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REVIEW

Biology of chronic graft-*vs*-host disease: Immune mechanisms and progress in biomarker discovery

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Abstract

Chronic graft-*vs*-host disease (cGVHD) is the leading cause of long-term morbidity and mortality following allogeneic hematopoietic stem cell transplantation. It presents as a chronic inflammatory and sclerotic autoimmune-like condition that most frequently affects the skin, oral mucosa, liver, eyes and gastrointestinal tract. Both clinical and animal studies have shown that multiple T cell subsets including Th1, Th2, Th17, T follicular helper cells and regulatory T-cells play some role in cGVHD development and progression; B cells also play an important role in the disease including the production of antibodies to HY and nuclear antigens that can cause serious tissue damage. An array of cytokines and chemokines produced by different types of immune cells also mediate tissue inflammation and damage of cGVHD target tissues such as the skin and oral cavity. Many of these same immune regulators have been studied as candidate cGVHD biomarkers. Recent studies suggest that some of these biomarkers may be useful for determining disease prognosis and planning long-term clinical follow-up of cGVHD patients.

Key words: Chronic graft-*vs*-host disease; Biomarker; Allogeneic hematopoietic stem cell transplantation; Cytokine

Core tip: Chronic graft-*vs*-host disease (cGVHD) is a frequent long-term medical complication of allogeneic hematopoietic stem cell transplantation which can have a devastating impact on overall health and quality of life. This immune-mediated disorder manifests as an inflammatory and autoimmune-like disorder that can affect multiple tissues in an individual patient. Both clinical and animal studies demonstrate that multiple T cell subsets, as well as B cells, and their secreted cytokines play important roles in cGVHD initiation and progression. In the last decade many molecular biomarkers have been identified that correlate with cGVHD onset and/or progression, and some might have applications clinically in the near future.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is utilized primarily as a curative treatment for both hematological and non-hematological malignancies[1], although it has been used successfully in small-scale clinical trials as a stem cell therapy for some inherited diseases such as Recessive Dystrophic Epidermolysis Bullosa[2]. In the case of hematologic malignancies, the graft-*vs*-leukemia or graft-*vs*-tumor (GVL or GVT) effect mediated by donor-derived T cells helps to eliminate malignant cells in the transplant recipient[3]. However, a major long-term complication of allo-HSCT is chronic graft-*vs*-host disease (cGVHD), which occurs in 30%-70% of patients, with adults more frequently affected than pediatric patients[4]. Chronic GVHD manifests as an autoimmune-like inflammatory disease that can affect a single organ, but more typically it presents as a multi-organ disease affecting the skin (75% of patients), oral mucosa (51%-63% of patients), liver, eyes and gastrointestinal tract (22%-51% of patients)[4]. Oral mucosal disease can include salivary gland pathology or sclerosis of the lamina propria or submucosa. Other tissues including the lung, esophagus, joints, muscles and genitalia can also be involved (Table 1). cGVHD is often preceded by acute GVHD, which typically occurs within 100 d after transplantation, although the acute form can persist longer.

In allo-HSCT patients, cGVHD is the most common cause of non-relapse mortality (NRM, which refers to mortality not related to the primary malignancy or disease) among patients surviving more than two years[5]. Other important contributing factors to patient mortality are viral or bacterial infection and secondary malignancies (Figure 1)[4,6]. A recent analysis by the Center for International Blood and Marrow Transplant Research (CIBMTR) of more than 26000 allo-HSCT patients demonstrated that the incidence of cGVHD is increasing worldwide, making it imperative that we fully understand the etiology of this disease[7].

This review will focus on the pathobiology of cGVHD, which has features of both alloimmune and autoimmune disease and involves altered activities and function of various T cell populations [T helper (Th) 1, Th2, Th17, T follicular helper cells and regulatory T-cells] as well as of B cells. Equally important are the various cytokines and chemokines produced by immune cells and their target tissues, which cause inflammation and tissue damage. A second productive area of cGVHD research is biomarker discovery; high-throughput approaches including mass spectrometry have led to the identification of a number of molecular markers from blood and saliva that correlate with active disease. Not surprisingly, many of these markers are associated with altered host immunity and/or tissue inflammation. This review will not discuss current primary and secondary therapeutic strategies for cGVHD; for an in-depth discussion of this topic, the reader is referred elsewhere[8-10].

