemia effects, possibly increasing the rate of graft failure[29]. Therefore, plasma concentrations of immunosuppressant are currently one of the critical factors to maintain the proper balance between aGVHD and graft-*vs*-leukemia effects. The lowest plasma cyclosporine concentration (< 200 ng/mL) in the third week after transplantation showed a high risk factor related to aGVHD (grades Ⅱ-Ⅳ) in HSCT patients[30]. Thus, assessment of cyclosporine levels is a valuable diagnostic tool to predict aGVHD. In the present study, we observed that the incidences of aGVHD (*i.e.*, cytopenia and delayed immune reconstitution) were not much different among the groups examined, which supports the appropriateness of the pharmacotherapy.

Patients currently meet McDonald's VOD-Seattle Criteria by exhibiting two or more of the following criteria: Hyperbilirubinemia > 2 mg/dL, tender hepatomegaly, and either ascites or weight gain (> 2%). A key finding of this study is that VOD occurrences were significantly higher in G#3. Similarly, the incidences of VOD increased in childhood age[31], whereas VOD was frequently observed day 18 (the median) after intravenous administration of cyclosporine[32]. When we compared the plasma levels of cyclosporine and other pharmacokinetic parameters, the turnover rate of cyclosporine seemed to vary in different age groups. Our finding showing lower plasma cyclosporine level with higher occurrence of VOD in G#3 differs from the previous report that high plasma concentrations or high doses of drugs in pediatric HSCT patients related to the frequent and severe VOD in different therapy in HSCT patients[33]. VOD occurrence seems to be associated with clearance of endogenous compounds as well as cyclosporine[34]. It has also been suggested that the clearance rate of cyclosporine may affect VOD and total bilirubin levels in blood[34]. This idea is consistent with the finding that the pharmacokinetic profile of cyclosporine was characterized by substantially faster elimination in children compared to adults, which necessitated more frequent dosing intervals and higher doses for younger children[7,35]. So, low plasma cyclosporine levels in G#3 may reflect its high turnover rate. Overall, our results and others support the contention that the turnover rate of cyclosporine is increased, particularly in HSCT patients of early childhood.

Our finding that HSCT patients of 8 years of age or under were more at risk for the reactions of VOD, which was distinctively characterized by the plasma BILmax level being ⩾ 1.4 mg/dL, indicates that plasma BILmax alone may serve as a valuable marker of VOD in this particular patient population. Since a large fraction of cyclosporine binds with erythrocytes (41%-50%)[36], cyclosporine-induced hyperbilirubinemia may result from destabilization and/or disruption of red blood cell membranes, with the consequent release of heme for biodegradation and excretion. It has also been shown that the clearance of red blood cells was slower, whereas the maturity and differentiation of red blood cells were lower in children compared to other groups[37]. Disruption of canaliculi in children has also been shown to increase, even at lower cyclosporine concentrations[38]. Therefore, the frequency of splenomegaly increases presumably due to the clearance of damaged red blood cells and debris, along with heme disposal, resulting in the subsequent production of bilirubin[39]. Consistently, red blood cells may be impaired after cyclosporine therapy, especially during radiation therapy[40].

Since cyclosporine is mainly oxidized *via* cytochrome P450s 3A4 (CYP3A4), followed by glucuronide conjugation *via* UDP-glucuronosyltransferase 1A1 (UGT1A1) and UGT2B7, total bilirubin levels in the blood would increase, enhancing the burden of detoxification[41]. Cyclosporine is primarily metabolized by CYP3A4 in the liver, 95% of which is excreted *via* the biliary route. The main reason for the low bioavailability of cyclosporine may be due to its extensive intestinal metabolism by CYPs[42]. The various rate and extent of cyclosporine metabolism, depending on age and drug interactions (60%-90%), may be related with polymorphisms of CYP3A4[43]. The genetic associations between UGT variations and cyclosporine pharmacokinetics in patients would also affect its efficacy and ADRs (*e.g.*, GVHD, hepatic and/or gastrointestinal disorders) presumably due to unpredictable cyclosporine concentrations[44]. Our results showed that plasma cyclosporine levels were significantly lower in G#3 despite the highest normalized dose. Clearance of endogenous bilirubin might also be reduced in the patients presumably due to relatively low rate of metabolism. Thus, cyclosporine biotransformation may change depending on the metabolic clearance of bilirubin, which would increase in early childhood compared to adolescents and/or adults[45]. Alterations in red blood cell turnover and/or interference of biliary excretion of glucuronidated cyclosporine would also contribute to total plasma bilirubin levels[46].

The value of pharmacist-provided drug-monitoring care to transplant recipients has been recognized as a beneficial service[47]. Considering the complexity of pharmacotherapy, pharmacists need to implement clinically relevant interventions on the transplant unit[48]. Although the dangers of ADRs are well recognized by clinicians and pharmacists, the efforts to elucidate the basis of ADRs still exist in clinical fields[49]. This situation stimulated attempts to validate ways of ADR monitoring by developing new and critical indicators, algorithms and analytical tools[50,51]. HSCT patients represent a population at high risk for drug-related problems[52]. Our

results demonstrate that HSCT patients 8 years of age or under are at higher risk for developing the reactions of VOD after cyclosporine therapy, which may be indicated by plasma BILmax levels being ⩾ 1.4 mg/dL, suggesting that this new criterion alone may be used as an indicator of VOD during cyclosporine therapy in HSCT patients of young childhood. A guideline for ADR-related problems and interventions may aid staffs working in the HSCT unit to optimize pharmaceutical care of patients, thereby reducing economic costs resulting from inappropriate drug utilization.

COMMENTS

Background

The incidence of veno-occlusive disease (VOD) differs from the ages of childhood, which is an obstacle of the use of cyclosporine, immunosuppressant for organ transplantation. Especially, the VOD incidence was higher in cyclosporine-treated neonates and children who underwent hematopoietic stem cell transplantation (HSCT). Therefore, the authors analyzed the association between plasma bilirubin levels and VOD in childhood patients undergoing HSCT during cyclosporine therapy.

Research frontiers

The sensitive indicator(s) would be of great help to avoid or minimize serious VOD and to accomplish successful cyclosporine therapy, especially in patients of the childhood population with higher VOD incidence. The results of this study contribute to clarifying the associations of bilirubin, VOD and cyclosporine concentrations.

Innovations and breakthroughs

Although age-different effects of cyclosporine therapy on various adverse drug reactions in HSCT patients are existing, the association between plasma bilirubin levels and VOD in non-adult patients undergoing HSCT during cyclosporine therapy was not reported yet. Thus, the authors report that marginally high levels of total plasma bilirubin reliably indicate VOD during cyclosporine therapy in the HSCT patient of early childhood.

Applications

A plasma BILmax levels being ⩾ 1.4 mg/dL may be used as an indicator of VOD during cyclosporine therapy in HSCT patients of young childhood. A guideline for adverse drug reaction-related problems and interventions may aid staffs working in the HSCT unit to optimize pharmaceutical care of patients, thereby reducing economic costs resulting from inappropriate drug utilization.

Terminology

A maximal total bilirubin level (BILmax) (-): Lower than 1.4 mg/dL of total bilirubin during cyclosporine therapy; BILmax (+): 1.4 mg/dL of total plasma bilirubin or higher during cyclosporine therapy.

Peer-review

This review is well written, presenting a very significant issue of "an increased risk for developing VOD after cyclosporine treatments in younger (< 8 years old) generations". Authors also claimed that the plasma BILmax levels being ⩾ 1.4 mg/dL would provide a useful indicator to recognize the development of VOD in those generations.

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P- Reviewer: Saeki K, Tanabe S, Zou ZM **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Transplantation

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5500/wjt.v6.i2.411

World J Transplant 2016 June 24; 6(2): 411-422
ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Study

Proposal of new expanded selection criteria using total tumor size and ^{18}F-fluorodeoxyglucose - positron emission tomography/computed tomography for living donor liver transplantation in patients with hepatocellular carcinoma: The National Cancer Center Korea criteria

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Author contributions: Lee SD designed study and wrote the paper; Lee B and Joo J performed the statistical analysis; Kim SH designed the study and performed research; Kim SK, Kim YK and Park SJ performed the study.

Institutional review board statement: This study was reviewed and approved by the National Cancer Center Institutional Review Board.

Informed consent statement: This is the retrospective study and we analyzed data using only medical records. Therefore, waiver of informed consent for this study subjects might be justifiable. In our institute IRB, waiver of informed consent in this study was approved.

Conflict-of-interest statement: The authors declare no potential conflicts of interest and funding resources.

Data sharing statement: Technical appendix, statistical code, and dataset available from the first author at 1sd@ncc.re.kr. Participent's consent was not obtained but the presented data are anonymized and risk of identification is low.

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Received: December 22, 2015
Peer-review started: December 23, 2015
First decision: January 15, 2016
Revised: January 18, 2016
Accepted: March 7, 2016
Article in press: March 9, 2016
Published online: June 24, 2016

Abstract

AIM: To expand the living donor liver transplantation (LT) pool of eligible patients with hepatocellular carcinoma (HCC) using new morphological and biological criteria.

METHODS: Patients with HCC who underwent living donor LT (LDLT) from March 2005 to May 2013 at the National Cancer Center Korea (NCCK) were enrolled. We performed the ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT)

before LDLT. Overall and disease-free survival analysis was done in patients to evaluate the usefulness of new NCCK criteria using PET/CT and total tumor size (10 cm).

RESULTS: We enrolled a total of 280 patients who pathologically confirmed to have HCC and performed the PET/CT before transplantation. Among them, 164 (58.6%) patients fulfilled the NCCK criteria and 132 patients (47.1%) met the Milan criteria. Five-year overall and disease-free survival rates for patients who fulfilled the NCCK criteria showed 85.2% and 84.0%, respectively, and were significantly higher than those beyond the NCCK criteria (60.2% and 44.4%, respectively; $P < 0.001$). The correlation analysis between preoperative imaging tests and pathologic reports using Cohen's Kappa demonstrated the better results in the NCCK criteria than those in the Milan criteria (0.850 *vs* 0.583). The comparison of disease-free analysis among the NCCK, Milan, and University of California, San Francisco (UCSF) criteria using the receiver operating characteristics curves revealed the similar area under the curve value criteria (NCCK *vs* Milan, $P = 0.484$; NCCK *vs* UCSF, $P = 0.189$ at 5-years).

CONCLUSION: The NCCK criteria using hybrid concept of both morphological and biological parameters showed an excellent agreement between preoperative imaging and pathological results, and favorable survival outcomes. These new criteria might select the optimal patients with HCC waiting LDLT and expand the selection pool.

Key words: Hepatocellular carcinoma; Living donor; Liver transplantation; Selection criteria

Core tip: National Cancer Center Korea criteria using positron-emission tomography/computed tomography positivity and total tumor size (cutoff 10 cm) expanded the pool of living donor liver transplantation for patients with hepatocellular carcinoma. Patient identification on the bases of the criteria showed an excellent agreement between preoperative imaging and pathological results and favorable survival outcomes.

Lee SD, Lee B, Kim SH, Joo J, Kim SK, Kim YK, Park SJ. Proposal of new expanded selection criteria using total tumor size and ^{18}F-fluorodeoxyglucose - positron emission tomography/computed tomography for living donor liver transplantation in patients with hepatocellular carcinoma: The National Cancer Center Korea criteria. *World J Transplant* 2016; 6(2): 411-422 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/411.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.411

INTRODUCTION

The application of selection criteria for liver transplantation (LT) in patients with hepatocellular carcinoma (HCC) has changed the HCC treatment algorithm over the past 20 years. The Milan criteria proposed by Mazzaferro *et al*[1]. helped to increase the number of LTs in patients with HCC and demonstrated remarkably good survival outcomes for these patients. In particular, the Milan criteria, which use both tumor size and number are very useful and have been adopted as selection criteria. Based on these criteria, the patients for whom HCC was identified early had the best chance of being cured of cancer following LT. In Asian countries such as South Korea and Japan, the number of deceased donors is limited and living donor LT (LDLT) has become an important option for treatment in patients with HCC[2,3]. As the amount of experience and evidence on LDLT for HCC has increased in recent years, the selection criteria for LT have gradually been expanded in large-volume centers. Various expanded criteria based on tumor number and size, such as the University of California, San Francisco (UCSF) criteria, have been proposed[4-9]. Some Japanese centers have demonstrated that preoperative tumor markers such as the des-gamma-carboxy prothrombin (DCP) level and tumor size were associated with higher recurrence rates[10,11]. These expanded criteria revealed that selected patients who did not fulfill the Milan criteria showed good overall survival (OS) and disease-free survival (DFS) rates compared with those who fulfilled the Milan criteria. Although the Milan criteria always guarantee the best survival rates in patients with HCC, they are too restrictive and use modalities.

In HCC patients, tumor characteristics, including differentiation grade and microvascular invasion, are well-known independent prognostic factors for OS and DFS following LT[12]. However, these factors cannot be evaluated by preoperative imaging studies, which reveal the morphological characteristics such as number and size. Recently, several studies using ^{18}F-fluorodeoxyglucose positron emission tomography/CT (^{18}F-FDG PET/CT) demonstrated that ^{18}F-FDG PET/CT findings were a powerful prognostic marker in patients with HCC after LT and showed good correlation with pathological tumor characteristics, such as microvascular invasion and differentiation[13-15].

In the present study, we performed a retrospective analysis to identify prognostic factors in patients with HCC who underwent ^{18}F-FDG PET/CT before LDLT. Based on this result, we developed new and simple expanded criteria [the National Cancer Center, Korea (NCCK) criteria], incorporating a hybrid concept of biological and morphological characteristics on PET/CT images, including total tumor size, and compared these criteria with the Milan criteria, which are based on only morphological evaluation.

MATERIALS AND METHODS

Patients

Patients who underwent LDLT due to HCC at NCCK

Table 1 Multivariable analysis of prognostic factors for overall and disease-free survival

Multivariable analysis		Overall survival			Disease-free survival		
		HR	95%CI	*P*	HR	95% CI	*P*
Variables							
AFP	> 400 ng/mL	1.145	0.543-2.418	0.722	1.003	0.556-1.811	0.991
PET/CT	Positive	2.652	1.384-5.085	0.003	2.517	1.481-4.279	0.001
Tumor number	> 3	0.647	0.294-1.425	0.280	0.814	0.425-1.557	0.534
Maximum tumor size	> 5 cm	0.696	0.307-1.580	0.386	1.551	0.836-2.877	0.164
Total tumor size	> 10 cm	2.909	1.230-6.880	0.015	3.003	1.536-5.870	0.001
Differentiation[1]	Ⅲ-Ⅳ	1.206	0.616-2.358	0.585	1.010	0.594-1.717	0.972
Microvascular invasion	Present	1.269	0.522-3.084	0.599	2.148	1.064-4.336	0.033
Capsule formation	Present	0.439	0.166-1.162	0.097	0.737	0.353- 1.542	0.418
Major vessel invasion	Present	2.017	0.829-4.905	0.122	1.712	0.850-3.449	0.132
Ductal invasion	Present	0.907	0.265-3.100	0.876	1.409	0.534-3.720	0.489
Serosal invasion	Present	1.463	0.670-3.195	0.339	1.047	0.553-1.984	0.887
Intrahepatic metastasis	Present	1.471	0.595-3.640	0.404	1.519	0.752-3.070	0.244
Dysplastic nodule	Present	0.744	0.365-1.514	0.414	0.840	0.478-1.479	0.546

[1]Edmondson-Steiner Grade. CT: Computed tomography; PET: Positronemission tomography; AFP: α-fetoprotein.

between March 2005 and May 2013 were collected using prospectively collected database. All patients were diagnosed as HCC by pathologic reports, and underwent ^{18}F-FDG PET/CT to check biologic status of the primary tumor and the presence of metastasis within 1 mo before LDLT. Routine preoperative imaging tools for clinical staging in patients with HCC before LDLT were ultrasonography, multi-detector CT (MDCT), and/or dual contrast-enhanced magnetic resonance imaging (MRI) including PET/CT without protocol tumor biopsy. We reviewed the medical records for clinicopathological data, including age, sex, serum α-fetoprotein (AFP), viral markers, C-reactive protein, Model for End-Stage Liver Disease (MELD) score, PET/CT reports, tumor maximum standardized uptake value (SUVmax), pre-transplant therapies, and pathologic data such as Edmondson and Steiner grade; vessel, serosa, and duct invasion; capsule formation; cirrhosis; intrahepatic metastasis; and dysplastic nodules. Prognostic factors using clinicopathological data were analyzed for their effect on OS and DFS. This study was approved by the institutional review board of NCCK.

Our policy for selecting recipients with HCC for LDLT was basically based on the Milan criteria by preoperative imaging tools such as MDCT, MRI, or PET/CT. However, considering the specificity of living related donation, we performed LDLT on patients without major vascular invasion and extrahepatic metastasis on preoperative imaging tools even though they do not satisfy the Milan criteria. We do not recommend the downstaging or bridging therapy before LDLT even though the patient had advanced HCC. The operative techniques, immunosuppression, and management for hepatitis virus of donor and recipient have been described in detail in previous our reports[16,17]. Patients were followed up periodically with interval 3 or 6 mo using imaging studies such as ultrasonography, abdomen, and chest MDCT with AFP and DCP level. As the tumor recurrence was suspected by imaging tools and serologic tests, additional PET/CT was performed to evaluate the recurrent tumor and distant metastasis. For one or two nodules in the liver, lung, bone, or brain, we performed the resections. However, in case of multiple metastases, we treated tumors with a multimodality approach such as radiofrequency ablation, transarterial chemoembolization (TACE), radiation therapy, or chemotherapy.

^{18}F-FDG PET/CT

Our protocol of ^{18}F-FDG PET/CT was described in detail previously[14]. In brief, ^{18}F-FDG PET/CT was performed using a PET/CT scanner (Biograph LSO; Siemens Medical Systems and Discovery LS; GE Healthcare, New Jersey, United States). The mean period between PET/CT and LDLT was 14.8 d. All PET/CT images were analyzed by experienced nuclear medicine physicians. SUV was calculated as (decay-corrected activity kBq/mL of tissue volume)/(injected FDG activity kBq/body mass gram). SUVs of the lesions were checked by placing a region of interest (ROI) at the site of the maximum FDG uptake in the PET images. The ROI was drawn to encircle the highest activity of each tumor, by the results of the CT scans that were acquired from PET/CT or MRI scans. PET/CT positivity was defined by experienced nuclear medicine physicians by checking whether the SUVmax of the tumor by CT or MRI scans was higher than that in the surrounding noncancerous hepatic tissue. Mean SUVmax of tumors for PET/CT positivity and negativity in this study was 4.46 and 3.08, respectively ($P < 0.001$).