CLINICAL FEATURES

According to the NIH consensus criteria published in 2005, cGVHD can be subclassified into: (1) Classic cGVHD presenting with manifestations that can be ascribed only to cGVHD; and (2) Overlap syndrome that has diagnostic or distinctive cGVHD manifestations together with features typical of acute GVHD[11]. Acute GVHD occurs in 40%-60% of patients receiving allo-HSCT and is one of the major risk factors for subsequent cGVHD. To improve cGVHD classification, the NIH severity score was developed which documents the number of organs involved and numerically scores the degree of functional impairment. Generally, patients are assessed as having mild, moderate or severe disease on a scale of 1 to 4 for each tissue[11-13]. However, clinical symptoms of cGVHD often overlap with other autoimmune diseases such as lichen planus and scleroderma and the degree of organ involvement is highly variable, which can make diagnosis challenging[8,14]. Table 1 lists signs and symptoms that are considered to be diagnostic of cGVHD as well as some of the commonly observed clinical features that are considered to be insufficient for disease diagnosis. As many as three or more tissues can be affected in a single patient, as reflected in the NIH global severity classification of cGVHD[13]. Skin manifestations that are considered diagnostic include poikiloderma (altered pigmentation with erythema), lichen planus-like lesions, sclerosis and morphea-like features (Table 1). Distinctive features (often observed in skin cGVHD but not sufficient for diagnosis) include depigmentation, papulosquamous lesions, ichthyosis and pruritis. Skin appendages are often targeted as well but these signs are not considered diagnostic: Symptoms can include scalp hair thinning or alopecia, sweat impairment and nail dystrophy or onycholysis (nail loss)[11,13].

Cutaneous cGVHD can occur in two forms termed lichenoid and sclerodermatous[15]. Lichenoid lesions usually occur early in the course of the disease, presenting as erythematous papules or plaques, with a squamous surface. Typical affected sites include the face, ears, palms and soles. Sclerodermatous cGVHD, which generally develops as a later complication, appear as sclerotic, shiny, white or yellow plaques with patchy hyperpigmentation or a poikilodermal appearance[15]. Sclerodermatous cGVHD can be localized or generalized and affect underlying tissues including the fascia, ligaments and peripheral nerves, causing pain and morbidity for the affected patient.

Oral symptoms vary but commonly involve lichenoid changes, xerostomia as a result of salivary gland damage,

Table 1 Signs, symptoms and prevalence of chronic graft-*vs*-host disease in selected organs and tissues

Organ or tissue	Prevalence, %[1]	Diagnostic features[2]	Distinctive features[3]
Skin	75%	Poikiloderma	Depigmentation
		Lichen planus-like features	Papulosquamous lesions
		Sclerosis	
		Morphea-like features	
Mouth	51%-63%	Lichen planus-like features	Xerostomia
			Mucoceles
			Mucosal atrophy
			Ulcers
			Pseudomembranes
Liver	29%-51%	None[4]	None[4]
Eye	22%-33%		Dry, gritty or painful eyes
			Cicatricial conjunctivitis
			Keratoconjunctivitis-Sicca syndrome
GI tract and esophagus	7%-45%	Esophageal web	
		Strictures or stenosis in upper esophagus	
Lung	4%-19%	Bronchiolitis obliterans	Air trapping and bronchiectasis on chest CT scan
Muscles, fascia and joints	6%	Fascitis	Myositis or polymyositis
		Sclerosis	
		Joint stiffness or contractures	
Genitalia	1%	Lichen planus-like features	Erosions, Fissures, Ulcers
		Lichen sclerosus-like features	

[1]Frequency of tissue involvement at initial cGVHD diagnosis (from Lee *et al*[4]); [2]Clinical symptoms that are sufficient for cGVHD diagnosis. Information adapted from references 8 and 13; [3]Clinical symptoms that are frequently seen in cGVHD, but insufficient for cGVHD diagnosis. Information adapted from references 8 and 13; [4]While no diagnostic or distinctive features have been identified for liver cGVHD, hepatitis is often seen (and also sometimes in acute GVHD) with elevated serum levels of bilirubin, alkaline phosphatase and alanine aminotransferase (ALT)[13]. cGVHD: Chronic graft-*vs*-host disease; CT: Computed tomography.