NCCK criteria

In a multivariable analysis of our data, we identified two significant prognostic factors by evaluating pathological examination results (Table 1). These were positive findings on PET/CT (HR = 2.652, 95%CI: 1.384-50.085, $P = 0.003$ for OS; HR = 2.517, 95%CI: 1.481-4.279, $P = 0.001$ for DFS) and total tumor size of > 10 cm (HR = 2.909, 95%CI: 1.230-6.880, $P = 0.015$ for OS; HR = 3.003, 95%CI: 1.536-5.870, $P = 0.001$ for DFS). Although microvascular invasion was a significant factor only for DFS (HR = 2.148, 95%CI: 1.064-4.336,

Table 2 Clinicopathologic characteristics of patients according to National Cancer Center Korea criteria

Variables		Within NCCK (*n* = 164)	Beyond NCCK (*n* = 116)	*P* value
Sex, *n* (%)	Male	138 (84.1)	97 (83.6)	1
	Female	26 (15.9)	19 (16.4)	
Age (yr), mean (SD)		54.2 (7)	54.7 (7.7)	0.561
MELD score, mean (SD)		14.4 (7.9)	12.5 (6.1)	0.029
C-reactive protein (mg/dL), mean (SD)		0.58 (1.11)	1.37 (2.67)	0.004
Tumor maximum SUV, mean (SD)		3.08 (0.64)	4.13 (1.79)	< 0.001
Tumor total size, *n* (%)	≤ 10 cm	164 (100)	56 (48.3)	< 0.001
	> 10 cm	0 (0)	60 (51.7)	
AFP, *n* (%)	≤ 400 ng/mL	151 (92.1)	88 (75.9)	< 0.001
	> 400 ng/mL	13 (7.9)	28 (24.1)	
PET/CT, *n* (%)	Negative	164 (100)	26 (22.4)	< 0.001
	Positive	0 (0)	90 (77.6)	
Pretransplant therapy, *n* (%)	No therapy	39 (23.8)	29 (25)	0.77
	Surgery only	8 (4.9)	4 (3.4)	
	TACE only	71 (43.3)	52 (44.8)	
	RFA only	7 (4.3)	2 (1.7)	
	Combination	39 (23.8)	29 (25)	
Viral hepatitis, *n* (%)	HBV	142 (86.6)	103 (88.8)	0.442
	HCV	9 (5.5)	8 (6.9)	
	NBNC	11 (6.7)	3 (2.6)	
	HBV + HCV	2 (1.2)	2 (1.7)	
Differentiation[1], *n* (%)	Ⅰ-Ⅱ	102 (62.2)	55 (47.4)	0.02
	Ⅲ-Ⅳ	62 (37.8)	61 (52.6)	
Microvascular invasion, *n* (%)	Absent	127 (77.4)	47 (40.5)	< 0.001
	Present	37 (22.6)	69 (59.5)	
Capsule formation, *n* (%)	No complete	134 (81.7)	94 (81)	1
	Complete	30 (18.3)	22 (19)	
Ductal invasion, *n* (%)	Absent	161 (98.2)	109 (94)	0.123
	Present	3 (1.8)	7 (6)	
Serosal invasion, *n* (%)	Absent	146 (89)	72 (62.1)	< 0.001
	Present	18 (11)	44 (37.9)	
Intrahepatic metastasis, *n* (%)	Absent	129 (78.7)	55 (47.4)	< 0.001
	Present	35 (21.3)	61 (52.6)	
Cirrhosis, *n* (%)	Absent	10 (6.1)	11 (9.5)	0.407
	Present	154 (93.9)	105 (90.5)	
Dysplastic nodule, *n* (%)	Absent	120 (73.2)	81 (69.8)	0.633
	Present	44 (26.8)	35 (30.2)	

[1]Edmondson-Steiner Grade. HBV: Hepatitis B virus; HCV: Hepatitis C virus; NBNC: Non-hepatitis B and non-hepatitis C virus; B + C: Hepatitis B and C virus; NCCK: National Cancer Center Korea; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation; PET/CT: Positron emission tomography/computed tomography; AFP: α-fetoprotein; MELD: Model for End-Stage Liver Disease; SUV: Standardized uptake value.

P = 0.033), it was not included because these data are typically not available before transplantation. We analyzed our data in comparison with the Milan and UCSF criteria using the NCCK criteria (negative findings on PET/CT and total tumor size < 10 cm *vs* others). The NCCK criteria were assessed both preoperatively and postoperatively.

Statistical analysis

Survival rates were estimated using Kaplan-Meier method, and survival curves were compared with log-rank test. Multivariable Cox proportional hazard regressions were fitted to identify factors that affected post-transplant survival. *T*-test and χ^2 test analyses were also used in comparing the differences between groups for continuous and categorical variables, respectively. Cohen's Kappa was used to assess classification consistency of each criteria. The prediction model of DFS using each criteria (the NCCK, Milan, and UCSF) adjusted for significant prognostic factors was developed using multivariable Cox proportional hazard regression. The receiver operating characteristic (ROC) curves and the associated area under the curves (AUC) of these models predicting 1, 3 and 5 years DFS rates were evaluated to compare the discrimination ability of different criteria. Differences in AUCs were tested using Delong's method[18]. All statistical analyses were performed using SAS software (9.2 version). *P*-value less than 0.05 was used to evaluate statistical significance.

RESULTS

Clinicopathological characteristics

During the study period, a total of 280 patients underwent LDLT for HCC. Among them, 116 (41.4%) patients did not fulfil the NCCK criteria. The comparisons of clinicopathological characteristics between patients who did and did not fulfill the NCCK criteria are presented in Table 2. C-reactive protein level, tumor SUVmax, total tumor size (> 10 cm), AFP (> 400 ng/mL), positive findings on PET/CT, differentiation (grade Ⅲ-Ⅳ), microvascular invasion, intrahepatic metastasis, and serosal

Table 3 Comparison between preoperative imaging and explant pathology by the Milan and National Cancer Center Korea criteria

Milan criteria		NCCK criteria		Preoperative imaging	
				Within	Beyond
Explant Pathology	Within			120 (42.86)	12 (4.29)
	Beyond			47 (16.79)	101 (36.07)
		Explant Pathology	Within	161 (57.50)	3 (1.07)
			Beyond	17 (6.07)	99 (35.36)

Cohen's Kappa = 0.850. NCCK: National Cancer Center Korea.

invaion were significantly greater in patients who did not fulfill the NCCK criteria compared with those who did. The mean C-reactive protein levels in two groups were 0.58 mg/dL and 1.37 mg/dL, and tumor SUVmax were 3.08 and 4.13, in patients who did and did not fulfill the NCCK criteria, respectively. On the other hand, patients who did not fulfill the NCCK criteria had significantly lower MELD scores compared to those within the NCCK criteria (12.5 *vs* 14.4, respectively, $P = 0.029$). Pre-transplant therapy type, viral hepatitis type, ductal invasion, capsule formation, dysplastic nodules, and cirrhosis were not significantly different between the two groups.

NCCK criteria: Survival rates and comparison between preoperative imaging and explant pathological reports

OS and DFS according to the NCCK criteria are presented in Figure 1. Patients fulfilling the NCCK criteria according to preoperative imaging findings revealed significantly higher OS and DFS than those who did not fulfill the NCCK criteria (five-year OS: 83.6% *vs* 59.8%, $P < 0.001$; five-year DFS: 80.7% *vs* 45.1%, $P < 0.001$). In patients who fulfilled the NCCK criteria according to explant pathological reports, five-year OS and DFS were 85.2% and 84.0%, respectively; these values were significantly higher than those among patients who did not fulfill the NCCK criteria (60.2% and 44.7%, respectively, $P < 0.001$).

The number of patients who fulfilled the NCCK criteria according to preoperative imaging and explant pathology reports were 178 (63.6%) and 164 (58.6%). According to the Milan criteria, these were 167 (59.6%) and 132 (47.1%) patients (Table 3). The NCCK criteria exhibited 95.0% accuracy of preoperative imaging and explant pathological reports; in contrast, the Milan criteria demonstrated only 78.9% accuracy. Compared with the Milan criteria, the NCCK criteria exhibited almost perfect agreement between preoperative imaging and explant pathological reports (Cohen's Kappa 0.850 *vs* 0.583).

Comparative survival analysis among the NCCK, Milan, and UCSF criteria

In a survival analysis including all patients, five-year OS and DFS were 75.2% and 67.7% (Figure 1). The patients who fulfilled the Milan criteria according to preoperative imaging and explant pathological reports showed good five-year OS and DFS (83.4% and 82.0% according to preoperative imaging; 85.5% and 84.4% by explant pathological reports, Figure 2). These survival results are very similar to those of patients fulfilling the NCCK criteria, particularly with regard to explant pathological reports. There were 34 (12.14%) patients who did not fulfill the NCCK criteria but fulfilled the Milan criteria according to preoperative imaging findings, and 22 (7.9%) according to explant pathological reports. This group showed a trend toward low five-year OS and DFS according to both preoperative imaging and explant pathological reports, compared with those who fulfilled the NCCK criteria; however, the differences between the two groups were not statistically significant ($P = 0.148$ in OS and $P = 0.212$ in DFS according to preoperative imaging findings; $P = 0.658$ in OS and $P = 0.376$ in DFS according to explant pathological reports, Figure 3).

Table 4 Area under the curves and 95%CI for the Milan, University of California, San Francisco, and National Cancer Center Korea criteria for the prediction of 1, 3, and 5 years disease-free survival

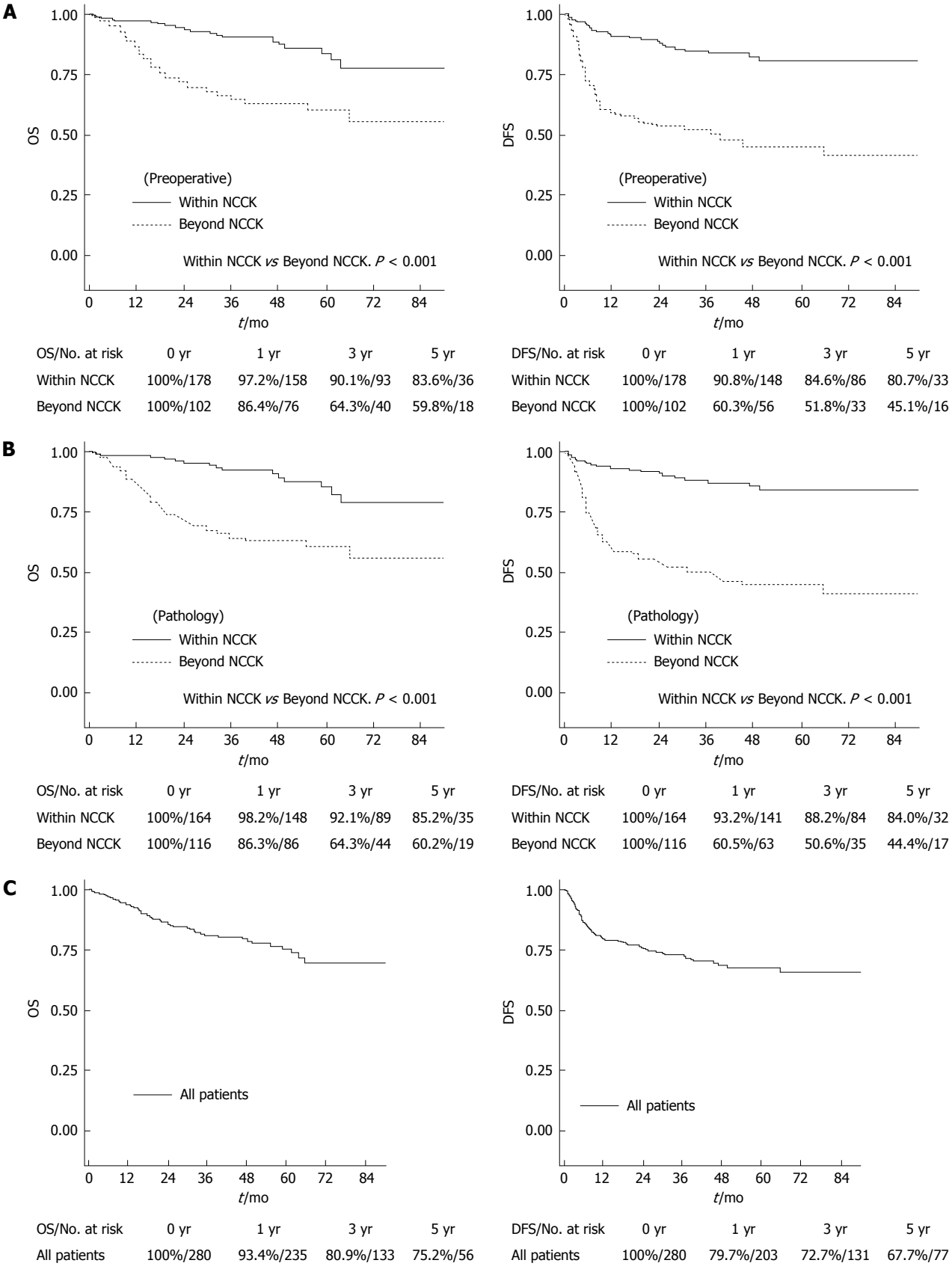
Diagnostic approach	Criteria	AUC (95%CI)		
		1 yr	3 yr	5 yr
Preoperative imaging	Milan[1]	0.814 (0.754, 0.873)	0.804 (0.750, 0.858)	0.799 (0.747, 0.851)
	UCSF[2]	0.812 (0.754, 0.871)	0.800 (0.747, 0.853)	0.793 (0.741, 0.844)
	NCCK[3]	0.810 (0.753, 0.867)	0.806 (0.755, 0.857)	0.802 (0.753, 0.852)
Explant pathology	Milan[4]	0.824 (0.767, 0.880)	0.815 (0.764, 0.866)	0.807 (0.757, 0.856)
	UCSF[5]	0.819 (0.761, 0.877)	0.811 (0.759, 0.863)	0.803 (0.752, 0.853)
	NCCK[6]	0.823 (0.769, 0.878)	0.817 (0.767, 0.866)	0.810 (0.762, 0.857)

[1]Adjusted by PET, X, Y and Z; [2]By PET, X and Y; [3]By maximum tumor size, X, Y, and Z; [4]By PET, total tumor size, X and Y; [5]By PET, X, Y, and Z; [6]By total tumor size, X, Y, and Z. X: Microvascular invasion; Y: Major vessel invasion; Z: Intrahepatic metastasis; AUC: Area under the curves; UCSF: University of California, San Francisco; PET: Positron emission tomography; NCCK: National Cancer Center Korea; 95%CI and *P* value were calculated by Cox PH regression analyses adjusted by the following covariates for each criteria.

ROC curve and AUC of the Milan, UCSF and NCCK criteria for the prediction of one, three, and five years DFS are presented in Figure 4 and Table 4. The value of AUC by three criteria was similar in both preoperative imaging and explant pathological reports, and there were no significant differences in the area under the ROC curve at one, three, and five years by three groups (five-year DFS, Delong's $P = 0.267$ for Milan *vs* NCCK, $P = 0.213$ for UCSF *vs* NCCK in preoperative imaging; $P = 0.484$ for Milan *vs* NCCK, $P = 0.189$ for UCSF *vs* NCCK in explant pathological reports).

DISCUSSION

In the present study, the NCCK criteria were associated



OS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within NCCK	100%/178	97.2%/158	90.1%/93	83.6%/36
Beyond NCCK	100%/102	86.4%/76	64.3%/40	59.8%/18

DFS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within NCCK	100%/178	90.8%/148	84.6%/86	80.7%/33
Beyond NCCK	100%/102	60.3%/56	51.8%/33	45.1%/16

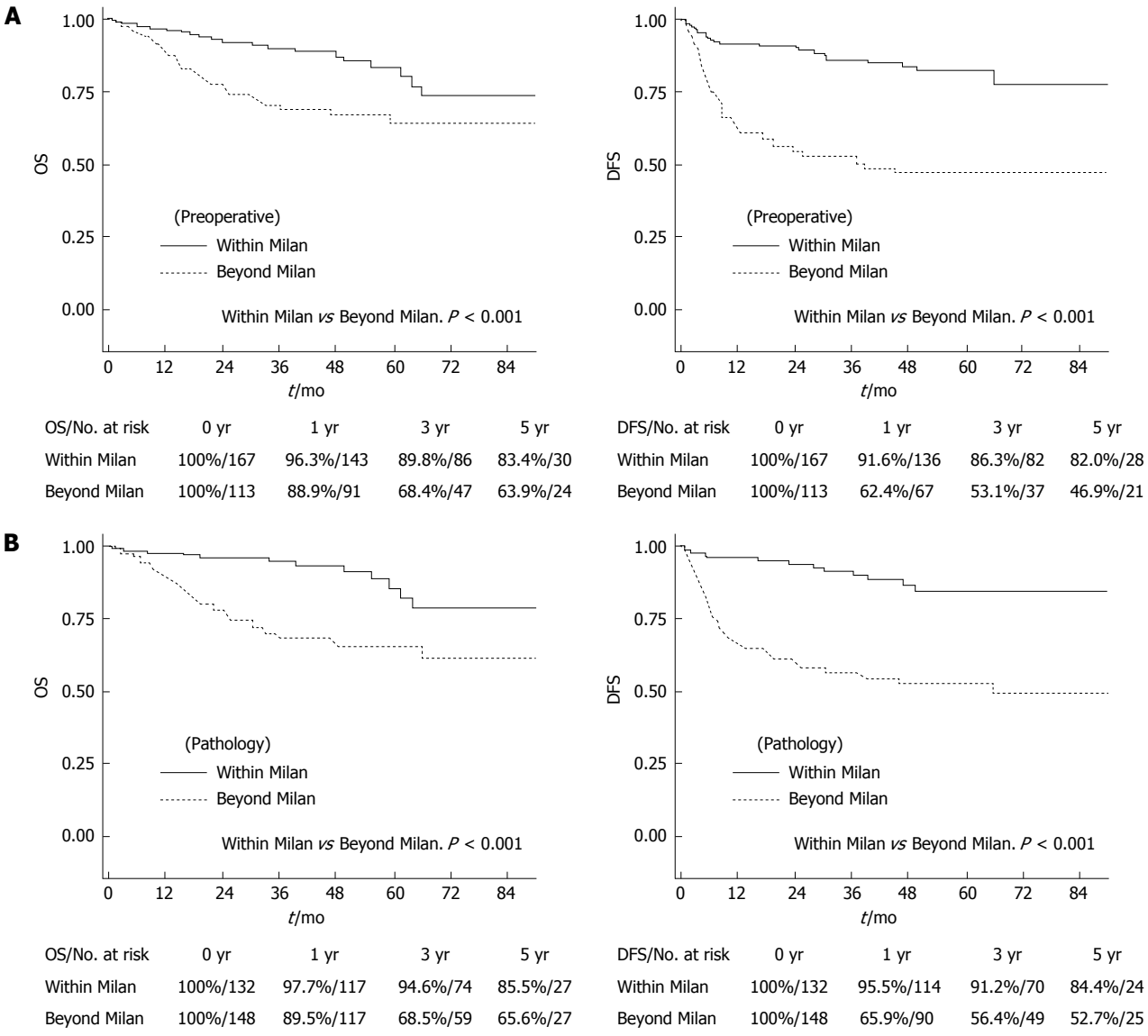
OS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within NCCK	100%/164	98.2%/148	92.1%/89	85.2%/35
Beyond NCCK	100%/116	86.3%/86	64.3%/44	60.2%/19

DFS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within NCCK	100%/164	93.2%/141	88.2%/84	84.0%/32
Beyond NCCK	100%/116	60.5%/63	50.6%/35	44.4%/17

OS/No. at risk	0 yr	1 yr	3 yr	5 yr
All patients	100%/280	93.4%/235	80.9%/133	75.2%/56

DFS/No. at risk	0 yr	1 yr	3 yr	5 yr
All patients	100%/280	79.7%/203	72.7%/131	67.7%/77

Figure 1 Overall and disease-free survival rates according to the National Cancer Center Korea criteria. A: By preoperative imaging; B: By explant pathology; C: OS and DFS rates for all patients. OS: Overall survival; DFS: Disease-free survival; NCCK: National Cancer Center Korea.