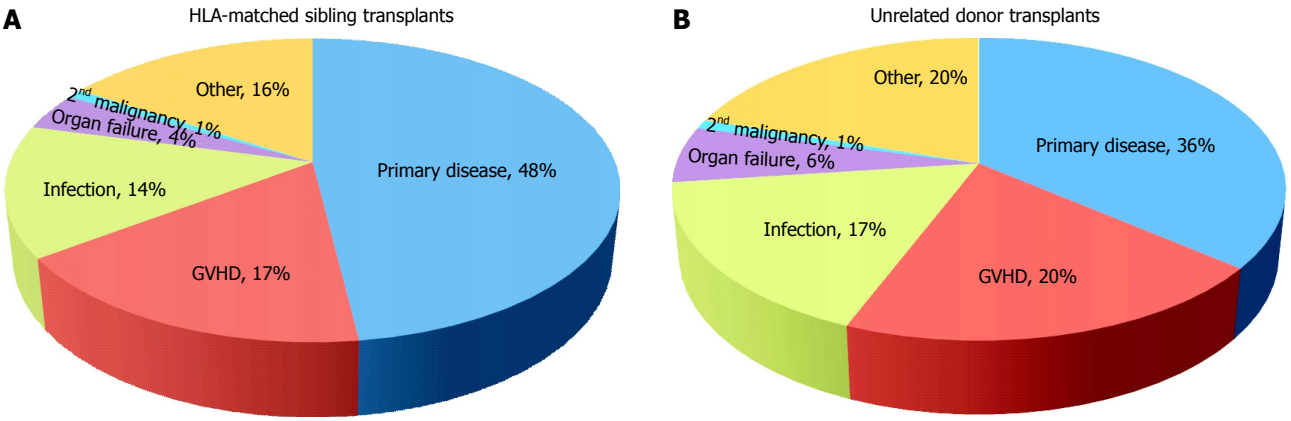

Figure 1 Causes of death among allogeneic hematopoietic stem cell transplantation patients. Pie charts show causes of death among patients who received a cell graft from (A) an HLA-matched sibling or (B) an unrelated donor. Data is from the Center for International Blood and Marrow Transplant Research, for allogeneic hematopoietic stem cell transplants performed in 2012-13[88] (Available from: URL: http//www.cibmtr.org/Data/Resources/pages/index.aspx). GVHD: Graft-*vs*-host disease.

mucositis, erythema, mucoceles and restricted mouth opening (trismus) due primarily to sclerosis[16-18]. However, under current guidelines only lichen planus-like features are considered to be diagnostic (Table 1)[13]. Oral sensitivity and pain are often observed, which in more severe cases manifests as dysphagia (difficulty with swallowing) and weight loss. In one recent study of 210 cGVHD patients, 29% of cases were classified as malnourished by measurement using the Patient-Generated Subjective Global Assessment tool. Malnutrition was correlated with a lower body-mass index and poorer overall survival[19]. Gingivitis and tooth decay also occur because of xerostomia and altered oral immunity related to immunosuppression and reduced salivary IgG production[20].

Clinical symptoms seen in other involved tissues such as the liver, eyes, gastrointestinal tract, lungs, muscles/fascia and genitalia are summarized in Table 1, and have been reviewed extensively elsewhere[9,13]. Neurological manifestations of cGVHD are rare, but when present can include Myositis and Myasthenia gravis that affect the peripheral nervous system, and less commonly, various complications that affect the central nervous system[21]. Clinical features of cGVHD do not seem to vary with patient age, graft source (typically either bone marrow or PBSCs) and type of pre-transplant conditioning[4,9].

Most cases of cGVHD occur 4-6 mo after allo-HSCT, but 5%-10% of patients are diagnosed more than one year following allogeneic transplantation.

RISK FACTORS

The best documented risk factors for cGVHD are a history of acute GVHD (seen in 40%-60% of cGVHD patients), the use of PBSCs for grafting, a female donor-male recipient combination, older patient age and the use of HLA-mismatched or unrelated donors[13,22,23]. The increasing use of PBSCs (which contain more T cells compared to aspirated bone marrow) is one factor that influences the incidence and severity of cGVHD, since alloreactive T cells are a major player in cGVHD pathobiology[7]. These risk factors appear to largely explain the increasing incidence of cGVHD worldwide in allo-HSCT; however, additionally, a significant decline in early NRM appears to be contributing to the increased incidence of cGVHD in long-term survivors[7]. Notably, the frequency of GVHD-associated mortality is similar in HLA-matched sibling transplants compared to transplants performed using an unrelated donor (Figure 1).

A number of studies have also implicated certain genetic polymorphisms in addition to HLA antigen disparity between donor and recipient in the risk of GVHD risk (reviewed in Pidala *et al*[24]). For example, polymorphisms in a considerable number of genes that encode cytokines, chemokines or their receptors are associated with increased risk of cGVHD. These include genetic variants in the donor and/or recipient *IL-10* genes[25-27], donor *IL-1α* gene[28], recipient *IL-6* gene[29], recipient MHC class I-related chain A (*MICA*) gene (Val allele)[30], and donor and recipient *IL-1* receptor antagonist (*IL-1ra*) genes[25,28]. For MICA, which acts as an activating ligand for the NKG2D receptor on certain types of T-cells, cGVHD incidence was positively correlated with serum MICA levels in patients post-HSCT; on the other hand, the presence of MICA antibodies prior to transplantation conferred protection against cGVHD[30]. A smaller number of genetic polymorphisms have been associated with decreased risk of cGVHD[24].

Baron *et al*[31] utilized gene expression profiling of donor $CD4^+$ and $CD8^+$ T cells to develop a "GVHD-predictive signature", demonstrating the central importance of the TGF-β signaling pathway in regulating donor T cell function[31]. Remarkably, the so-called "dangerous donor" trait derived from T cell gene expression profiling not only predicted early (acute) GVHD, but also cGVHD occurrence in the recipient at one year post-transplantation. These observations reinforces other studies in humans and mice showing that the growth factor TGF-β has pleiotropic effects on T cells, including inhibition of Th1 cell differentiation and promoting expansion of regulatory T cells that are protective against cGVHD[9,32,33]. It also suggests that the grafted stem cells can have a long-term, dominant influence on the transplant recipient's T cell profile and consequently the overall health of the patient.

PATHOBIOLOGY OF CHRONIC GVHD: ROLE OF T CELLS, B CELLS AND THEIR CYTOKINES

T cells

While the mechanisms that cause the inflammation and tissue damage of acute GVHD are now quite well understood, the pathobiology of cGVHD is more complex and less well understood. Many investigators believe that the destructive immunological and autoimmune mechanisms that cause cGVHD are distinct from acute GVHD, irrespective of whether or not the cGVHD evolves from acute GVHD[6,34]. Activated donor T cells are the most important cell population in cGVHD, since T cell depletion from the graft prevents cGVHD in both human and animal studies[35]. The use of rabbit anti-thymocyte globulin (ATG) in conditioning regimens prior to transplant reduces the risk of subsequent acute and cGVHD, either by depleting donor T cells or by interfering with their activation by recipient alloantigens[22,36]. The major T cell subsets proposed to be involved in cGVHD include $CD4^+$ T cells, $CD4^+$ regulatory T cells (Tregs) and $CD8^+$ T cells (Table 2).

Th1, Th2 and Th17 cells: Alloreactive $CD4^+$ T cells that react to foreign (donor-derived) antigens include several Th cell subsets, primarily Th1, Th2 and Th17 cells. A central role for Th1 cells in acute GVHD is well established[6]; however, the importance of Th1 (and Th2) cells in cGVHD is still a matter of debate, even though Th1 cytokines such as interferon-γ (IFN-γ) can be found in skin and other tissues of affected patients[37]. Infusion of murine IFN-γ-null donor T cells reduced cGVHD symptoms in skin and salivary glands, indicating a role for Th1 cells in certain tissues[38]. A role for Th2 cells has been suggested because of the role of Th2 cytokines such as IL-4 and IL-13 (Table 2) in the production of antibodies to both self and non-self-antigens in patients; murine studies support the involvement of the Th2 cytokines IL-4 and IL-10 in stimulating B cell expansion in cGVHD[39].

Th17 cells produce several cytokines including IL-17, IL-21 and IL-22, which have potent pro-inflammatory functions in cGVHD[40]. IL-17A, produced mainly by $CD8^+$ T cells, stimulates scleroderma which is an important feature of cutaneous cGVHD; however, current data suggests that co-expressed Th1 cytokines such as tumor necrosis factor-α (TNF-α) contribute to the observed pathology[41]. Improvement in cGVHD symptoms correlates with a reduction in Th-17 cell numbers in peripheral blood[42]. In liver cGVHD, there are increased numbers of Th17 cells and an increased Th17/Treg ratio observed in liver biopsies, suggesting that Th17 cells are an important driver of clinical liver disease[43].

T follicular helper cells: T follicular helper (TFH) cells promote differentiation of naïve B cells into memory B

Table 2 Immune cell types and their function in chronic graft-*vs*-host disease

Cell type	Subtypes	Key cytokines or markers	Brief summary of disease involvement
$CD4^+$ T cells	Th1	IFN-γ, TNF-α	Pro-inflammatory. Important in acute GVHD, but role in cGVHD unclear
	Th2	IL-4, IL-13	Stimulate antibody production. Role in clinical cGVHD poorly defined
	Th17	IL-17; also IL-21, IL-22, TNF-α	Pro-inflammatory. IL-17 levels correlate with disease severity; IL-17 induces scleroderma of skin and lung
	Tregs	TGF-β, required for Treg proliferation and differentiation	Produced mostly in thymus. Suppress autoreactive T cells. Lower levels of Tregs present in cGVHD patients, associated with thymic damage and loss of self-tolerance in cGVHD
	T follicular helper cells[1]	Express CCR5, PD-1 and ICOS	Promote abnormal B cell maturation into long-lived active plasma cells, and IgG secretion
$CD8^+$ T cells		CXCL9, CXCL10	Mediate graft-*vs*-tumor effect of transplant. Serum CXCL9 levels elevated in cGVHD patients
B cells (total)		Increased BAFF/B-cell ratio, elevated serum BAFF levels	Decreased in active cGVHD. Remaining B cells are resistant to apoptosis
Naïve and transitional B cells		CD19	Decreased in active cGVHD
Memory B cells (total)		CD19, CD27	Decreased in active cGVHD. Cells essential for a normal immune response to bacterial pathogens or opportunistic infections
Regulatory B cells		IL-10	Decreased in active cGVHD. Function to maintain tolerance and help prevent autoimmune disease
Plasma cells		CD27, CD38	Increased in active cGVHD. Cells secrete immunoglobulins including IgGs and are resistant to apoptosis