OS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within Milan	100%/167	96.3%/143	89.8%/86	83.4%/30
Beyond Milan	100%/113	88.9%/91	68.4%/47	63.9%/24

DFS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within Milan	100%/167	91.6%/136	86.3%/82	82.0%/28
Beyond Milan	100%/113	62.4%/67	53.1%/37	46.9%/21

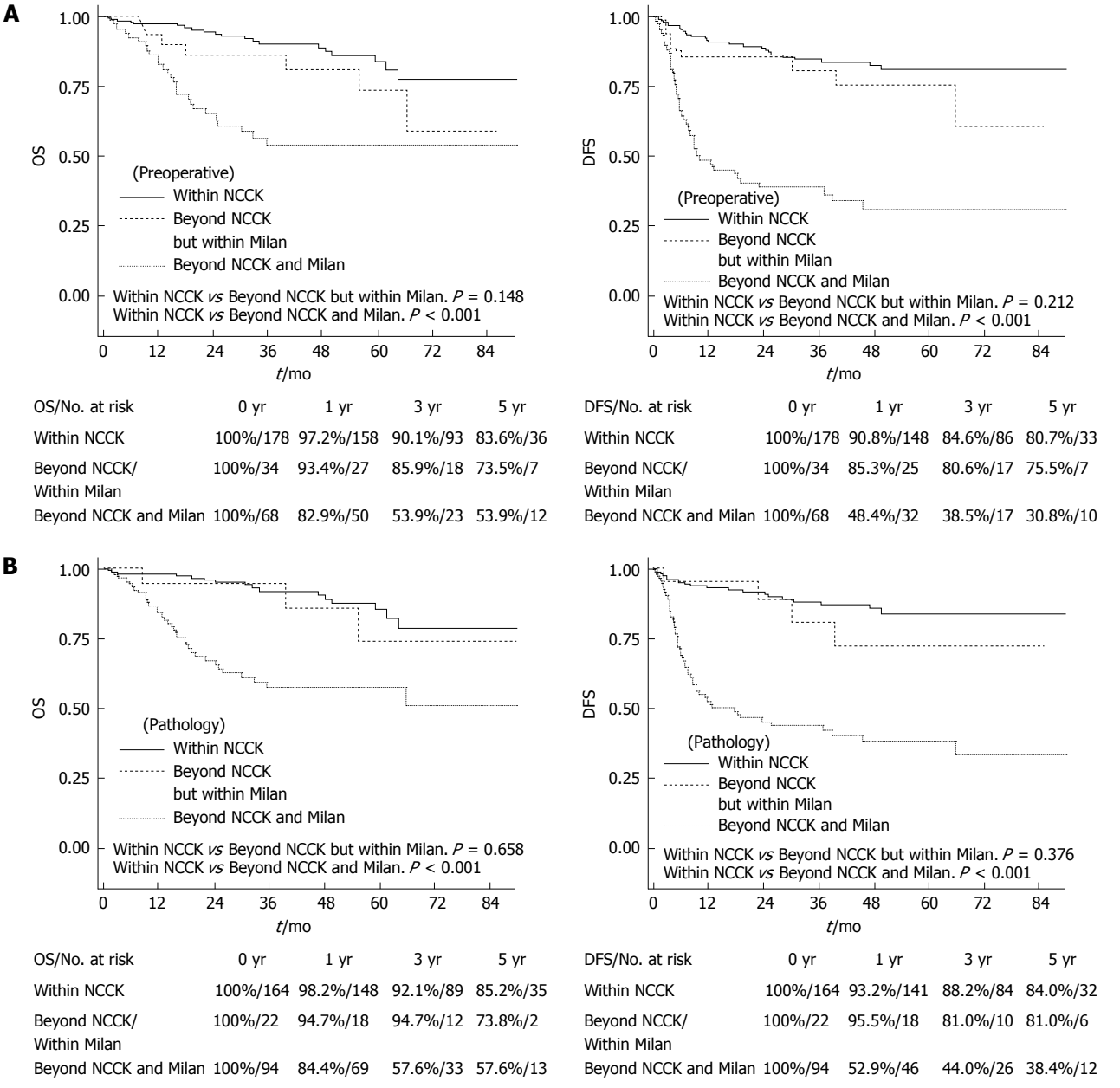
OS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within Milan	100%/132	97.7%/117	94.6%/74	85.5%/27
Beyond Milan	100%/148	89.5%/117	68.5%/59	65.6%/27

DFS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within Milan	100%/132	95.5%/114	91.2%/70	84.4%/24
Beyond Milan	100%/148	65.9%/90	56.4%/49	52.7%/25

Figure 2 Overall and disease-free survival rates according to the Milan criteria. A: By preoperative imaging; B: By explant pathology. OS: Overall survival; DFS: Disease-free survival.

with favorable survival outcomes and expanded the selection pool for LDLT among patients with HCC. Over the past 10 years, the Milan criteria have been regarded as a well-established tool for assessing the prognosis of HCC for LT. However, limited selection and inaccurate assessment using preoperative imaging modalities, such as CT, have been constantly recognized as a limitation of the criteria. Tumor biological characteristics, such as microvascular invasion and differentiation, are strong predictive factors for HCC recurrence. ^{18}F-FDG PET/CT findings are a useful marker to predict these factors before LT, as well as to detect extrahepatic metastases. Furthermore, total tumor size itself can be simple and relatively accurate measure rather than using both tumor number and size which are used in the Milan and UCSF criteria. The proposed NCCK criteria, therefore, presented with better correlation with preoperative imaging and explant pathological reports than the Milan criteria.

There were several expanded criteria for patients with HCC beyond the Milan criteria. The main factors that were present in these criteria were tumor size and number. The UCSF, Tokyo, and "up-to-seven" criteria are based on tumor morphological characteristics using preoperative imaging or explant pathological reports[4,8,19]. However, recent studies reported the expanded criteria using markers of tumor aggressiveness as well as tumor morphological characteristics. These included responses to TACE, the degree of differentiation, the gene-expression profile, the presence of microvascular invasion, and the levels of tumor markers, including AFP or DCP[11,20-24]. In particular, it is well known that microvascular invasion and the degree of differentiation are associated with decreased survival and an increased risk of recurrence following LT. However, these pathological examination results are not routinely available before LT because fine-needle biopsy before surgery has not shown significant correlations with explant



OS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within NCCK	100%/178	97.2%/158	90.1%/93	83.6%/36
Beyond NCCK/ Within Milan	100%/34	93.4%/27	85.9%/18	73.5%/7
Beyond NCCK and Milan	100%/68	82.9%/50	53.9%/23	53.9%/12

DFS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within NCCK	100%/178	90.8%/148	84.6%/86	80.7%/33
Beyond NCCK/ Within Milan	100%/34	85.3%/25	80.6%/17	75.5%/7
Beyond NCCK and Milan	100%/68	48.4%/32	38.5%/17	30.8%/10

OS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within NCCK	100%/164	98.2%/148	92.1%/89	85.2%/35
Beyond NCCK/ Within Milan	100%/22	94.7%/18	94.7%/12	73.8%/2
Beyond NCCK and Milan	100%/94	84.4%/69	57.6%/33	57.6%/13

DFS/No. at risk	0 yr	1 yr	3 yr	5 yr
Within NCCK	100%/164	93.2%/141	88.2%/84	84.0%/32
Beyond NCCK/ Within Milan	100%/22	95.5%/18	81.0%/10	81.0%/6
Beyond NCCK and Milan	100%/94	52.9%/46	44.0%/26	38.4%/12

Figure 3 Overall and disease-free survival rates according to three groups (within the National Cancer Center Korea criteria, Beyond the National Cancer Center Korea but within the Milan criteria, Beyond both the National Cancer Center Korea and Milan criteria). A: By preoperative imaging; B: By explant pathology; C: The diagram of the portion of patients in Milan and NCCK criteria by preoperative imaging. OS: Overall survival; DFS: Disease-free survival; NCCK: National Cancer Center Korea.

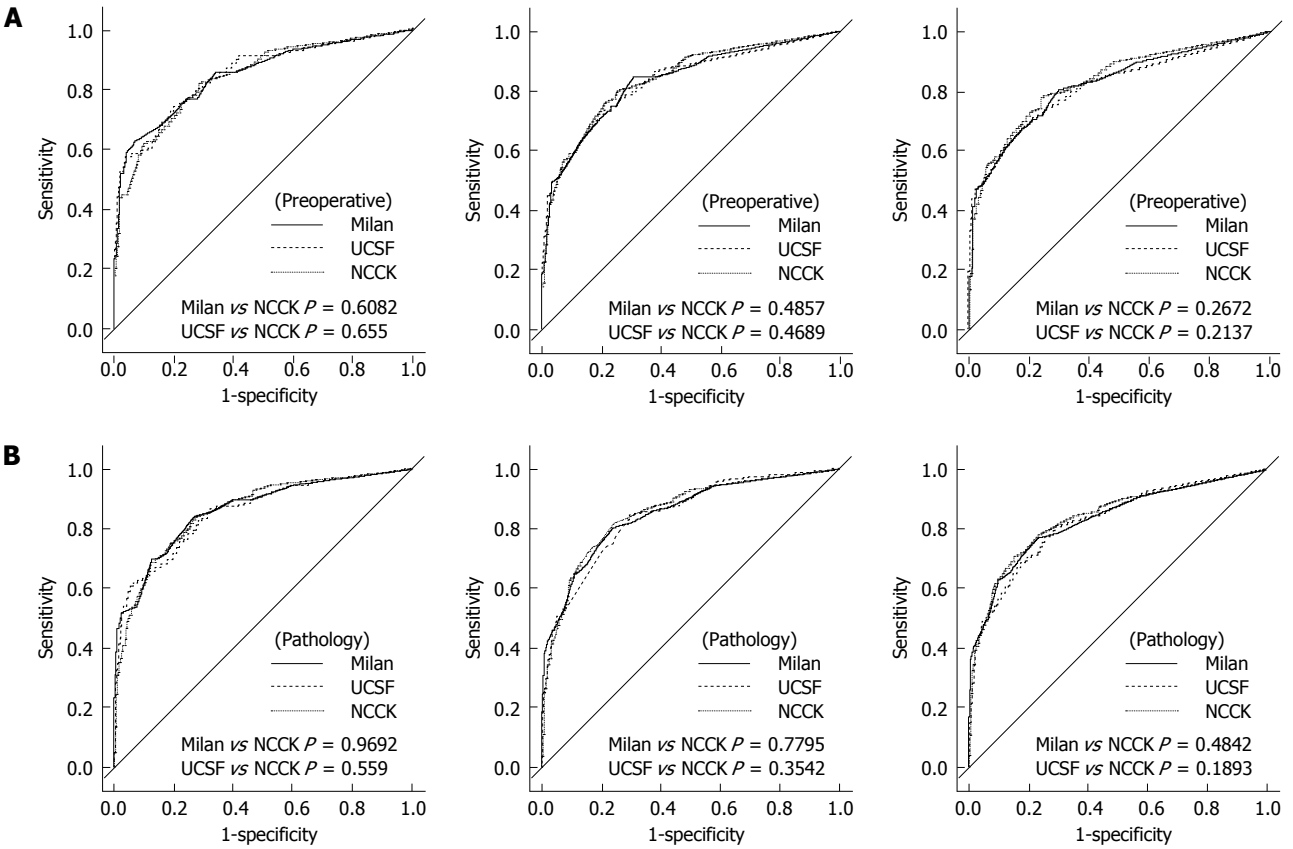


Figure 4 Receiver operating characteristic curves of three criteria (the National Cancer Center Korea, Milan and University of California, San Francisco) at 1, 3, and 5 years. A: By preoperative imaging; B: By explant pathology. UCSF: University of California, San Francisco; NCCK: National Cancer Center Korea.

pathological reports[25]. Some promising attempts to identify microvascular invasion before LT through ^{18}F-FDG PET or PET/CT have been reported[13,14,26]. Moreover, positive findings on PET/CT in patients with HCC predicted the prognosis and tumor recurrence after LT[13-15]. In the present study, the patients beyond the NCCK criteria, including positive findings on PET/CT, showed more microvascular invasion (59.5% *vs* 22.6%, $P < 0.001$) and poor differentiation (52.6% *vs* 37.8%, $P = 0.02$). One concern regarding the use of PET/CT in patients with HCC is that the sensitivity is low for the primary detection of HCC compared with many other cancers, because glucose metabolism is high in liver tissue[27,28]. On the other hand, PET/CT has been shown to differentiate between well-differentiated and poorly-differentiated HCC, and is useful in the detection of extrahepatic metastases and recurrence of HCC after transplantation[29].

The concept of the NCCK criteria began from the observation that good survival rates without recurrence could occur in patients who did not fulfill the Milan criteria. In our data, patients beyond the Milan criteria who also had negative findings on PET/CT showed significantly better survival rates than those who had positive findings on PET/CT (five-year OS, 74.6% *vs* 51.4%, $P < 0.001$; five-year DFS, 73.3% *vs* 37.5%, $P < 0.001$). When another significant factor for survival in multivariable analysis (total tumor size < 10 cm) was considered, patients who did not fulfill the Milan criteria with negative findings on PET/CT and total tumor size < 10 cm showed similar OS and DFS compared with those who met the Milan criteria (OS: mean 90.7 mo *vs* 83.8 mo, $P = 0.235$; DFS: mean 94.4 mo *vs* 84.4 mo, $P = 0.076$). Furthermore, positive findings on PET/CT and total tumor size were significant prognostic factors of OS and DFS for all patients (Table 1). Therefore, we applied the NCCK criteria to all patients and analyzed their usefulness and associated survival rates as new expanded criteria that could be used instead of the traditional Milan criteria.

Numerous expanded criteria based on tumor number and size have been reported, but are not used widely due to limited clinical usefulness. The major reason for this is that the risk of underestimating tumor status is considerable regardless the recent developments of new technologies in radiological assessment of liver tumors[30]. Freeman *et al*[31] studied the results from the United Network for Organ Sharing database on 789 LT recipients to analyze the accuracy of imaging findings compared with the explant pathological reports. In that report, radiological imaging underestimated tumor staging in 26.6% of cases, and the risk of overestimation was almost 30%. The overall preoperative accuracy was approximately 50%, regardless of the radiological technique used. In our data, among 167 patients who fulfilled the Milan criteria according to preoperative

imaging modalities, 47 patients (28.1%) were found as not fulfilling the Milan criteria in explant pathological reports. Therefore, some authors proposed that total tumor volume or size was more likely to result in accurate staging before LT[32-34]. We also used the total tumor size (cutoff 10 cm), which was a significant prognostic factor in multivariable analysis for the NCCK criteria. In our study, among a total of 243 patients with preoperative total tumor size < 10 cm measured with imaging modalities, only 27 patients (11.1%) were confirmed to have a total tumor size of > 10 cm according to pathological reports. Compared with the Milan criteria, the percentage of underestimation in the NCCK criteria using total tumor size (cutoff 10 cm) was lower (9.6%), and Cohen's Kappa was high (0.850), explaining the near-perfect agreement between preoperative imaging and explant pathological reports (Table 3).

In particular, the survival rates of patients who fulfilled the NCCK criteria were quite good and showed similar outcomes compared with the Milan and UCSF criteria (five-year DFS; 80.7% according to preoperative imaging findings, 84.0% in explant pathological reports, Figure 2). Furthermore, the number of patients who fulfilled the NCCK criteria was higher than the Milan criteria [preoperative imaging findings, 178 (63.6%) *vs* 164 (58.6%) patients; explant pathological reports, 167 (59.6%) *vs* 132 (47.1%) patients]. The patients who did not fulfill the NCCK, but fulfilled the Milan criteria did not show statistically significant differences compared with those who fulfilled the NCCK criteria; however, a trend toward low five-year OS and DFS according to both preoperative imaging and explant pathological reports was observed (Figure 3). This result was likely because of the fact that the Milan criteria are too restrictive and limited. There was no significant difference observed when the values of AUC and ROC curves for predicting DFS at one, three, and five years were compared among the three criteria (NCCK, Milan, and UCSF) (Figure 4 and Table 4).

There are some limitations to the present study. First, we analyzed LDLT patients without including deceased donor LT patients; therefore, comparison with other studies that included deceased donor LT patients was not possible. However, we included a considerable proportion of patients who were beyond the Milan criteria; thus, the dilution effect on the analysis was less than that in other studies. Second, the present study was retrospective in nature, and selection bias could have influenced the survival analysis. However, we enrolled all consecutive cases and performed routine PET/CT before LDLT in patients with HCC. Therefore, exclusions during the study period were rare.