[1]Mainly classified into Th2 and Th17 subtypes[44]. cGVHD: Chronic graft-*vs*-host disease; BAFF: B-cell activating factor; CD3: Cluster of Differentiation molecule 13, 19, 27 and 38; CCR5: Chemokine (C-C Motif) receptor 5; CXCL: Chemokine (C-X-C motif) ligand; ICOS: Inducible T-Cell Co-Stimulator; IFN-γ: interferon gamma; IL: Interleukin; PD-1: Programmed cell death 1; Th: T helper cell; TGF-β: Transforming growth factor beta; TNF-α: Tumor necrosis factor alpha.

cells and class switching of *IgG* genes in the germinal center, within secondary lymphoid organs. A recent study showed that TFH cells were unusually active with prolonged survival in cGVHD patients, which correlates with the aberrant survival of B cells and hypersecretion of immunoglobulins[44]. The increased survival of Th2- and Th17-type TFH cells was correlated with increased cellular expression of the pro-survival marker Bcl-2. Overall, the study by Forcade *et al*[44] suggests that aberrant B cell activity including production of antibodies is driven, at least in part, by abnormal TFH cell activity. Studies using a murine model have confirmed the importance of TFH cells in cGVHD pathogenesis, particularly for the development of bronchiolitis obliterans syndrome which is a signature feature of lung cGVHD[45] (Table 1).

Tregs: Tregs, which are $CD4^+$ $CD25^+$ and also express the transcription factor FOXP3, suppress autoreactive T cells and are important for immune system homeostasis. Specifically, Tregs are essential for the establishment and maintenance of tolerance after allo-HSCT[46]. Tregs are depleted in both acute and cGVHD, demonstrating their importance as suppressors of inflammation and disease development[23]. Impaired Treg production and function has been linked to thymic damage as a result of $CD4^+$ lymphopenia following allo-HSCT, at least in myeloablative patients[47]. The presence of Tregs in the skin and oral mucosa of cGVHD patients in a functional (*e.g.,* $CXCR3^+$) state suggests they may play a role in limiting tissue damage by alloreactive T cells[48]. Pharmacological approaches used to treat steroid-refractory cGVHD that increased Treg cell numbers have shown promise clinically in treating cutaneous cGVHD[49]. Further, in a study of allo-HSCT patients with acute leukemia, direct infusion of Tregs together with conventional T cells protected against GVHD in almost 90% of engrafted patients, while still maintaining the GVT anti-tumor effect conferred by conventional T cells[50]. These studies suggest that manipulation of Tregs might be a feasible approach to reducing or preventing GVHD without compromising the anti-tumor surveillance capacity of the patient's immune system.

$CD8^+$ T cells: $CD8^+$ T cells are another immune cell population present in tissues affected by cGVHD, including the skin and oral mucosa[17]. Donor $CD8^+$ cells mediate the GVT effect of allo-HSCT that typically results in the eradication of malignant cells from the patient. Among the cytokines produced by $CD8^+$ cells are CXCL9 and CXCL10; CXCL9 is elevated in the serum of early-stage cGVHD patients, with CXCL9 levels being correlated with disease severity[51] (Tables 2 and 3).

B cells

In addition to T cells, there is increasing evidence that B cells play a number of important roles in cGVHD pathogenesis[52]. Patients with active cGVHD consistently have

Table 3 Candidate biomarkers of chronic graft-*vs*-host disease[1]

Gene/protein	Function	Biofluid[2]	Ref.
BAFF, soluble; BAFF/B cell ratio	Growth factor, promotes B cell expansion and activation	Blood	[59,60,77]
CXCL9	Chemokine produced by activated T cells	Blood	[51,79]
CD-13, soluble	Antigen presentation	Blood	[59]
C-reactive protein[3]	Acute phase protein	Blood	[12]
Cystatin B	Inhibitor of cathepsin proteases	Saliva	[80]
IL-1ra	Inhibitor of IL-1 receptor signaling	Saliva	[80]
IL-2R, soluble	IL-2 receptor, marker of activated T cells	Blood	[59,76]
IL-6	Pro-inflammatory Th2 cytokine	Blood	[42,75]
IL-10	Th2 cytokine	Blood	[73]
IL-15	Enhances anti-tumor function of $CD8^+$ T cells	Blood	[78]
Lactoperoxidase	Anti-microbial enzyme	Saliva	[81]
Lactoferrin	Iron-binding glycoprotein	Saliva	[81]
MICA, soluble	Stimulates T cell activity *via* NKG2D receptor	Blood	[30]
TGF-β	Anti-inflammatory cytokine; stimulates activity of Tregs	Blood	[33]
TNF-α	Pro-inflammatory Th1 cytokine	Blood	[73-75]