In conclusion, our data show that the NCCK criteria, utilizing total tumor size and PET/CT findings, successfully expanded the recipient pool and demonstrated better ability of tumor assessment before LT and similar survival rates compared with the well-known criteria, such as the Milan and UCSF. These criteria represent a new approach to selection for LT that incorporates both tumor biological and morphological characteristics. Therefore, the NCCK criteria are simple and useful expanded criteria for LDLT in HCC, showing excellent agreement between preoperative imaging and explant pathological reports and favorable survival outcomes.

COMMENTS

Background

Several expanded criteria based on morphological features have been proposed to identify appropriate candidates for liver transplantation (LT). However, the definitions are still complex, and the benefit of expanding the pool remains controversial. In this study, the authors evaluated the new criteria using positron-emission tomography/computed tomography (PET/CT) and total tumor size, called as National Cancer Center Korea criteria.

Research frontiers

The expanding criteria for living donor liver transplantation (LDLT) for hepatocellular carcinoma (HCC) is issued recently. The results of this study contribute to clarifying exact criteria using PET/CT and tumor morphologic characteristics.

Innovations and breakthroughs

In this study, they used the PET/CT for all patients underwent LDLT in their institute before transplantation. These results are so unique and included relatively large number of patients. PET/CT is very useful tool for selecting recipients with HCC in LDLT.

Applications

This study suggested that PET/CT is useful for selecting recipient and total tumor size is simple for marker in preoperative imaging tests. If a patient is diagnosed with HCC and waiting the LDLT, PET/CT can be chosen for diagnostic metastasis and prediction of prognosis.

Peer-review

The author this paper evaluated the usefulness of PET/CT and total tumor size for predicting the prognosis after LDLT for HCC, and showed the expanded criteria using these tools. Further trials using these criteria in large population of LDLT will be valuable.

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P- Reviewer: Chiu KW, Goral V, Kabir A, Mihaila RG, Mizuguchi T
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Transplantation

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5500/wjt.v6.i2.423

World J Transplant 2016 June 24; 6(2): 423-428
ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Observational Study

Deceased donor organ procurement injuries in the United States

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Author contributions: Taber TE, Neidlinger NA and Paramesh AS contributed to the conception and design of the study; Taber TE and Mujtaba MA contributed to the acquisition and analysis of the data; all of the authors contributed to the drafting and critical revisions of the manuscript and gave final approval of the article to be published.

Institutional review board statement: As the study was done on deceased donors, no institutional review board approval was obtained (see body of manuscript).

Informed consent statement: As this was a study looking at deceased donor procurements, there are are no patients to consent as the subjects are legally dead per US law. Hence, we are asking for a waiver of informed consent requirements.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at Dryad repository, who will provide a permanent, citable and open-access home for the dataset. In addition, a copy of the signed statement should be provided to the BPG in PDF format.

Manuscript source: Invited manuscript

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Received: January 30, 2016
Peer-review started: February 1, 2016
First decision: March 15, 2016
Revised: April 26, 2016
Accepted: May 17, 2016
Article in press: May 27, 2016
Published online: June 24, 2016

Abstract

AIM: To determine the incidence of surgical injury during deceased donor organ procurements.

METHODS: Organ damage was classified into three tiers, from 1-3, with the latter rendering the organ non-transplantable. For 12 consecutive months starting in January of 2014, 36 of 58 organ procurement organization's (OPO)'s prospectively submitted quality data regarding organ damage (as reported by the transplanting surgeon and confirmed by the OPO medical director) seen on the procured organ.

RESULTS: These 36 OPOs recovered 5401 of the nations's 8504 deceased donors for calendar year 2014.

A total of 19043 organs procured were prospectively analyzed. Of this total, 59 organs sustained damage making them non-transplantable (0 intestines; 4 pancreata; 5 lungs; 6 livers; 43 kidneys). The class 3 damage was spread over 22 (of 36) reporting OPO's.

CONCLUSION: While damage to the procured organ is rare with organ loss being approximately 0.3% of procured organs, loss of potential transplantable organs does occur during procurement.

Key words: Organ procurement; Deceased donations; Organ procurement organization; Organ injury; Organ transplantation

Core tip: This study represents a unique report looking into the incidence of surgical injuries during deceased donor organ procurement. There is no other large scale study reporting this. This represents a multi-organizational study, collecting data prospectively over a period of a year. This study will hopefully help define the problem and contribute to the development of basic standards that organ procurement organizations can follow across the country.

Taber TE, Neidlinger NA, Mujtaba MA, Eidbo EE, Cauwels RL, Hannan EM, Miller JR, Paramesh AS. Deceased donor organ procurement injuries in the United States. *World J Transplant* 2016; 6(2): 423-428 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/423.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.423

INTRODUCTION

Organ transplantation remains one of the enduring miracles of modern medicine. The ability to replace a dysfunctional organ with a functional allograft that returns the recipient to health is truly an impressive feat. Human organ transplantation essentially started in 1954 with the first successful kidney transplant[1]. Early transplant successes were limited by the lack of availability of adequate immunosuppression. With the advent of cyclosporine in 1983, the modern era of transplantation began[2]. Organ transplantation, since that time, has been limited less by the ability to maintain viability of allografts post-transplant than by the supply of transplantable organs[3]. As most organ transplants are deceased organs, the willingness of potential donor families to agree to organ donation has become paramount. Despite the altruism of these families, over time, there has developed a mismatch of supply and demand with the current waitlist (April 2016) of patients for a solid organ transplant exceeding 121000. Hence, there has been an imperative to ensure that any organ procured should be uninjured during the procedure in order to maximize utilization. Little data exists in the literature regarding procurement injury. The aim of this study was to determine the incidence of procurement injury in the United States. Organ procurement organization (OPO) system and to further stratify the impact of these injuries by developing a graded scoring system directly linked to the extent of loss.

MATERIALS AND METHODS

There are currently 58 OPOs in this country performing organ procurement. The Association of OPOs (AOPO) serves to unify these individual OPO's and to assist in the sharing of knowledge of best practices in the many tasks performed by the OPOs. Within each OPO, organ procurement is overseen by a medical director to whom each is extended an offer for membership within the AOPO medical council. It is within this medical council that, in 2013, a discussion culminated in the desire to ensure that the "gift of life" of an organ donation should be protected. The medical directors agreed upon a national standard of measurement of organ damage. These levels of damage were agreed upon and range from a level of "0" (no damage); level "1" (minimal damage sustained upon procurement requiring no intervention); level "2" (damage sustained upon procurement requiring some surgical repair but not rendering the allograft non-transplantable); and finally level "3" (damage sustained upon procurement rendering the allograft non-transplantable). These levels of damage would be reported by the transplanting surgeon and reviewed and agreed upon by the medical director of the procuring OPO in consultation with the medical advisory board within that OPO (as deemed necessary by the individual medical director).

After the aforementioned preliminary agreement was reached, this study commenced and included all deceased donors from whom solid organs were procured for transplantation from January 1, 2014 through December 31, 2014. All 58 OPOs were encouraged to prospectively collect data during this period. Data was sent to the AOPO national office where it was transferred to a database and separated by month and OPO. Data was collected for transplantable solid organs: Heart, lung, liver, kidney, pancreas and intestine. Data was subsequently analyzed in an organ-specific fashion. Only data collected for the entire 12 mo of the study was included for evaluation. As noted above, levels of damage were defined as class 1, class 2 or class 3. For each level 3 injury, a written description of the injury was provided to AOPO.

All data for this analysis were collected prospectively in our OPO database. Continuous variables are presented as mean/median. Number and type of organ procured at each OPO and class of injury were reviewed. Class of injury was expressed as 1, 2 or 3 and reported as a frequency at each OPO. Chi square test was used for categorical variables. A two-tailed P value of < 0.05 was considered to be significant. The program - graph pad

Table 1 Participating organ procurement organizations

Arkansas Regional Organ Recovery Agency
Donor Network West
Life Sharing - A Donate Life Organization
Donor Alliance Inc.
Life Choice Donor Services
Washington Regional Transplant Community
Life Alliance Organ Recovery Agency
Life Quest Organ Recovery Services
LifeLink of Florida
Legacy of Life Hawaii
Indiana Donor Network
Louisiana Organ Procurement Agency
New England Organ Bank
The Living Legacy Foundation of Maryland
Gift of Life Michigan
Life Source
Mid-America Transplant Services
Mississippi Organ Recovery Agency
Midwest Transplant Network
Carolina Donor Services
Nebraska Organ Recovery System
New Jersey Organ and Tissue Sharing Network
Live-On-NY
Lifebanc
Life Connection of Ohio
Lifeline of Ohio
Life Center Organ Donor Network
Life Share Transplant Donor Services of Oklahoma
Pacific Northwest Transplant Bank
Center for Organ Recovery and Education
Tennessee Donor Services
Life Gift Organ Donation Center
Southwest Transplant Alliance
Life Center Northwest
Wisconsin Donor Network
UW Organ and Tissue Donation

prism - was used to perform statistical evaluation.

The need for consent in the United States is regulated by local Institutional Review Boards. The consent for brain dead (BD) donors for research is not legally required when no additional tissue, *etc*., is taken from the donor[4]. For that reason this study was Institutional Review Board (IRB) exempt and IRB consent was not requested.

RESULTS

A total of 36 OPOs (out of a potential 58) participated in the prospective collection of data (Table 1). An additional 3 OPOs submitted data but were not included in the analysis as this data was not a complete years' collection. By excluding partial year's data, we aimed to minimize selection bias. OPO size (donors/year) varied from 43 to 305 donors/year (mean 147.5; median 141). These 36 OPOs recovered a total of 5401 of the nation's 8594 deceased donors in 2014. From these donors, 19043 procured organs' data was analyzed. Of the donors, 4347 were BD donors and 870 were donation after cardiac death (DCD) donors. Data was reported in terms of both recovered and transplanted organs. The most frequent type of injury was class 1 (Table 2). Class 2 injuries were usually but not always intermediate in number between class 1 and class 3 injuries. In order of increasing incidence of injury, type 3 injuries were compared to recovered organs and occurred in the following frequencies: Intestine: 0/128 (0%); heart 1/1726 (0.05%); liver: 6/4396 (0.14%); lung: 5/2437 (0.21%); kidney: 43/9501 (0.42%); pancreas: 4/855 (0.47%). A total incidence then of class 3 injury in the 19043 organs procured was 0.3%. Among individual OPOs, there were a total of 22 OPOs that reported at least one type 3 injury (Table 3). The median number of class 3 injuries per OPO (in OPOs that had at least 1 injury) was 2.0 with a mean of 2.7 and a mode of 1. One OPO reported 10 class 3 injuries during the year of data collection, one OPO reported 6 and 2 OPOs reported 4 class 3 injuries. The remaining OPOs reporting class 3 injuries fell in the range of 1-3 injuries for the year (#18).

Table 2 Recovery data with Injuries

Recovered intestine	128
Transplanted intestine	77
Type 1	2
Type 2	0
Type 3	0
Recovered pancreas	855
Transplanted pancreas	648
Type 1	7
Type 2	3
Type 3	4
Recovered heart	1726
Transplanted heart	1617
Type 1	6
Type 2	2
Type 3	1
Recovered lung	2437
Transplanted lung	2004
Type 1	16
Type 2	1
Type 3	5
Recovered liver	4396
Transplanted liver	3928
Type 1	58
Type 2	16
Type 3	6
Recovered kidney	9501
Transplanted kidney	7889
Type 1	156
Type 2	86
Type 3	43

Table 3 Number of type 3 injuries (one year) by organ procurement organization

OPOs with 1 injury	7
OPOs with 2 injuries	5
OPOs with 3 injuries	6
OPOs with 4 injuries	2
OPOs with 6 injuries	1
OPOs with 10 injuries	1

OPO: Organ procurement organization.

Table 4 Causes of class 3 injury

Organ	# injuries	Cause
Intestine	0	N/A
Pancreas	4	Vascular injury (2)
		Traction injury to organ (2)
Heart	1	Vascular injury (1)
Lung	5	Vascular injury (2)
		Inadequate trachea for anastomosis (1)
		Not specified (1)
Liver	6	Vascular injury (3)
		Capsular tear (2)
		Not specified (1)
Kidney	43	Vascular injury (27)
		Capsular tear (7)
		Ureteral transection (5)
		Not specified (3)
		Failure to flush artery adequately (1)

In looking at OPO size as being predictive of the number of class 3 injuries, 3 of 4 of the OPOs having at least 4 class 3 injuries were larger than the median OPO size in the total cohort (147.5 donors/OPO) but this did not reflect their frequency. The incidence of class 3 injury within this subset of OPO's having at least 4 injuries ranged from 1.3% (of procured organs) to 4.4% with the highest incidence occurring in the OPO with 10 class 3 injuries. Further evaluation of this subgroup of 22 OPOs with class 3 injuries, 7 had only 1 and 5 only had 2. In the subgroup of OPOs with at least 3 class 3 injuries (#10), only 6 of the OPOs had an incidence of over 2.1%. In looking at the highest incidence of injury, 4 OPOs had an incidence of at least 3.9% (range 3.9%-4.7%). In contrast to that noted above in regards to total injuries and OPO size, 3 of these 4 OPOs were smaller OPOs as defined by annual donor numbers (< 147.5 donors/year). Finally, arbitrarily using a 2% injury rate irrespective of number of injuries, there were 7 OPOs that fell within this parameter. Of those OPOs, 5 were in the smaller OPO group (again - as defined as < 147.5 donors/year) and 2 were in the larger group. From a statistical analysis standpoint, using chi square testing, a higher incidence of class 3 injury was observed in the smaller OPOs (grouped together: < 147.5 donors/year) *vs* larger OPOs (> 147.5 donors/year) with a *P* value of 0.044.

As class 3 injuries rendered the allograft unable to be transplanted, a summary was received for each lost organ (Table 4). In all allografts (with the exception of pancreas that sustained a "traction" injury) vascular damage was the most common injury rendering the organ non-transplantable. The total BD *vs* DCD donors were noted but the only data regarding donor type supplied on failed organs was in the narrative. Of note, however, 2 of 6 livers felt to be non-transplantable were noted to be DCDs and 4 of the 43 kidneys. Unfortunately, this data was gleaned from the narrative and not specifically collected so a comparison of DCD *vs* BD donors reflecting the likelihood of class 3 injuries cannot be made.

DISCUSSION

There have been retrospective reviews regarding surgical damage during procurement but to our knowledge, this is the first prospective look at the surgical outcome of organs procured from deceased donors gathered at the United States OPO level[5,6]. For that reason, an acceptable degree of surgical damage seen during procurement could not be known. The technique required in procuring organs for donation requires the skills of a vascular surgeon and the insights of a transplant surgeon. Surgical damage may be related to the procurement procedure itself or may be related to the cause of death of the donor (trauma). Damage rendering the organ non-transplantable may be related to parenchymal damage, injury to the vasculature or other parts of the organ (ureter, *etc*.). The surgeon is required to procure the organ without injury to any of these structures[7]. In addition, they must obtain enough of the vasculature to allow for anastomosis into the recipient. This desire for adequate vessel length, though, must be balanced with the needs of the other procuring surgeons. Frequently vessel lengths are shared between donor surgeons and a degree of communication and cooperation is required and almost always achieved. Anomalous anatomy also may play a part in organ injury[8]. This is especially true in the procurement of small organs (pancreas)[9]. Finally, the insight of the transplanting surgeon should not be overlooked in the determination of transplantability of the organ. If a marginal organ is procured and found to have a significant injury that could potentially impact its function, the transplanting surgeon might be more disinclined to transplant this organ. This could especially be the case in the procurement of a marginal or DCD organ as has been seen previously in DCD kidneys[10]. Unfortunately, this study was not designed to compare damage seen in BD *vs* DCD donors. In some cases this information was contained in the narrative describing the injury but as this was not consistent, that information is not reported here.

Despite all the enumerated pitfalls involved in organ procurement, the frequency of organ injury during procurement is rare. The motivation and the skill of the procuring and transplanting surgeon combine to make this outcome predictable. In looking for trends within class 3 injuries, the very scarcity of these injuries made such efforts difficult. What was seen, however, in the OPOs with the highest levels of class 3 injuries was that the injuries tended to cluster within months and then disappear in the months following. In reading the narrative associated with injuries, it was evident that procurement injuries resulted in feedback to the procuring surgeons. It was likely then that such feedback either improved the future focus of the procuring surgeon or resulted in a change or a call for mentorship (in at least one case) in the procuring team. This study would then support the importance of a collegial

discussion with the procurement team in the instance of organ injury.

In looking at class 3 injuries, as noted previously, 14 of the 36 participating OPOs had no such injuries while 3 of 4 of the highest raw number of injuries occurred in larger OPOs (> 147.5 donors/year). However in looking at the frequency of injury of > 2%, smaller OPOs made up the majority of this subset (see above). It does appear then that smaller OPOs by size tend to have a statistically significantly higher likelihood of having a greater frequency of class 3 injury - again arbitrarily defined as a frequency of > 2%. At least one of the reasons for this can be the smaller margin for error when fewer donors are procured. Other potential causes for this would be speculative without further data collection.

Certainly the vast majority of this discussion has been focused on class 3 injuries. The numbers of class 1 and 2 injuries certainly exceed class 3 but, as these do not result in a lost allograft, there is a lessened imperative to examine these events. However, it is likely that these events may be harbingers of class 3 injuries. As no narrative was provided for class 1 and class 2 injuries, it is unknown as to whether OPOs have these discussions after these events. By providing feedback to individual procuring surgeons not just in class 3 but also in the event of a class 1 or 2 injury, there would seem to be potential for improving an individual's procurement surgeon's skills and so avoid future type 3 injuries. These events therefore should continue to be reviewed on an individual OPO level.

Finally, the collection of this data provides OPOs a perspective on their effectiveness in organ procurement. Individual OPOs can, by continuing to follow their surgical injury rate, have an idea as to where their injury rate falls within the national benchmarks. While the goal for surgical damage continues to be the lack of damage, careful review of the frequency of different damage levels will give individual OPOs continuous feedback on at least one aspect of their quality.

The strengths of this study include the prospective data collection, the inclusion of 36 of 58 OPOs as well as the use of the entire 12 mo of data during the collection period. The inclusion and review of the narrative also gave insight into the individual OPOs efforts in enhancing quality. The weaknesses of this study include the lack of participating would have shown a higher level of surgical injury but that outcome again would not be a fait accompli. Additionally, expanding data collection to include determination of BD *vs* DCD donors, names of procurement teams and levels of experience of these teams would have been helpful in interpreting the data. Finally, as the degree of damage was first quantitated by the transplanting surgeon, there is a potential for under-reporting type 1 and 2 injuries if the procuring team were from the transplanting center. This degree of underreporting should not be seen, however with type 3 injuries as the loss of an organ would be evident to the on-site OPO coordinators. Taking all of these concerns into account, the goal of this study was to establish a standard in the description of procurement surgical damage and a baseline of injury rate. Examined in this light, this study achieved its goals.