[1]This table only includes proteins identified in human biofluids. Antibodies are discussed in the text; [2]Blood markers were measured in either plasma or serum isolated from peripheral blood, depending on the study. For saliva, whole unstimulated saliva collected from oral cGVHD patients was used; [3]Increased CRP levels were especially associated with joint/fascia and skin involvement, compared to the non-cGVHD control group. cGVHD: Chronic graft-*vs*-host disease; BAFF: B-cell activating factor; CXCL: Chemokine (C-X-C motif) ligand; CD-13: Cluster of Differentiation molecule 13 (or aminopeptidase N); IL-1ra: Interleukin 1 receptor antagonist; IL-2R: Interleukin 2 receptor; MICA: MHC class I-related chain A; TGF-β: Transforming growth factor beta; TNF-α: Tumor necrosis factor alpha; CRP: C-reactive protein.

lower numbers of naïve and transitional B cells as well as total B cells[53,54] (Table 3). Regulatory B cells that secrete the anti-inflammatory cytokine IL-10 (and form a subpopulation within the transitional and memory B cell compartments) were also less frequent in cGVHD patients and displayed a deficiency in IL-10 production[55]. Together with Tregs, regulatory B cells play a central role in graft tolerance and the prevention of autoimmune disease and hence represent a topic worthy of further investigation in relation to cGVHD[56]. CGVHD patients are susceptible to pneumococcal infection which can cause severe or fatal infections in long term transplant survivors[57]. This susceptibility to infections is associated with the abnormal B cell profile, including decreased numbers of memory B cells that are critical for a normal immune response including IgG production[53,58]. Like many other autoimmune conditions, cGVHD patients frequently produce allo- and auto-antibodies to DNA and/or other antigens such as male HY antigen, which can correlate with disease onset and severity (see below). Activated B cells secrete an array of Th1 and Th2 cytokines that can regulate the function of T cell populations including Tregs. Levels of B cell activation factor (BAFF), a cytokine that promotes the survival and differentiation of activated B cells, are consistently increased relative to B cell numbers in patients with cGVHD[59,60]. As discussed above, the increased activity of TFH cells appears to play a significant role in producing the abnormal B cell profile characteristic of cGVHD[44].

Perhaps the best evidence that B cells are functionally important in human cGVHD are the numerous clinical observations with Rituximab, a humanized monoclonal antibody that targets the membrane protein CD20 of B cells, causing their cell death. Rituximab (and other anti-CD20 drugs) are effective in the treatment of steroid-refractory cGVHD, resulting in rapid and selective depletion of B cells and diminished activation of cytotoxic T cells; concurrently, the number and activity of Tregs are elevated[52,61,62]. In one study, the prophylactic use of Rituximab after allo-HSCT significantly reduced the incidence of both acute and cGVHD as well as NRM[63]. Hence, inhibiting B cell function has profound effects on both B and T cell homeostasis, with significant benefits to cGVHD patients especially in cases where other primary and/or secondary treatments have been unsatisfactory.

ANIMAL MODELS OF CHRONIC GVHD

Several types of murine models have been utilized for studies of GVHD pathobiology including: (1) a bone marrow transplantation (BMT) model involving lethal radiation (total body irradiation, TBI) and transplantation of syngeneic marrow into treated mice[64]; (2) a parent-into-F1 model where donor spleen cells are infused into non-irradiated mice[39]; and (3) a transgenic model utilizing a self-antigen, membrane-associated chick ovalbumin, expressed under the control of the K14 promoter (K14-mOVA), where autoreactive skin disease is promoted by adoptive transfer of CD8 T cells from a second mouse strain, OT-1, that has an engineered T cell receptor specific for an ovalbumin peptide[65,66]. These animal models each demonstrate one or more manifestations of clinical cGVHD including the presence of anti-DNA antibodies, sclerosis, weight loss and chronic inflammation of skin and mucosal tissues associated with elevated Th1, Th2 and/or Th17 cytokines. The K14-mOVA adoptive transfer model has been used to test the efficacy of novel anti-inflammatory biologics that target the Janus kinase (JAK)[67] and Histone Deacetylase 6[68] enzymes, which were shown to be effective at suppressing and/or

reversing cutaneous disease. Tofacitinib, the JAK inhibitor, blocked the expansion and activation of $CD8^+$ cells thereby reducing IFN-γ secretion by $CD8^+$ cells and keratinocytes as well as preventing the downstream consequences of interferon signaling such as chemokine production and keratinocyte apoptosis[67]. Another JAK inhibitor, Ruxolitinib (INCB018424), reduced murine GVHD (acute GVHD) symptoms and the levels of pro-inflammatory cytokines by both impairing differentiation of $CD4^+$ T cells into IFN-γ and IL-17-producing cells, and by promoting the production of protective Tregs[69]. Notably this JAK 1/2 inhibitor reduced GVHD symptoms and improved overall animal survival while still maintaining the anti-tumor (GVT) effect[70].

While these pre-clinical models have been valuable in defining the immune mediators of cGVHD, the animal models do not typically parallel the evolution of the human disease, especially the common clinical presentation of classic cGVHD[8]. Additionally, in mice receiving intensive conditioning regimens (especially radiation), there is a well characterized scenario of inflammatory cytokine release, T cell activation and homing to target organs where tissue destruction occurs through the action of PBMCs and their associated cytokines. However, in humans the preparative regimen is only one factor involved in GVHD initiation, and its influence may be diminished in patients who now receive reduced-intensity conditioning prior to allo-HSCT[8]. Some recently described animal models exhibit systemic disease with multi-organ involvement including the lung, which appears to more closely resemble human cGVHD[71]. Despite some weaknesses, animal models will undoubtedly continue to provide insight into specific aspects of cGVHD pathobiology and will be essential for preclinical testing of new therapies for acute and cGVHD.

CHRONIC GVHD BIOMARKERS

An emerging area of cGVHD research involves the discovery and validation of biomarkers that might eventually be used in clinical diagnosis or treatment planning. To date, most studies have focused on protein and immune cell biomarkers, even though RNAs (including mRNAs and micro RNAs) might also have utility as disease biomarkers[54,60]. As defined at the first meeting of the NIH Biomarker Working Group in 2006, cGVHD biomarkers could be used in disease management or clinical trials to: (1) predict response to therapy; (2) measure disease activity; (3) predict the risk of developing cGVHD; (4) diagnose cGVHD or predict prognosis; and (5) serve as a surrogate end point for therapeutic response[37].

To date, researchers have utilized mass spectrometry-based discovery approaches as well as Luminex and antibody arrays to screen clinical samples for potential serum and saliva protein biomarkers. Biomarkers identified to date can be broadly divided into proteins that function as cytokines and chemokines, immune (*e.g.,* cytokine) receptors and other types of immune or non-immune proteins (Paczesny *et al*[72] for a recent review). Identified serum biomarkers that might indicate overall disease (and/or altered immune cell) activity include B cell activation factor (BAFF), MICA and anti-MICA antibodies, TNF-α, IL-15 and Chemokine (C-X-C motif) ligand 9 (CXCL9). Salivary biomarkers, associated mainly with oral cGVHD, include IL-1ra, cystatin B, lactotransferrin and lactoperoxidase (Table 3). Cellular markers primarily comprise immune cell populations that are altered in cGVHD (Table 2).

Serum biomarkers

Chronic cGVHD onset and/or persistence is associated with increased levels of TNF-α, BAFF, IL-6, sIL-2R (soluble IL-2 receptor alpha), and IL-10, and decreased levels of TGF-β and IL-15 (Table 3). Several studies have reported elevated levels of the pro-inflammatory cytokine TNF-α in acute and cGVHD, with measured levels correlating with cGVHD severity[73-75]. IL-6 shows a similar trend and correlation with cGVHD severity[42,75]. Soluble IL-2 receptor alpha (sIL-2Rα) is another example of a serum marker that is increased in pediatric and adult patients with cGVHD[59,76]. BAFF, a growth factor that promotes B cell differentiation and immunoglobulin production, is increased in both pediatric and adult patients with cGVHD; levels of this growth factor are often reported relative to the number of B cells in blood samples[51,59,60,77]. High levels of BAFF protein were present in allo-HSCT patients who subsequently developed cGVHD, confirming its role in alloimmunity[77]. CXCL9 levels are also elevated in newly diagnosed cGVHD patients and were correlated with disease severity in three different cohorts studied at two transplant centers[51].

Other markers besides BAFF and CXCL9 have been shown to have potential predictive value in allo-HSCT patients for determining future disease. For example, elevated levels of soluble MICA protein post-allo-HSCT were associated with an increased risk of cGVHD (by contrast, as stated above, the presence of MICA antibodies before transplantation conferred some protection from cGVHD)[30]. Similarly, Pratt *et al*[78] have shown that patients with low serum levels of IL-15 at day 7 post-transplant had 3-fold higher risk of developing cGVHD subsequently. IL-15 levels were observed to be inversely correlated with CD8 T cell levels, which are important for the GVT effect but also influence the development of cGVHD (see above).

In addition to intrinsic, host-dependent (*e.g.,* immune) factors, the levels of biomarkers such as BAFF and CXCL9 can be modified by extrinsic factors including immunosuppressive drugs such as corticosteroids[51,54]. Hence, as recognized by many investigators, independent validation of promising biomarker candidates is essential. CXCL9 was recently validated as a cGVHD biomarker in a multicenter United States study of allo-HSCT patients[79].