A 12 mo collection of surgical damage data from 36 of 58 OPOs in the United States was reviewed. In the entire group, surgical damage was a rare event with the loss of allograft seen in less than 0.5% of procured organs. The majority of the surgical damage seen was related to vascular injuries. Incidence of class 3 injury appears to be higher in OPOs with smaller donor volumes.

COMMENTS

Background

There is a paucity of information about organ injuries during deceased donor procurements. This has significant importance into the numbers of organs that are transplanted every year. This study set out to document and report, for the first time, the incidence and grades of surgical injury to organs during their procurement across the United States. This has the potential to set national standards for quality and offer future ideas for research.

Research frontiers

As the waiting list for organ transplants gets bigger, there has been recent impetus for research to look at more ways to obtain such organs. One such important way would be to identify the numbers of organs that are lost to injury during procurement.

Innovations and breakthroughs

There have been no previous large scale reports of this kind, hence the novelty of this study.

Applications

This study offers for the first time, a national perspective on procurement organ injuries. It helps define a national problem, which the authors know little about. This offers the potential to establish national standards for organ procurement organizations, training of transplant surgeons, and even insurance and regulatory purposes.

Terminology

OPO: Organ procurement organizations. These are independent organizations, contracted with the United Network of Organ Sharing in the United States. Their purpose is the responsibility of procurement and distribution of organs from deceased donors in their assigned geographic area; AOPO: Association of OPOs. A national organization representing all of the 58 OPOs across the United States.

Peer-review

This study is a large scale multi-organizational report looking into the incidence of surgical injuries during deceased donor organ procurement, collecting data prospectively over a period of a year. Organ damage was classified into three tiers, with the latter rendering the organ non-transplantable.

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P- Reviewer: Kita K, Kin T, Marino IR, Yildiz B **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Transplantation

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5500/wjt.v6.i2.429

World J Transplant 2016 June 24; 6(2): 429-436
ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Randomized Controlled Trial

Exercise manual for liver disease patients

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Supported by Fapesp n° 2010/19326-5.

Institutional review board statement: The study was reviewed and approved by the Ethics Committee of the Medical Sciences Faculty, Unicamp, CEP: 922/2009.

Clinical trial registration statement: This study is registered at http://www.ensaiosclinicos.gov.br/rg/RBR-8fz3mj/ the registration identification number is RBR-8fz3mj.

Informed consent statement: All participants of the study, or their legal guardian, provided informed written consent prior to the study enrollment.

Conflict-of-interest statement: No conflicts of interest to declare.

Data sharing statement: No additional data are available.

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Received: January 14, 2016
Peer-review started: January 15, 2016
First decision: February 2, 2016
Revised: February 18, 2016
Accepted: April 14, 2016
Article in press: April 18, 2016
Published online: June 24, 2016

Abstract

AIM: To increase inspiratory muscle strength and improve the quality of life of candidates for liver transplantation.

METHODS: Twenty-three candidates for liver transplantation participated in the control group and 14 made up the intervention group. The control group consisted of 18 men and 5 women, body mass index (BMI) 27.3 ± 4.5 kg/m^2 and Model for End-Stage Liver Disease (MELD) 18.2 ± 6.1. The intervention group consisted of 11 men and 3 women, BMI 28.6 ± 5.4 kg/m^2 and MELD 18 ± 4.5. The presence or absence of ascites was identified in the first patient evaluation and after three months. We evaluated maximal inspiratory pressure (MIP) and maximal expiratory pressure, spirometry, root mean square (RMS) of diaphragm and rectus abdominis, and the quality of life. The exercises were performed daily by patients at home for three months and were supervised at distance monthly. The manual consisted of diaphragmatic breathing exercises, diaphragmatic isometric exercise, Threshold IMT®, lifting upper limbs with a bat and strengthening the abdomen.

RESULTS: There was significant difference (P = 0.01) between the first (initial) and the third month (final) MIP in the control group and in the intervention group, but there was no difference (P = 0.45) between the groups.

The RMS of the diaphragm was lower (P = 0.001) and the functional capacity was higher (P = 0.006) in the intervention group compared to the control. The general health and mental health domains received higher scores after three months in the control group (P = 0.01) and the intervention group (P = 0.004), but there was no significant difference between them. The comparison between the presence of initial ascites with the presence of ascites was performed after three months in the control group (P = 0.083) and intervention group (P = 0.31). There was no significant difference, in relation to the presence of ascites after three months between groups (P = 0.21). In the intervention group, patients with ascites at the end of the time period had decreased scores on the social aspects SF-36 domain (P = 0.023) compared to those who had no ascites.

CONCLUSION: The proposed exercises provide an increase in the inspiratory muscle strength and improve functional capacity, consequently bettering the quality of life of liver disease patients.

Key words: Respiratory muscles; Pre-operative period; Electromyography; Muscle strength; Breathing exercises

Core tip: Studies on the effects of exercises, mainly those on breathing for liver transplant patients on the waiting list, are rare in the literature. This study proposes a manual of exercises for this group in order to increase muscle strength and improve their quality of life, as sarcopenia found in these patients contributes to a worsening of quality of life and is associated with mortality. The results are encouraging and may represent the beginning of further studies in the area and the establishment of exercise protocols for liver diseases.

Limongi V, Dos Santos DC, Oliveira da Silva AM, Boin IFSF, Stucchi RSB. Exercise manual for liver disease patients. *World J Transplant* 2016; 6(2): 429-436 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/429.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.429

INTRODUCTION

Liver transplantation means a chance of survival for individuals with advanced chronic liver diseases or acute liver failure when there are no clinical treatments available[1-3]. However, there is a disproportion between the supply of organs[4] and those in need of transplantation, increasing the time on the waiting list and the chance of complications[5] such as fatigue, decreased aerobic capacity, malnutrition, sarcopenia[6] and impaired ventilator mechanics due to ascites[7].

Sarcopenia may be associated with mortality in cirrhotic patients[8,9] and contributes to an impaired of quality of life in these patients[10-13].

Probably also due to loss of muscle mass, according to the authors Oliveira da Silva *et al*[7], and da Silva *et al*[14], the liver disease patients showed on average higher RMS of the diaphragm when compared to healthy subjects. This means that the respiratory muscles of patients with liver disease should try harder to gain the best resistance in the basal ventilation profile, in order for the electrical activity of the diaphragm to be higher.

Studies of Dharancy *et al*[15], Pieber *et al*[16] and Wiesinger *et al*[17], suggest that a change from the predominance of aerobic metabolism to anaerobic metabolism occurs early during exercise in individuals with cirrhosis compared to healthy subjects[8].

The findings in cardiopulmonary exercise testing, early termination of exercise with low peak VO_2 (oxygen consumption), hyperventilation precocious and reduced or unattainable ventilatory threshold[15,18] may correspond to a fatigue at the beginning of exercise or indicate deconditioning thus hampering the exercise. This reflects the difficulty that cirrhotic patients have to performing everyday activities, as well as feeling fatigue[17] even when they are hospitalized.

All these complications in the preoperative period, which also influence the recovery after transplantation, can be mitigated with well-defined and specific intervention programs for this group.

Therefore, the aim of the study was to increase inspiratory muscle strength and improve quality of life for liver disease patients with the proposed manual of breathing exercises.

MATERIALS AND METHODS

In this prospective, randomized and controlled trial, data collection was performed at the Unit of Liver Transplantation, Hospital de Clinicas, State University Campinas (Unicamp). The study protocol followed the Ethics Committee of the Medical Sciences Faculty, Unicamp, CEP: 922/2009. Each study participant signs the Informed consent statement.

Liver disease patients were included, men and women, aged over 18 years, with or without a diagnosis of cardiorespiratory disease and those with any Model for End-Stage Liver Disease (MELD) score obtained. All patients filled out a form for identification, age, gender and diagnosis of liver disease. The MELD and body mass index (BMI) were calculated. The presence or absence of ascites was identified in the first patient evaluation and after three months.

Exclusion criteria were: The inability to understand verbal commands, patients with poor general condition (for example, bed reset condition), the failure to perform the evaluations and acute liver failure diagnosis.

The study population was selected from the liver transplant waiting list from August 2012 to February 2014. From the 49 patients evaluated, 27 individuals were chosen through a random draw for participation in the control group. However, four patients were

Table 1 Demographic and baseline characteristics of the patients

Features	Control (n = 23)	Intervention (n = 14)	P
Male/female	18 (78.3%)/5 (21.7%)	11 (78.6%)/3 (21.4%)	1.00
Age (yr)	55.4 ± 9.9	55.8 ± 5.4	0.97
BMI (kg/m^2)	27.3 ± 4.5	28.6 ± 5.4	0.58
Diagnosis			
HCV	5 (21.7%)	4 (28.6%)	
HCC + HCV	4 (17.4%)		
Alcohol	3 (13%)	3 (21.4%)	
HCC	3 (13%)		
Alcohol + HCV	3 (13%)	2 (14.2%)	
Alcohol + HCC	1 (4.3%)		
Alcohol + HCV + HCC	1 (4.3%)	2 (14.2%)	
Autoimmune hepatitis	1 (4.3%)		
Polycystic liver disease	1 (4.3%)		
Cryptogenic cirrhosis		1 (7.1%)	
Sclerosing cholangitis		1 (7.1%)	
HBV + HCV		1 (7.1%)	

BMI: Body mass index; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma.

excluded; three died and one was submitted to a liver transplantation. Thus, 23 patients made up the control group. Twenty-two patients were randomly picked to take part in the intervention group, through a random draw. However, eight patients were excluded as three died, two had liver transplant operations and three individuals declined to perform the exercises. Thus, 14 patients constituted the intervention group. Software for randomization and allocation was not used; the names of the patients were placed in identical envelopes and drawn by the researcher, one by one, to make up the control group and intervention.

The control group was composed of 18 men (78.3%) and five women (21.7%) and the intervention group consisted of 11 men (78.6%) and three women (21.4%). Table 1 shows the demographic and baseline characteristics of the patients.

The respiratory pressures, maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) were measured using an analog manometer Gerarmed® (SP, Brazil), with unit scale in cmH_2O, coupled to a mouthpiece and nose clip, always with the patient seated. The data were always collected by the same researcher.

To measure the MIP was requested a maximum exhalation until residual volume and after, a maximal inspiratory effort. To measure the MEP a maximal inspiratory effort was asked for in order to achieve the level of total lung capacity, and then a maximum expiratory effort. The maneuvers were repeated three to five times at intervals of 30 s and it was considered the highest value obtained[19].

The surface electromyography EMG System of Brazil Ltda®, Series 00405, Model 210C (SP, Brazil) was used to obtain the electrical activity of the diaphragm and rectus abdominis, represented by the root mean square (RMS). Electrodes 3M Brazil® (Sumare, SP, Brazil) were used for the study of electrical activity in these muscles.

The electronic circuit acquisition captures and processes the signals, making them available to the EMG System of Brasil® software, it was installed on a computer Intelbras I21® (SP, Brazil).

The participants were positioned at 45° in order to study the electrical activity of the diaphragm. A passive electrode was adapted in the paraxiphoid position about 5 cm from the xiphoid process and another 16 cm from the right costal margin. To measure the rectus abdominis an electrode was adapted in the rectus abdominis muscle 5 cm away from the umbilicus and another about 15 cm along the involved muscle[7]. On the left hand side was positioned a ground electrode. Participants breathe normally while the electrical activity was recorded for ten seconds. A heavy breathing was requested every three seconds. For rectus abdominis was used 500 Hz of frequency and 500 μV of the sensitivity of signal amplitude[7]. For the diaphragm was used 300 Hz frequency and 300 μV of the sensitivity of signal amplitude[7].

Through EasyOne Diagnostic Espirometer World® (Zurich, Switzerland), it was possible to perform spirometry, following the Guidelines for Pulmonary Function Tests[20]. Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and forced expiratory flow rate at 25%-75% of FVC curve ($FEF_{25\%-75\%}$) were measured.

The "short form 36" (SF-36)[21] was used to evaluate the quality of life of the participants. The questionnaire consisted of 36 items related to eight domains covering different concepts of health, functional capacity, physical role, pain, general health, vitality, social aspects, emotional role and mental health.

Participants in the intervention group received a manual with illustrations and explanations to be held at home for three months and they received orientation from the therapist at the time of the delivery of the material. The first evaluation was made at this time; the second was made after three months. Figure 1 shows the prepared manual.

The therapist remained available for any questions

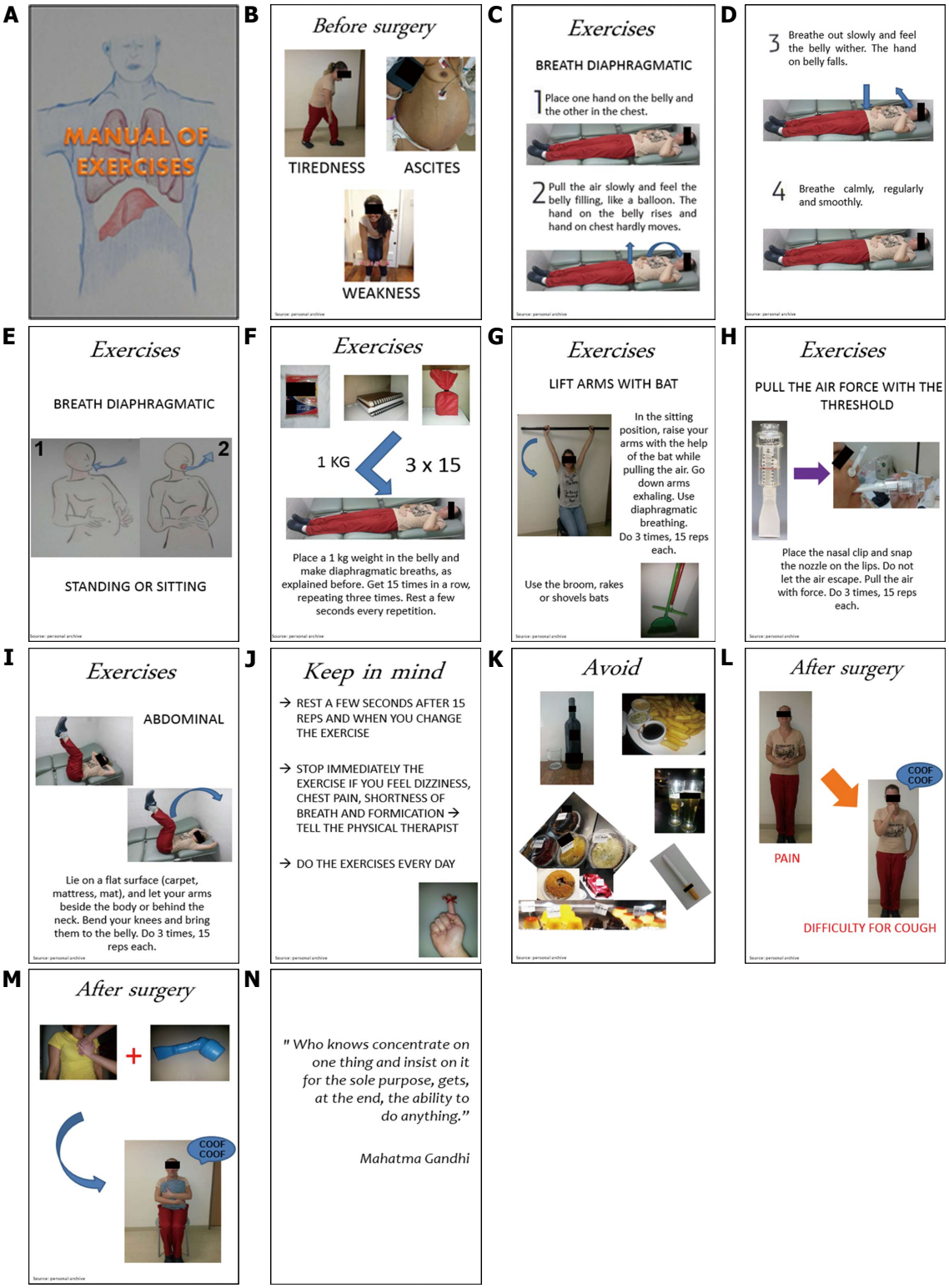

Figure 1 Manual of exercises for liver disease patients. A: Manual of exercises; B: Complications before the liver transplantation; C: How to do breath diaphragmatic; D: How to do breath diaphragmatic; E: Breath diaphragmatic standing or sitting; F: Breath diaphragmatic with weight on the belly; G: Lift warms with bat; H: Training with Threshold IMT®; I: How to do abdominal exercises; J: Instructions for patients; K: To avoid alcoholic beverages, tobacco, frying and pastries; L: Complications after liver transplantation; M: Physical therapy after surgery; N: Incentive phrase for patients.

and followed up these patients monthly by phone.

Patients were aware regarding diaphragmatic breathing and instructed to perform this breathing in all the exercises.

In addition to the diaphragmatic breathing, the exercises described in the manual were: Diaphragmatic isometric exercise with the patient in the supine position and 1kg of weight placed on the diaphragm muscle, exercise with Threshold inspiratory muscular training (IMT)® (Philips Respironics®), elevation of upper limbs

Table 2 Comparison between the control and intervention groups

	Control (n = 23)		Intervention (n = 14)		P
	Initial	Final	Initial	Final	
MIP (cmH_2O)	88.5 (44.1)	98.3 (39.2)	101.1 (34.4)	117.9 (43)	0.45
MEP (cmH_2O)	108.3 (46.3)	116.5 (51.8)	113.6 (31)	128.2 (35)	0.61
EMG rectus (μV)	52.9 (51.1)	46.1 (29.7)	32.5 (12.4)	28.8 (7.9)	0.65
EMG diaphragm (μV)	43.8 (14.9)	53.8 (22.4)	55.7 (34.7)	35.6 (15.8)	0.001[1]
FVC (%)	84.6 (13.9)	85.5 (16.1)	88.3 (14.5)	92.6 (14.2)	0.42
FEV_1 (%)	84.6 (15.1)	85.4 (14.5)	88.6 (20)	90 (14.1)	0.5
$FEF_{25\%-75\%}$ (%)	92.4 (31.2)	94.7 (24.6)	100.7 (47.1)	102.9 (44.2)	0.72
Funcional capacity	68.5 (24.5)	71.7 (21.7)	69.3 (21.4)	84.6 (14.5)	0.006[2]
Physical role	52.2 (39.1)	45.7 (38.9)	60.7 (38.9)	55.4 (38.2)	0.92
Pain	61 (32)	61.3 (21.1)	62 (27.9)	56.7 (30.1)	0.78
General health	52.8 (26.2)	58.4 (26.3)	59.3 (20.1)	68.4 (19.3)	0.4
Vitality	61.7 (23.9)	59.8 (23.2)	58.9 (15.2)	65 (25.2)	0.33
Social aspects	58.2 (34.9)	67.9 (29.9)	68.8 (37.3)	75.9 (30)	0.93
Emotional role	60.8 (39.8)	56.5 (44.3)	45.1 (44.5)	61.9 (36.6)	0.16
Mental health	59.1 (26.4)	64.5 (24)	64.9 (20.7)	78.3 (22)	0.14

[1]Difference between groups; [2]Difference in the intervention group after three months. MIP (cmH_2O): Maximal inspiratory pressure; MEP (cmH_2O): Maximal expiratory pressure; EMG: Electromyography; FVC: Forced vital capacity; FEV_1: Forced expiratory volume in one second; $FEF_{25\%-75\%}$: Forced expiratory flow rate at 25%-75%.

with the help of a bat and strengthening the abdominal muscles.

The manual contained information of the possible complications during the postoperative period. It was highlighted the importance of bronchial hygiene and the proper way to cough in the postoperative period. Patients were instructed to perform the exercises in three sets of fifteen repetitions.

The control group did not perform the exercises; the second evaluation was done three months after the first.

The patients' tolerance was a parameter for the choice of load for training with the Threshold IMT®, since it is already established in the literature that liver disease patients have fatigue[17,22,23] and interrupt the exercise early with low peak VO_2[15,18].

The manual also contained orientation for patients regarding the avoidance of alcohol, cigarettes, sweets and fried foods and had information about the importance of exercises during postoperative recovery from liver transplantation, especially concerning bronchial hygiene and effective cough.

Statistical analysis

The Statistical Analysis System (SAS) System for Windows (SAS Institute Inc, Cary, NC, United States), version 9.2 was used for statistical analysis.

Position and dispersion measures were used for numerical variables and frequency tables for categorical variables, for descriptive analysis.

For comparison of proportions, the χ^2 test or Fisher's exact test were used when necessary. For comparison of numerical measurements between two groups the exact Mann-Whitney test was used. For comparison of measurements between groups and times, ANOVA for repeated measurements was employed or post hoc transformation. To compare changes in proportions, the *McNemar* test was used. The significance level used was $P < 0.05$.

RESULTS

There was significant difference (P = 0.01) between the first (initial) and the third month (final) MIP in the control group and in the intervention group, but there was no difference (P = 0.45) between the groups.

After three months, the electromyography of the diaphragm represented by RMS decreased in the intervention group (P = 0.001) compared to that of the control group.

The score of the domain functional capacity (SF-36) was not statistically different between the groups; however, in the intervention group there was a significant increase in the score (P = 0.006) after three months.

The general health and mental health domains received higher scores after three months in the control group (P = 0.01) and the intervention group (P = 0.004), but there was no significant difference between them.

The descriptive analysis and comparison between groups are detailed in Table 2.

In the first evaluation, 10 patients had ascites in the control group and 3 had ascites in the intervention group. After three months, 13 patients had ascites in the control group and 5 patients had ascites in the intervention group.

The comparison between the presence of initial ascites with the presence of ascites was performed after three months in the control group (P = 0.083) and intervention group (P = 0.31). There was no significant difference in relation to the presence of ascites after three months between groups (P = 0.21).

The presence or absence of ascites three months after the first assessment was compared with age, BMI, MIP, MEP, RMS of the diaphragm and rectus abdominis,

FVC, FEV_1, $FEF_{25\%-75\%}$ and the SF-36 domains.

There was no significant difference between the variables in the control group. In the intervention group, patients with ascites at the end of the time period had decrease of scores on the social aspects domain (P = 0.023) compared to those who had no ascites.

DISCUSSION

Patients on the waiting list for liver transplantation waiting a long time for the new organ and consequently, there may be complications in this period, such as sarcopenia. Therefore, rehabilitation becomes an important alternative in order to reduce inactivity, increase muscle performance, as well as exercise tolerance, and to avoid complications in the post-operative period[24].

The results of this study showed that most patients were men, aged above 50 years and BMI revealing overweight. These findings are consistent with other studies[9,25].

Several authors[26,27] have recommended IMT in order to minimize respiratory muscle dysfunction in the postoperative period of cardiac, thoracic and abdominal surgery. Despite the literature employing a 40%[28] initial MIP load for IMT and increasing it over time training, patients' tolerance has been responsible for the choice of load for training with the Threshold IMT®, since it is already established in the literature that liver disease patients have fatigue[20-23].

In the current study, there was a significant increase of MIP in the intervention group after the final evaluation.

In the study of Gosselink *et al*[29], a meta-analysis was performed on the effects of IMT in patients with chronic obstructive pulmonary disease. The study revealed better results in the inspiratory muscle strength, functional capacity and dyspnea after strength training.

In the study by Serón *et al*[28], the Threshold IMT® was effective for strengthening inspiratory muscles.

One possible explanation for the non-significant increase in MEP in the present study is that the main focus of the prepared manual was to strengthen the inspiratory muscles. Unlike what was expected, the control group also showed a significant increase in MIP after three months. One possible explanation is that the patients were not discouraged from performing physical activities or were advised to stop exercising because they were participating in the research.

The intervention proved to be effective in this study; after three months there was a reduction of the RMS of the diaphragm in the intervention group, and due to the increase of the inspiratory muscle strength, the diaphragm needed to perform less force in order to overcome the same resistance. In other words, the action's potential decreased since only a small amount of fibers were needed to be recruited during normal breathing. No articles on the effects of inspiratory muscle training on electromyography of the diaphragm have been found; therefore, further studies are required for a broader discussion on the issue.

The exercise program also provided relevant improvement in functional capacity domain. This means that the difficulty in performing daily life activities decreased, and individuals became more active and willing. Regarding liver transplant, two authors[30,31] proved that the quality of life can be improved with physical exercises.

The general and mental health areas received higher scores after three months in the intervention and control groups, demonstrating that the patients' perception of their health improved. The control group may have presented positive changes in the mentioned aspects for the same reasons already explained above.

Also, one must consider, on average, an increase of some values of variables (FVC, FEV 1, FEF 25%-75%, vitality, social aspects and emotional role) at the end time, in the intervention group, showing the positive effects of the intervention performed.

The two groups were not ideally matched, because the incidence of ascites was lower in the intervention group in the first evaluation and after three months. However, the presence of ascites did not affect the respiratory variables evaluated. In the intervention group, patients with ascites had worse scores on the social aspects domain. In the final stage of cirrhosis, ascites causes the appearance of symptoms that can impair the performance of activities of daily living[32]. All these factors contribute to social isolation being away from work and low self-esteem. According to Saab *et al*[33], the ascites, associated or not with encephalopathy, was associated with poorer quality of life.

Certainly, new studies on the benefits of breathing exercises will be necessary after liver transplantation. However, the results of the present study are satisfactory regarding the improvement of quality of life as well as the electrical diaphragm activity result using the exercises learnt in the preoperative manual. This study is the beginning of exercise protocols developed specifically for this group, and it may prompt new research with a larger population sample.

ACKNOWLEDGMENTS

The authors also thank Philips Respironics® who kindly provided the Threshold IMT® for the study. Our acknowledgments to Stephen A Shaw for the English revision.

COMMENTS

Background

Some changes usually affect the quality of life of patients with chronic liver disease, such as fatigue, malnutrition and predominance of anaerobic metabolism. In order to improve the functionality, muscle strength and physical conditioning of the liver disease patients and physically prepare them for transplantation, minimizing possible postoperative complications, specific preoperative rehabilitation programs for this population become necessary.

Research frontiers

The liver transplant waiting list patients belong to the Unit of Liver Trans-

plantation at the Hospital de Clinicas/Unicamp, and are from several cities in the state of Sao Paulo, and other regions of Brazil. Therefore, it was difficult to weekly or even monthly require patients to participate in evaluations or in the respiratory intervention group at the Unit of Liver Transplantation. In addition, these patients are constantly doing exams, have difficult schedules, and often need help for locomotion. As a result, these patients were followed up by phone each month. Despite these hardships, in the current study, only three participants were excluded from the trial, since they declined to perform the exercises. The other patients satisfactorily agreed to the exercises. Due to lack of financial resources, the authors used the analog manometer and some Thresholds inspiratory muscular training (IMT)® were donated by Philips Respironics®. Each participant remained with the Threshold IMT® for three months. This contributed to the reduced sample in the intervention group, in addition to other factors, such as death, abandonment or the transplant itself.

Innovations and breakthroughs

An illustrative and explanatory manual was prepared with breathing exercises to be performed by patients at home, for a period of three months. Monthly, they were accompanied by the same researcher by telephone, and doubts were resolved.

Applications

The results found in the group that performed the exercises were encouraging; there was a decrease in the electrical activity of the diaphragm and increase some scores of the short form 36 domains. These results represent a start for new rehabilitation programs which are developed preoperatively. Still, the proposed manual in this article may be used in other studies, with extended samples, and further positive results may be found.

Terminology

The manual of the exercises was prepared by the researchers and consisted of breathing exercises, including the Threshold IMT®. The Threshold is a device designed for respiratory muscle training in which the load is independent of the air flow. It consists of a chamber where at the distal end there is a valve which is held closed by the positive pressure (graduated in cmH_2O) of a spring. If a negative pressure with an absolute value greater than the spring pressure is generated, the valve will open and allow the air passage.

Peer-review

Studies on the effects of exercises, mainly those on breathing, in liver disease awaiting transplantation are rare in the literature. This issue can spark interest in other researchers who want to study the manual exercises in an enlarged sample of liver disease patients and who also want to follow up these patients postoperatively, evaluating the effects of exercises in this period. Another possibility is to use this study as a basis for development of new specific exercise programs before surgery for this population.

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P- Reviewer: Bramhall S, Marino IR, Qin JM, Salvadori M, Sugawara Y
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Transplantation

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DOI: 10.5500/wjt.v6.i2.437

World J Transplant 2016 June 24; 6(2): 437-441
ISSN 2220-3230 (online)

CASE REPORT

Islet autotransplantation in a patient with hypercoagulable disorder

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Author contributions: Desai CS designed the research, performed research, gathered data, wrote paper; Khan KM wrote paper, did critical revision of manuscript, and contributed to ideas; Cui W gathered data, structured manuscript and help writing paper.

Institutional review board statement: This study is under IRB approved protocol (ID: 2015-1379) of Georgetown University.

Informed consent statement: This study is under IRB approved protocol (ID: 2015-1379) for de-identified patient information.

Conflict-of-interest statement: The authors have no potential conflict of interest.

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Received: February 3, 2016
Peer-review started: February 14, 2016
First decision: March 1, 2016
Revised: April 4, 2016
Accepted: April 21, 2016
Article in press: April 22, 2016
Published online: June 24, 2016

Abstract

Total pancreatectomy and islet auto transplantation is a good option for chronic pancreatitis patients who suffer from significant pain, poor quality of life, and the potential of type 3C diabetes and pancreatic cancer. Portal vein thrombosis is the most feared complication of the surgery and chances are increased if the patient has a hypercoagulable disorder. We present a challenging case of islet auto transplantation from our institution. A 29-year-old woman with plasminogen activator inhibitor-4G/4G variant and a clinical history of venous thrombosis was successfully managed with a precise peri- and post-operative anticoagulation protocol. In this paper we discuss the anti-coagulation protocol for safely and successfully caring out islet transplantation and associated risks and benefits.

Key words: Islet transplantation; Autoislet transplant; Pancreatectomy; Chronic pancreatitis; Hypercoagulable disorder; Heparin

Core tip: Total pancreatectomy and islet auto-transplantation is an option for select patients with chronic pancreatitis. Portal vein thrombosis is the most feared surgical complication and chances are increased if the patient has a hypercoagulable disorder. The paper describes important topics like the management of the anticoagulation in the peri-operative period.

Desai CS, Khan KM, Cui W. Islet autotransplantation in a patient with hypercoagulable disorder. *World J Transplant* 2016; 6(2): 437-441 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/437.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.437

INTRODUCTION

Patients with chronic pancreatitis suffer from significant pain and associated decrease in the quality of life and also a potential of forming type 3C diabetes and the pancreatic cancers[1-4]. It is an inflammatory disease, which is characterized by irreversible, morphological changes that cause permanent loss of function, and fibrosis and development of severe pain and complications. Over time, fibrosis in the pancreas, results in destruction of the islet cells, and patients are at risk of diabetes[1,3,4]. The risk of pancreatic cancer is 10 to 15 fold higher in chronic pancreatitis patients and if it is associated with hereditary pancreatitis with genetic mutations, then the lifetime risk is 75%[2,5]. Many surgical, medical, endoscopic and intervention radiological treatments are applied to these patients, despite which many still suffer from continuous dependence on narcotics and bad quality of life.

Removal of the pancreas followed by autologous islet cell transplantation is a great option for selected patients with chronic pancreatitis[6-14]. Islet auto transplantation helps to take care of 3 Ps that are necessary for this disorder: (1) Pain relief; (2) Prevention of the brittle diabetes mellitus; and (3) Prevention of pancreatic cancer[15]. At times, the results of the autologous islet cell transplantation are criticized because the variable insulin independence rate reported[16,17]. We have previously argued that the insulin independence is not the only marker of the success, the wide marker of the success would be euglycemia, preventing cancer and having better quality of life[15].

Good outcomes of islet auto transplantation are based on various factors from selection of the case to performing safe surgery, good isolation and safe injection of the cells followed by good engraftment of the islet cells. Once the islets are isolated and brought back to the patient, a small angiocatheter is introduced in one of the vessels either the splenic vein stump or any vessels draining into the superior mesenteric vein to infuse these cells into the portal vein so that they can flow to the liver. Safety is important in terms of decreasing the risk of thrombogenesis in these vessels by paying attention to the details of the procedure, the physiology of the patient, and the liver pathology[18]. Surgical complications are most dreaded compared to the long-term outcome and insulin dependency because they can add to significant morbidity and therefore poor quality of life to the patient. Porto-venous thrombosis would arguably be the most important complication. It can vary in magnitude from a segmental vein to thrombosis of the main portal vein and potentially complete thrombosis of the superior mesenteric access requiring a bowel resection and consequent problems[19,20]. The risk of portal vein thrombosis will be increased if the patient has a hypercoagulable disorder.

We report a case from our new program with physiological challenge in the context of issues described. These include a case of islet autotransplantation performed in a patient with a hypercoagulable disorder. To our knowledge, it is the first such case in the literature.

CASE REPORT

The patient was a 29-year-old lady (body weight 83 kg, body mass index 29.3 kg/m^2) with a history of chronic abdominal pain related to chronic pancreatitis. At the time of her initial visit she was in the emergency room or hospitalized on a weekly basis. Her history dated back 13 years and she had been on narcotics for 6 years. She had undergone 7 endoscopic retrograde cholangiopancreatographys over the years and magnetic resonance imaging (MRI) had shown pancreas divisum. Our own MRI scoring system[21] indicated minimal pancreatic damage (atrophy, 1/6). The pre-operative C-peptide was 1.75 ng/mL and hemoglobin A1c was 5.5%. We also considered gall stone disease, alcohol and completed a genetic analysis for common hereditary gene mutations that are causally associated with chronic pancreatitis. She had also reported having developed thrombosis related to PICC line placement on multiple occasions at an outside institution. During her evaluation we obtained hypercoagulability studies, which included factor V Leiden mutation, prothrombin gene mutation, plasminogen activator inhibitor-1 *(PAI-1*) gene mutation and level, clotting factor Ⅶ, protein C, protein S levels, methylenetetrahydrofolate reductase *(MTHFR*) gene mutations and an autoimmune thrombophilia screen. She was found to be homozygous for the 4G variant of the *PAI-1* gene and heterozygote for the *MTHFR A1298C*.

Her surgery was performed using the technique described earlier[22] and islet infusion was also done through splenic vein stump. Islet preparation was performed at the current good manufacturing practice facility in the Islet Cell Laboratory at the Georgetown University Hospital.

The pancreas was explanted post 1 and half min of warm ischemia time and placed immediately into an ice-cold Viaspan solution in a sterile container and delivered to the lab on ice. On arrival of the lab, the pancreatic duct was cannulated after trimming. The pancreas was then divided into two portions at the neck. On the cut surface both openings of the pancreatic duct were cannulated with a 14-gauge cannula. An enzyme solution containing collagenase HA and Thermolysin (Vitacyte, Indiana, United States) was infused into the pancreas through the cannula and connected with a 60 cc syringe through an extension tube. In addition, the parenchyma was then repeatedly injected with the enzyme solution using a 60 cc syringe. The thoroughly distended pancreas was then digested using the semi-automated method of Ricordi[23]. The pancreas weighed 65.9 g. The total cold ischemia time from removal of the pancreas to completion of trimming was 51 min. The digestion rate was 92.2% post 18 min of digestion. After purification using a modified continuous density gradient method with cell processor COBE2991[24], the

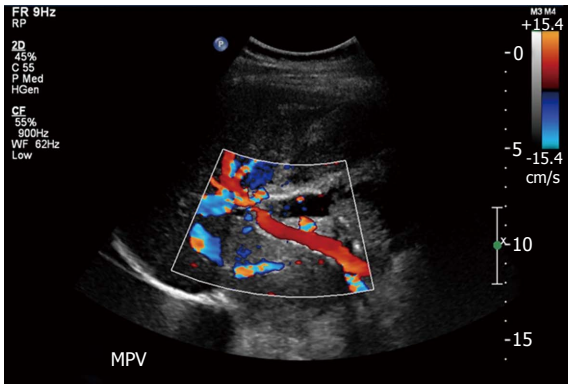

Figure 1 Post-operative Doppler ultrasound of the liver demonstrating widely patent portal vein with normal direction of blood flow. MPV: Main portal vein.

final pellet was reduced from 36 to 12 mL[25]. The total islet yield was 459164 islet equivalents (IEQ) which was quantified as IEQ by normalizing the islet mass to an islet size of 150 μm diameter. The islet recovery was 7552 IEQ/g of pancreas tissue. The final pellet was suspended in the transplantation media (5% human serum albumin) containing 35 units of Heparin per kilogram of patient body weight. In total, 5532 IEQ per kilogram recipient body weight (IEQ/kg) of islets were available.

The islet infusion in to the liver involved a venous catheter placed in a splenic vein stump and advanced intravenously towards the portal vein. In order to reduce complication rates of acute portal hypertension and thrombosis in this case at the most, low-volume (12 mL pellet) prepared through purification procedure, was infused. We gave the patient 35 U/kg intravenously in addition to the 35 U/kg of Heparin along with islet infusion; the patient therefore received a total dose of 70 U/kg of heparin. Portal pressures were closely monitored during infusion, because of an established tenfold (1.52%-15.2%) increase in the risk of thrombosis with portal pressure changes above 25 cm H_2O[25]. The pre infusion portal pressure was 4.5 cm/saline and the post infusion pressure was 15 cm/saline.

Heparin was started intra-operatively. Fifty IU/kg of body weight bolus before the infusion of islet cells followed by 25000 IU mixed with 500 mL of D5 1/2 normal saline at the rate of 10 IU/kg per hour. Postoperatively, the patient was continued on a heparin drip according to our protocol and activated thromboplastin time was maintained in the range of 50 to 60 s. At the end of three days when she started on clear liquid diet, we continued the patient on low molecular weight heparin and monitored with anti-Xa activity factors maintained between 0.6 to 1 international units/mL. Postoperative Doppler ultrasound of the liver was performed on day 1, 2 and 5 and once weekly for one month and biweekly for another two months. Specifically, the doppler studies during the first week demonstrated patency and normal flow in the portal veins, hepatic arteries and veins; the main portal vein peak velocities ranged between 25-38 cm/s, left and right portal vein velocities ranged from 11-27 cm/s (Figure 1). The patient was discharge home after 14 d. At three months the patient was off insulin with a C-peptide of 1.95 ng/mL. At the end of three months, the dose of low molecular weight heparin was reduced to maintain anti-Xa level between 0.3 to 0.6 international units/mL. Six months after the surgery, the low molecular weight heparin was discontinued after consultation with hematology. The patient did not develop venous thrombosis of any form during follow-up and was able to resume a normal life.

DISCUSSION

Total pancreatectomy and islet auto transplantation has been described by some as a radical option though it has a clear role for patients with chronic pancreatitis. Patients undergo multiple endoscopic procedures and fail to get a satisfactory outcome and all the time their narcotic requirement keeps escalating. This definitive procedure is feared because of surgical complications like portal vein thrombosis and also the failure of the islets to prevent diabetes.

Hypercoagulability is a significant risk factor for portal vein thrombosis. In one study 28% of patients with portal vein thrombosis had an inherited thrombophilic disorder[26]. Of this factor Ⅴ Leiden mutation was the most common (11%) followed by anti-thrombin Ⅲ deficiency (11%) and protein c deficiency (8%). Prothrombin gene mutations are also commonly implicated in venous thrombosis[27]. The PAI 4G variant and MTHFR mutations are considered less severe though do have an increased risk for venous thrombosis after major surgery including transplantation. Such situations are challenging because of the post-operative risk of thrombosis leading to graft failure or bleeding from anticoagulation. However, many such transplants are carried out in a safe manner. Our patient had a *PAI-1* gene mutation, which was only diagnosed after diligent history taking helped us to obtain the risk in this case. The authors have previously worked at different auto islet cell transplantation centers and as with other surgeries it was not routine to do a hypercoagulable workup since obtaining this panel in every patient is very expensive and may not be cost effective[15,18,22].

Portal vein thrombosis after islet auto-transplant though uncommon, can be risky and life threatening. There are few previous individual reports of portal vein thrombosis after islet auto-transplantation[20] and one series that indicated a prevalence of 3.7% after clinical islet transplantation[28]. There is however no systematic study of the cause of thrombosis in such cases. In a previous publication we have noted that there may be unrecognized mild fibrosis and or steatosis[18]. We were however unable to show that any specific histologic pattern was more susceptible to venous thrombus formation. To prevent portal venous thrombosis in patients such as ours above with pre-existing risk factors it is imperative to identify at risk patients and manage these

patients with therapeutic anticoagulation with heparin. Heparin also has advantage in the islet engraftment process and hence it has dual advantage, but has a significant risk of post-operative bleeding and hence it is very important that the surgery is performed with good hemostasis. Heparin is given by almost all the centers performing auto-islet cell transplant to their patients. However, there are no consensus guidelines on the amount and duration it needs to given. We adapted an approach in which we start a heparin drip in operating room at the time of starting islet infusion after giving bolus. It is continued for the next three days maintaining the activated thromboplastin time in the range of 50 to 60 s. At the end of three days when the patient starts taking clears, we continue with low molecular weight heparin two times a day dose based on patient's weight with anti-Xa activity factors maintained between 0.6 to 1 international units/mL. Patient's postoperative Doppler ultrasound on the liver is done on postoperative day 1, 2 and 5 and subsequently was done once weekly for one month and then twice weekly for another two months if they are at high risk. High risk is defined by three main factor: (1) hypercoagulable disorder; (2) previous history of deep venous thrombosis other than segmental splenic vein thrombosis related to chronic pancreatitis (even if the hypercoagulable panel is normal); and (3) high portal pressure after infusion (more than 25 cm of saline). If the patient is high risk then at the end of three months, low molecular weight heparin dose is reduced to maintain anti-Xa level to be between 0.3 to 0.6 international units/mL. Six months after the surgery, the low molecular weight heparin is discontinued after consultation with hematology. If the patient is not at high risk then after two weeks dose is reduced and then stopped after another two weeks.

In summary, islet auto transplantation in itself is a challenging procedure and even more challenges can arise medically if there are physiological challenges like a hypercoagulable disorder. Despite all these challenges with careful teamwork and experience, these patients can be safely managed.

Islet auto transplantation is a challenging procedure and even more challenges can arise medically; if there are physiological challenges like a hypercoagulable disorder. Despite all these challenges with careful teamwork and experience, these patients can be safely managed.

COMMENTS

Case characteristics

Total pancreatectomy and islet autotransplantation complicated by primary hypercoagulability that presented as repeated thrombosis of indwelling venous lines.

Clinical diagnosis

The presentation was characterized by symptoms of chronic pancreatitis and a history of deep venous thrombosis.

Differential diagnosis

An alternative explanation to a primary hypercoagulability to account for thrombosis if intravenous lines would be that the presence of intravenous lines themselves was the cause of catheter thrombosis.

Laboratory findings

Screening for hypercoagulability included plasma proteins, genetic defects and autoimmunity as potential causes of thrombosis with the patient having a plasminogen activator inhibitor-1 variant.

Imaging diagnosis

Serial ultrasounds were used to monitor for portal vein thrombosis after islet infusion in to the portal vein after total pancreatectomy.

Pathological diagnosis

Confirmation of chronic pancreatitis as the cause for abdominal pain.

Treatment

Heparin infusion followed by low molecular weight heparin and aspirin as prophylaxis for a prothrombotic state.

Related reports

There are previous cases of a hypercoagulability giving rise to deep venous thrombosis, most notably with factor V Leiden mutation.

Term explanation

Hypercoagulability refers to a pathological increase in the tendency to form intravascular clots. Patients undergoing major intraabdominal operations should be screened for a hypercoagulable state if there is any history of abnormal venous clot formation.

Peer-review

This a successful case of islet autotransplantation performed in a chronic pancreatitis patient suffered from significant pain with a hypercoagulable disorder. It is imperative to identify at risk patients and manage these patients with therapeutic anticoagulation with heparin to prevent portal venous thrombosis in patients with pre-existing risk factors. The author's careful teamwork and experience is helpful for safely managing these patients.

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P- Reviewer: Fu D, Kin T, Kleeff J **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Transplantation

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5500/wjt.v6.i2.442

World J Transplant 2016 June 24; 6(2): 442-446
ISSN 2220-3230 (online)

CASE REPORT

Acute bacterial sternoclavicular osteomyelitis in a long-term renal transplant recipient

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Author contributions: All authors contributed to the acquisition of data, writing, and revision of this manuscript.

Institutional review board statement: This case report was exempt from the Institutional Scientific Committee standards at University Hospital of Ioannina, Ioannina, Greece.

Informed consent statement: The patient involved in this study gave his written informed consent authorizing use and disclosure of his protected health information.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

Manuscript source: Invited manuscript

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Received: March 29, 2016
Peer-review started: March 29, 2016
First decision: April 15, 2016
Revised: May 2, 2016
Accepted: May 17, 2016
Article in press: May 27, 2016
Published online: June 24, 2016

Abstract

Kidney transplantation is the treatment of choice for a significant number of patients with end-stage renal disease. Although immunosuppression therapy improves graft and patient's survival, it is a major risk factor for infection following kidney transplantation altering clinical manifestations of the infectious diseases and complicating both the diagnosis and management of renal transplant recipients (RTRs). Existing literature is very limited regarding osteomyelitis in RTRs. Sternoclavicular osteomyelitis is rare and has been mainly reported after contiguous spread of infection or direct traumatic seeding of the bacteria. We present an interesting case of acute, bacterial sternoclavicular osteomyelitis in a long-term RTR. Blood cultures were positive for *Streptococcus mitis*, while the portal entry site was not identified. Magnetic resonance imaging of the sternoclavicluar region and a three-phase bone scan were positive for sternoclavicular osteomyelitis. Eventually, the patient was successfully treated with Daptomycin as monotherapy. In the presence of immunosuppression, the transplant physician should always remain alert for opportunistic pathogens or unusual location of osteomyelitis.

Key words: Bacterial infections; Immunosuppression; Renal transplantation; Osteomyelitis

Core tip: Although immunosuppression therapy improves kidney allograft and patient's survival, it is a major risk factor for infection following kidney transplantation, altering the clinical manifestations of the infectious diseases and complicating both the diagnosis and management of renal transplant recipients (RTRs). Existing literature regarding osteomyelitis in RTRs is very limited while sternoclavicular osteomyelitis is a rare entity presenting with its own unique set of risk factors and complications. Infections caused by unconventional

pathogens with unconventional infection sites are being increasingly diagnosed in RTRs and the physician should always remain alert when dealing with these patients.

Dounousi E, Duni A, Xiromeriti S, Pappas C, Siamopoulos KC. Acute bacterial sternoclavicular osteomyelitis in a long-term renal transplant recipient. *World J Transplant* 2016; 6(2): 442-446 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/442.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.442

INTRODUCTION

Kidney transplantation is the treatment of choice for a significant number of patients with end-stage renal disease. Renal transplant recipients (RTRs) benefit from a longer life expectancy and a better quality of life. Despite, recent accomplishments in the field of kidney transplantation, both short- and long-term medical complications still exist. Infectious diseases constitute one of the most common complications after kidney transplantation and the second most common cause of death among RTRs with a functioning graft[1]. Even though immunosuppressive therapy improves graft and patient survival, it has been reported that the increasing load of maintenance immunosuppression predisposes RTRs to clinically important infectious sequelae. The plethora, diversity and consequences of infectious complications in kidney transplantation have led to the accumulation of a growing amount of evidence describing the problem and trying at the same time to establish guidance for optimal management and support of these patients[1].

Existing literature comprises of a very small number of cases reporting osteomyelitis in RTRs. Traditional risk factors for osteomyelitis include trauma to the bone and trauma near a site of infection, the presence of sickle-cell disease, rheumatoid arthritis, diabetes mellitus, dialysis and related procedures, as well as immunosuppression. Most cases of osteomyelitis in adults are of hematogenous origin and primarily affect the spine[2]. The sternoclavicular joint is less commonly associated with osteomyelitis but presents its own unique set of risk factors and complications. We present a rare case of an adult long-term RTR who was diagnosed with acute, hematogenus sternoclavicular osteomyelitis due to streptococcus bacteremia whereas remarkably a portal entry site was not identified.

CASE REPORT

A 50-year-old male RTR, presented at the emergency department of our Tertiary University Hospital complaining about fever, chills and pain over the left sternoclavicular area, radiating to the shoulder and neck for the last two days. He denied any recent history of trauma, intravenous drug administration or dental procedure. Physical examination revealed pyrexia and marked tenderness over the left sternoclavicular area which appeared warm, red and swollen. Laboratory exams showed an elevated white blood cell count and C-reactive protein (Table 1), while the cervical spine and chest X-rays were unremarkable. The patient was directly admitted to the Renal Unit Ward and serial blood cultures were taken.

The patient was a long-term RTR regularly followed up at the renal transplant outpatient clinic (OC) of our Hospital during the last year. On his last visit a month ago, he was asymptomatic with unremarkable clinical findings and stable renal function, with an estimated glomerular filtration rate (eGFR) of 41 mL/min per 1.73 m^2 (Modification of Diet in Renal Disease equation). Maintenance immunosuppression therapy included cyclosporine (75 mg bid, C2 levels of 436 ng/mL), mycophenolate mofetil (1 g bid) as well as prednisolone (5 mg qd). The patients was diagnosed with chronic kidney disease of unknown etiology more than twenty years ago, was treated with hemodialysis for approximately 7 years and subsequently received a renal allograft from a cadaveric donor 14 years ago. Three months after the transplantation, the patient had suffered an acute rejection episode, which was successfully treated with intravenous pulses of steroids. The rest current medical history included well controlled arterial hypertension (antihypertensive treatment: Amlodipine 10 mg qd) and hip osteopenia diagnosed by a Dual-energy X-ray absorptiometry scan (DEXA) (Alfacalcidol 0.25 μg qd).

Immediately after admission, imaging of the sternoclavicular area excluded the presence of a fluid collection that could be aspirated. Considering the patient's clinical findings and his long-term immunocompromised status, empirical treatment for septic arthritis with Vancomycin (dose adjusted to eGFR) and Ciprofloxacin was commenced. Further diagnostic workup included a dental examination which did not reveal a possible portal entry site for the bacteria. Abdominal ultrasound findings were unremarkable while ultrasound of the renal allograft was within normal. Urine cultures were negative. Transthoracic echocardiography revealed mild mitral regurgitation and calcifications of the aortic cusps and mitral annulus. A transesophageal ultrasound was subsequently performed, ruling out concomitant endocarditis.

All blood cultures became positive within 48 h for *Streptococcus mitis* (Viridans group streptococcus) with a good sensitivity profile, including Glycopeptides (Vancomycin minimum inhibitory concentration < 1 mg/L) and Daptomycin. Vancomycin treatment, targeting trough blood levels of 15-20 mg/L, was continued whereas Ciprofloxacin was stopped. In order to further evaluate the sternoclavicular joint and differentiate between septic arthritis and osteomyelitis, magnetic resonance imaging (MRI) (no gadolinium administration) of the region was performed. The MRI showed bone edema of the left intraarticular surface of the sternum and the clavicle together with soft tissue

Table 1 Patient's laboratory findings at admission and on discharge

	At admission	On discharge
Hemoglobin (g/dL)	13.3	11.3
WBC (/μL)	11760	11900
Neutro-Lympho-Mono (%)	83-6-10	83-7-7
PLT (/μL)	163000	290000
ESR (mm/h)	41	15
CRP (mg/L)	130	17
eGFR (mL/min per 1.73 m^2)	36	40
Urea (mg/dL)	86	76
Sodium (mEq/L)	136	139
Potassium (mEq/L)	4.2	4.3
PTH (pg/mL)	75	80
Phosphate (mg/dL)	3.9	2.7
Albumin (g/L)	3.4	3.4

WBC: White blood count; PLT: Platelets; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; eGFR: Estimate glomerular filtration rate (calculated by CKD-EPI formula); PTH: Parathyroid hormone.

edema, findings suggestive of acute sternoclavicular osteomyelitis (Figure 1). No intraarticular fluid collection was observed. A three-phase whole body bone scan (technetium-99m methylene diphosphonate) was subsequently performed, which showed focally intense, increased activity over the left sternoclavicular area, a finding positive for osteomyelitis (Figure 2).

A week from admission the patient continued to have low grade fever and was dependent on analgesics for pain control, despite achieving adequate Vancomycin trough levels. Considering the diagnosis of acute bacterial osteomyelitis with an unconventional location, the patient's clinical course, the need of long-term intravenous antibiotic treatment, the difficulties of Vancomycin treatment (monitoring levels and possible related nephrotoxicity) and practical issues (patient's residence was far from the hospital), the decision of switching antimicrobial treatment to Daptomycin as monotherapy (dose 4 mg/kg per 24 h) was taken. The patient became afebrile within a few days, inflammatory markers gradually declined and his physical status progressively improved (Table 1). No surgical debridement was performed as there was no evidence of a soft tissue abscess or subperiosteal collection, and no concomitant joint infection was diagnosed. The patient was discharged from the hospital a fortnight after admission, with recommendations for continuation of antimicrobial treatment for a total period of 6 wk and close medical follow-up at the renal transplant OC.

The patient remains asymptomatic and with preserved renal function six months after the completion of the antimicrobial treatment.

DISCUSSION

In the modern era of renal transplantation infectious diseases remain a major cause of morbidity and mortality in RTRs[1]. The introduction of new immunosuppressant agents in renal transplantation along with the increasing

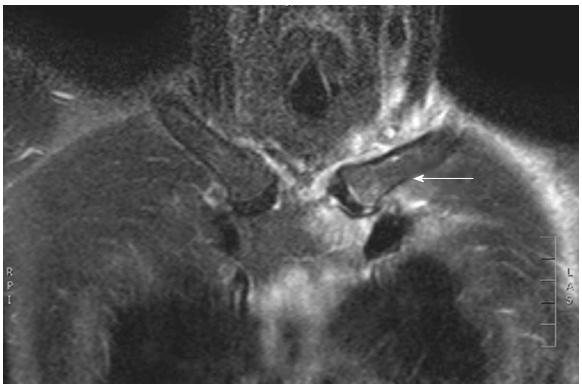

Figure 1 Magnetic resonance imaging of the sternoclavicular area showing edema on the left intraarticular surface of the sternum and the clavicle together with edema of the surroundings soft tissues (arrow).

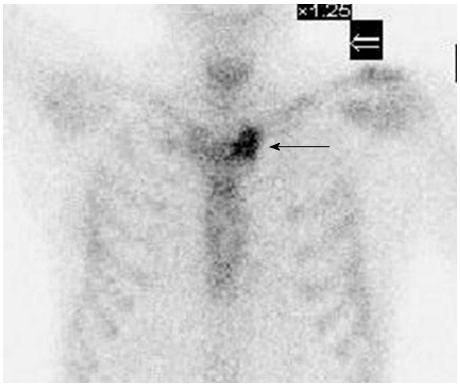

Figure 2 Three-phase bone scan (technetium-99m methylene diphosphonate) showing intense increased focal activity uptake over the left sternoclavicular area (arrow).

resistance of pathogens to antimicrobial agents worldwide are partially responsible for the emergence of rare infectious clinical cases which constitute a major challenge for the transplant clinicians. Here, we report an interesting, noteworthy case of acute bacterial sternoclavicular osteomyelitis in a long-term adult RTR, with no portal entry site for the bacteria which was successfully treated with Daptomycin as monotherapy.

In general, traditional risk factors for osteomyelitis include trauma to the bone and trauma near a site of infection, the presence of sickle-cell disease, rheumatoid arthritis, diabetes mellitus, dialysis and related procedures, as well as immunosuppression. Most cases of osteomyelitis in adults are of hematogenous origin and primarily affect the spine[2]. The clavicle contains scanty red marrow and sparse vascular supply. It is an exceedingly rare site for osteomyelitis, especially of hematogenous origin[3]. Clavicular osteomyelitis is rare and has been mainly reported after contiguous spread of infection or direct traumatic seeding of the bacteria[4]. Thus, there are reports in the literature of sternoclavicular osteomyelitis following central line placement[5], major head and neck surgery and radiation therapy to head and neck tumors[6]. Intravenous drug abusers are an especially high risk group for clavicular

osteomyelitis and septic arthritis[7].

With regard to the responsible pathogens, *S. aureus* is the most commonly isolated organism in most types of osteomyelitis, affecting 50%-70% of cases, while other gram positive cocci and gram negative bacilli are identified less often, accounting for approximately 20%-25% of acute osteomyelitis cases respectively[8,9]. Treatment of osteomyelitis requires prolonged antimicrobial therapy and frequently adjunctive surgical therapy for the debridement of necrotic material in order to eradicate the infection. Antibiotic therapy should be adjusted to culture and susceptibility results. If culture results are not obtainable, broad spectrum empiric therapy, including Vancomycin together with an agent with activity against gram negative organisms, should be administered[10-12].

Regarding selection of antimicrobial treatment in our patient, Daptomycin was finally chosen as it has exhibited activity in the treatment of gram positive bone and joint infections[13]. It is rapidly bactericidal and appears effective against multidrug-resistant gram positive pathogens, commonly found in osteomyelitis and joint infections, even when other first-line antibacterial treatments have failed[14-16]. Daptomycin is well tolerated; it has a relatively safe side effect profile, no interactions with calcineurin inhibitors, and a low risk of spontaneous resistance. The mode of action, rapid *in vitro* bactericidal activity against growing and stationary-phase bacteria, a once-daily dosing regimen, and no requirement for drug monitoring contribute to its potential therapeutic utility[17].

Existing literature comprises of a very small number of cases reporting osteomyelitis in RTRs, which involve locations such as the ankle, the symphysis pubis or the vertebral column[13,18-21], whereas there are no reports in the literature regarding sternoclavicular osteomyelitis in RTRs. The additive effect of long-term immunosuppresion treatment and possibly osteopenia (although previous routine DEXA scans revealed only hip localized osteopenia) rendered our patient among patients' subgroups with increased risk for osteomyelitis. Remarkably, a portal entry site for the bacteremia was not identified. Finally, the sternoclavicular bone was the solitary site of infection as demonstrated from the imaging studies.

Considering the immune suppressed status as a predisposing factor for infections as well as the growing number of RTRs, we might come across more cases of unconventional pathogens and sites of infection in the future[22]. Prevention, vigilance and deep knowledge of the diagnostic and therapeutic management of infections could potentially mitigate the consequences for RTRs.

COMMENTS

Case characteristics

A 50-year-old male renal transplant recipient complained about fever, chills and pain over the left sternoclavicular area, radiating to the shoulder and neck for the last two days.

Clinical diagnosis

Physical examination revealed pyrexia and marked tenderness over the left sternoclavicular area which appeared warm, red and swollen.

Differential diagnosis

Differential diagnosis was between septic arthritis and osteomyelitis.

Laboratory diagnosis

Laboratory exams showed an elevated white blood cell count and C-reactive protein and all blood cultures became positive within 48 h for *Streptococcus mitis*.

Imaging diagnosis

Magnetic resonance imaging (no gadolinium administration) of the region showed bone edema of the left intraarticular surface of the sternum and the clavicle together with soft tissue edema, without intraarticular fluid collection and a three-phase whole body bone scan [technetium-99m methylene diphosphonate (^{99}Tc-MDP)] showed focally intense, increased activity over the left sternoclavicular area, findings positive for osteomyelitis.

Treatment

Empirical treatment for septic arthritis with Vancomycin and Ciprofloxacin was commenced and subsequently switched to treatment with Daptomycin as monotherapy.

Related reports

Clavicular osteomyelitis is rare and has been mainly reported after contiguous spread of infection or direct traumatic seeding of the bacteria as occurs following central line placement, major head and neck surgery and radiation therapy to head and neck tumors.

Term explanation

A three-phase whole body bone scan is a ^{99}Tc-MDP based diagnostic test is used in nuclear medicine in order to detect different types of pathology in the bones. The three phases are the flow phase, the blood pool image and the delayed phase. Differential diagnosis is based on differential image processing from the three phases; Calcineurin inhibitors: Cyclosporine and Tacrolimus - are a class of immunosuppressive drugs which are used as first line agents for maintenance therapy after kidney transplantation.

Experiences and lessons

Considering the immune suppressed status as a predisposing factor for infections as well as the growing number of renal transplant recipients, the authors might come across more cases of unconventional pathogens and sites of infection in the future.

Peer-review

The authors describe a very unusual complication occurring late after renal transplantation. The case report is well written and useful for the reader just bacause of the unusual complication.

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P- Reviewer: Guerado E, Mu JS, Shrestha BM, Salvadori M
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Transplantation

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5500/wjt.v6.i2.447

World J Transplant 2016 June 24; 6(2): 447-450
ISSN 2220-3230 (online)

CASE REPORT

Cavitary lung lesion 6 years after renal transplantation

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Author contributions: All authors contributed to the management of the patient, conceptualizing, writing and revising the manuscript.

Institutional review board statement: The case report was exempted from ethics approval by the Institute Ethics Committee, AIIMS.

Informed consent statement: The patient involved in this study gave his informed consent authorizing use and disclosure of his anonymized health information.

Conflict-of-interest statement: All authors have no conflicts of interests to declare.

Manuscript source: Unsolicited manuscript

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Received: January 13, 2016
Peer-review started: January 15, 2016
First decision: March 1, 2016
Revised: March 29, 2016
Accepted: May 10, 2016
Article in press: May 11, 2016
Published online: June 24, 2016

Abstract

The differential diagnoses of a cavitary lung lesion in renal transplant recipients would include infection, malignancy and less commonly inflammatory diseases. Bacterial infection, Tuberculosis, Nocardiosis, fungal infections like Aspergillosis and Cryptococcosis need to be considered in these patients. Pulmonary cryptococcosis usually presents 16-21 mo after transplantation, more frequently in patients who have a high level of cumulative immunosuppression. Here we discuss an interesting patient who never received any induction/anti-rejection therapy but developed both BK virus nephropathy as well as severe pulmonary Cryptococcal infection after remaining stable for 6 years after transplantation. This case highlights the risk of serious opportunistic infections even in apparently low immunologic risk transplant recipients many years after transplantation.

Key words: Lung cavity; Immunosuppression; Renal transplantation

Core tip: Here we discuss an interesting patient who never received any induction/anti-rejection therapy but developed both BK virus nephropathy as well as severe pulmonary Cryptococcal infection after remaining stable for 6 years after transplantation. This case highlights the risk of serious opportunistic infections even in apparently low immunologic risk transplant recipients many years after transplantation.

Subbiah AK, Arava S, Bagchi S, Madan K, Das CJ, Agarwal SK. Cavitary lung lesion 6 years after renal transplantation. *World J Transplant* 2016; 6(2): 447-450 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i2/447.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i2.447

INTRODUCTION

Fungal infections causing cavitary lung lesions usually manifest in transplant recipients who have received a high level of cumulative immunosuppression. We describe an unusual case, where a low risk transplant recipient who had been stable for 6 years developed severe pulmonary Cryptococcal disease and BK virus nephropathy.

CASE REPORT

A 40-year-old Indian man was admitted with low grade fever and dry cough for one month. He had end stage renal disease due to unclassified primary disease and had a live related renal transplantation with his sister as the donor in 2009. He was detected hepatitis B surface antigen (HBsAg) positive before transplantation and has been on Tenofovir since then. He received no induction and was initially maintained on Tacrolimus, Mycophenolate Mofetil (MMF) and Steroids. After a year, MMF was changed to Azathioprine due to financial constraints. He received Trimethoprim-Sulfamethoxazole for 6 mo after transplantation but no primary prophylaxis for Cytomegalovirus (CMV), Tuberculosis (TB) or fungal infection. His postoperative course was uneventful and he maintained serum creatinine of 1.1-1.2 mg/dL. He is a non smoker.

Clinically, the patient was febrile, hemodynamically stable and hypoxemic (SPO_2 92% on room air) requiring oxygen by mask. Investigations revealed pancytopenia (Hb 7.4 g/dL, total leucocyte count -3400/cu mm, platelet count -87000/cu mm) and high serum creatinine (2.5 mg%). Azathioprine was stopped. Tacrolimus trough level was 3.7 ng/mL. Urinalysis was unremarkable. Graft biopsy showed BK virus (BKV) nephropathy and serum BKV plasma load was more than 10^4 copies/mL.

He was started empirically on broad spectrum antibiotics. Blood and urine cultures and quantitative CMV PCR assay were non-contributory. A non-contrast CT thorax showed bilateral, multiple, diffuse centrilobular and peribronchovascular cavitating nodules coalescing to form areas of consolidation with a larger cavity in apico posterior segment of upper lobe of left lung (Figure 1). Bronchoscopy with bronchoalveolar lavage (BAL) fluid cultures was unrevealing. Serum Cryptococcal antigen was negative. Serum and BAL fluid galactomannan were negative.

Since patient continued to be febrile, computed tomography guided biopsy of the cavitary lesion in the left lung was done and the histopathology (Figure 2) showed Cryptococcal infection. He was treated with liposomal Amphotericin for 6 wk and given Fluconazole prophylaxis. Flucytosine was not available at that time. Patient showed clinical as well as radiologic improvement and was discharged on oral fluconazole. His pulmonary infection has subsequently recurred and now he is being treated with a combination of Amphotericin and Flucytosine.

DISCUSSION

A renal transplant recipient may present with a cavitary lung lesion due to infection, malignancy (post-transplant lymphoproliferative disorder) or inflammatory disease, though infections are the predominant causative factor[1-3]. TB is the commonest cause of cavitary lung lesions in endemic areas like India and patients may receive empiric anti-TB therapy if the index of suspicion for rarer infections is not high and investigations are non-contributory. Aspergillosis (either angioinvasive or chronic necrotizing form) is the most common fungal infection associated with cavitation. Other causes are Nocardiosis, Cryptococcosis, Actinomycosis and rarely Legionella pneumophila. In a sick patient, the possibility of septic emboli has to be kept in mind[1,2].

Cryptococcosis is the third most common fungal infection seen in transplant recipients[4,5]. It typically occurs late with median time to onset being 16 to 21 mo after renal transplantation. However our patient presented very late - 6 years after transplantation. So besides TB and fungal infection, post-transplant lymphoproliferative disease was an important differential diagnosis considered. All factors which increased the cumulative immunosuppression in patients increase the risk of disseminated Cryptococcal disease. Presence of chronic liver disease and use of steroids, T cell depleting antibodies and Alemtuzumab are specifically associated with increased risk of Cryptococcosis. Calcineurin inhibitor based regimens are believed to be protective, being associated more commonly with Cryptococcosis limited to lungs with less likelihood of dissemination[5,6].

Our patient is HBsAg positive. But he had not received induction, had no history of rejection requiring pulse steroid therapy and has not been on MMF for 5 years. Though the apparent dose of immunosuppressive drugs given seems to be low, his cumulative immunosuppression level is definitely high as is suggested by the onset of late BKV associated nephropathy.

Cryptococcal infection commonly presents with neurologic disease (meningitis) or pneumonia. But it may also involve the skin and soft tissue, bones, joints and other organs like the liver and the kidney. Isolated pulmonary disease is uncommon seen in only 33% of the patients. Serum Cryptococcal antigen has 90% sensitivity in disseminated disease but may be

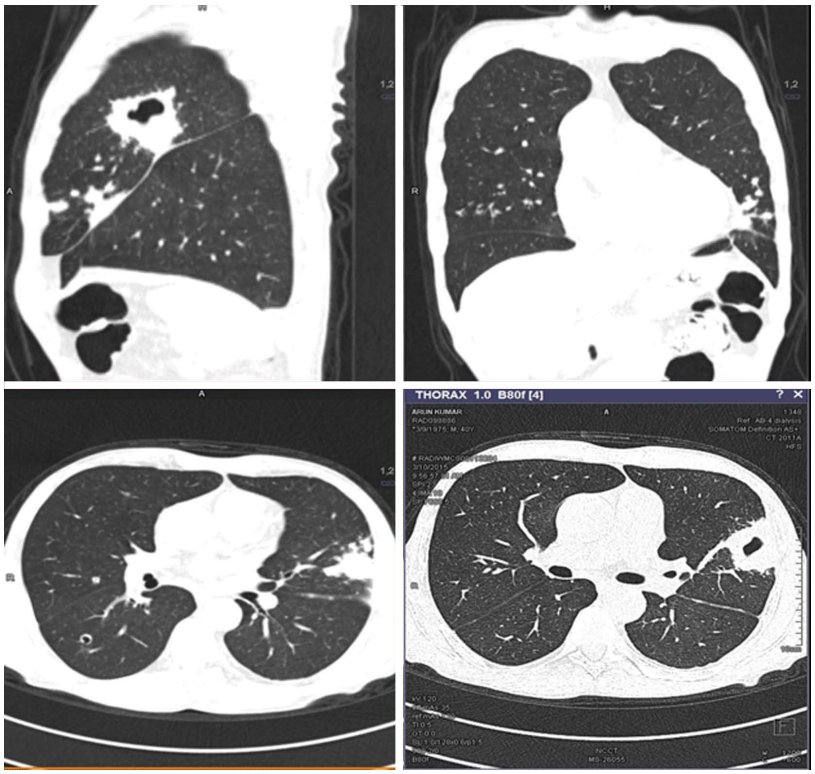

Figure 1 Multiple diffuse bilateral centrilobular and peribronchovascular cavitating nodules coalescing to form areas of consolidation with larger cavity in apico posterior segment of upper lobe of left lung.

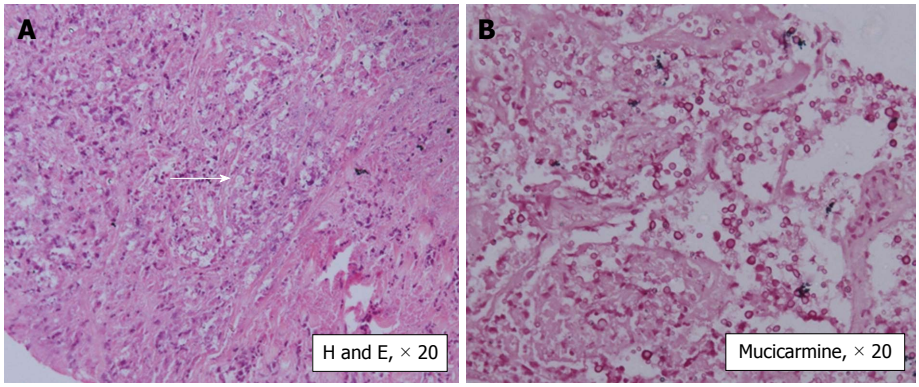

Figure 2 The histopathology showed cryptococcal infection. Histopathology of the lung lesion shows: A: Large area of necrosis with numerous capsulated yeast forms of fungi (arrow) morphologically resembling Cryptococcus; B: Special histochemical stain (Mucicarmine) highlights its polysaccharide capsule.

negative in immunosuppressed patients especially with isolated pulmonary disease[5] as seen in our patient. The final diagnosis is by tissue biopsy and/or culture. The organism can be recognized by its oval shape, and narrow-based budding on histopathology. With the use of mucicarmine staining, the Cryptococcal capsule will stain rose to burgundy in color and help differentiate *Cryptococcus neoformans* from other yeasts, especially *Blastomyces dermatiditis* and *Histoplasma capsulatum*[5].

Choice of antifungal therapy depends on the severity and extent of the disease. In patients with severe pulmonary infection, neurological involvement and disseminated disease, combination of liposomal Amphotericin with Flucytosine for 2 wk followed by Fluconazole for 12 mo is recommended. If Flucytosine is not available, which was the case initially in our patient, Amphotericin should be given for a minimum of 4-6 wk[5].

Cryptococcal infection has an overall mortality of 14% in solid organ transplant recipients[6]. Early diagnosis and initiation of treatment is the key to survival. A high index of suspicion and step-wise approach to diagnosis including a lung biopsy is required as the duration of therapy differs significantly from other fungal infections.

COMMENTS

Case characteristics

A 40-year-old male renal transplant recipient presented with low grade fever

and dry cough for one month.

Clinical diagnosis
A febrile patient with respiratory symptoms.

Differential diagnosis
Chest infection-baterial/Tuberculosis/fungal.

Laboratory diagnosis
Pancytopenia with high serum creatinine.

Imaging diagnosis
Non contrast computed tomography scan of chest showed bilateral, multiple, diffuse centrilobular and peribronchovascular cavitating nodules coalescing to form areas of consolidation with a larger cavity in apico posterior segment of upper lobe of left lung.

Pathological diagnosis
Biopsy from the lung lesion showed Cryptococcal infection and graft kidney biopsy showed BK virus associated nephropathy.

Treatment
He was treated with liposomal Amphotericin and Flucytosine.

Related reports
A renal transplant recipient may present with a cavitary lung lesion due to infection, malignancy (post-transplant lymphoproliferative disorder) or inflammatory disease, though infections are the predominant causative factor.

Term explanation
Cryptococcal infection is the third most common fungal infection seen in transplant recipients. It commonly presents with neurologic disease (meningitis) or pneumonia, but may also involve the skin and soft tissue, bones, joints and other organs like the liver and the kidney.

Experiences and lessons
Tissue biopsy or culture is required to diagnose isolated pulmonary cryptococcosis. Early diagnosis and initiation of treatment is essential for survival.

Peer-review
The case discusses an important issue in patients with kidney transplantation.

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P- Reviewer: Ali-El-Dein B, Mahmoud KM, Sheashaa HA
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Liu SQ

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