Salivary biomarkers

Two recent studies utilizing mass spectrometry approaches identified a total of 82 and 102 salivary proteins, re-

spectively, that showed altered expression in oral cGVHD[80,81]. IL-1 receptor antagonist (IL-1ra) exhibited reduced expression in patients with oral cGVHD[80]. The changes in IL-1ra expression coupled with higher levels of IL-1 family cytokines[16] in saliva likely enhance oral inflammation and subsequent tissue damage. In particular, IL-6 levels have been shown to correlate with oral cGVHD severity[16]. Changes in expression of salivary lactoperoxidase and lactotransferrin have also been reported, indicative of impaired innate immunity in oral cGVHD[81] (Table 3). The alterations in the salivary proteome among proteins involved in innate and acquired immunity are consistent with the clinical features of oral cGVHD, in particular patient susceptibility to bacterial and viral infections[4,18]. Changes in inorganic salivary components, especially Na^+ and Cl^- ions and inorganic phosphate, also occur in concert with cGVHD onset, correlating with hyposalivation and damage to the salivary glands[82,83].

Other biofluids

Certain Th2 and Th17 cytokines, in particular IL-6, IL-10, IL-17A and TNF-α are elevated in the tear fluid of cGVHD patients and correlated with systemic cGVHD regardless of ocular symptoms; levels of three of these cytokines (IL-6, IL-10 and TNF-α) also were significantly correlated with ocular cGVHD parameters[84].

Cellular biomarkers

In addition to protein biomarkers, a large number of immune cell populations have been studied as potential cGVHD biomarkers. Some of the best studied are listed in Table 2. As discussed in the Pathobiology section above, $CD4^+$ $IL\text{-}17^+$ Th17 cells are elevated in active cGVHD while Tregs that express the markers CD4, CD25 and FoxP3 are typically decreased[42,47]. There are also complex changes in the B cell population of cGVHD patients. Overall, total B cell counts are decreased in cGVHD patients as are the levels of naïve, transitional and regulatory B cells. In contrast, differentiated $CD38^+$ $CD27^+$ IgG-secreting plasma cells are increased in patients with active cGVHD (Table 2)[53,54].

Antibodies

Antibodies including autoantibodies are another group of well-studied potential biomarkers that are produced in cGVHD patients by an aberrant B cell population. Up to 80% of allogeneic transplants involving a female donor-male recipient combination produce antibodies against Y-chromosome-encoded HY proteins, and these antibodies appear to predict the development of cGVHD[8,37]. Antibodies to Platelet-derived Growth Factor (PDGF) Receptor, double stranded DNA and anti-nuclear antibody (ANA) are also common in cGVHD patients[59]. Anti-PDGF receptor antibodies cause accumulation of reactive oxygen species and stimulate type Ⅰ collagen expression, suggesting a role for these antibodies in skin and lung fibrosis[85,86]. Patients with classic cGVHD were found to have higher levels of ANA and anti-DNA antibodies compared to patients who had a prior history of acute GVHD where B cells have limited involvement[59].

CONCLUSION

CGVHD is a chronic inflammatory and autoimmune-like condition that involves a complex interplay between the immune systems of the transplant donor and recipient. Despite significant progress in understanding the risk factors, and the development of effective second-line treatments for steroid-refractory cGVHD, the incidence of cGVHD is increasing worldwide[7]. While donor-derived T cells are still considered to be the preeminent mediators of cGVHD, aberrant B cells clearly play a significant role in promoting autoimmunity and inflammation, and conferring susceptibility to serious, often life-threatening infections. The enhanced activity of T follicular helper cells in cGVHD also appears to play a key role in the aberrant B cell activity and the resulting autoimmune-like features of cGVHD, including the presence of antibodies that target HY and nuclear proteins[44].

In addition to the significant progress in our understanding of cGVHD immunobiology and pathobiology, guidelines for biomarker development and validation were recently updated. The updated guidelines include recommendations for biomarker identification, verification, qualification, and application with terminology based on Food and Drug Administration and European Medicines Agency guidelines[72]. Suggested areas of focus for validation include biomarkers that are prognostic, stratify cGVHD risk or are predictive of future disease. Biobank repositories that can serially collect peripheral blood and cell samples from allo-HSCT patients in a standardized format will also be an important tool for pre-clinical biomarker validation[72]. The French National Cryostem Project is one example of such a national effort[87,88] which, together with multicenter collaborations[51,79], should enable protein biomarkers to be added to the clinician's toolkit for cGVHD patient care in the not-too-distant future.

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REVIEW

Organ transplantation and drug eluting stents: Perioperative challenges

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Abstract

Patients listed for organ transplant frequently have severe coronary artery disease (CAD), which may be treated with drug eluting stents (DES). Everolimus and zotarolimus eluting stents are commonly used. Newer generation biolimus and novolimus eluting biodegradable stents are becoming increasingly popular. Patients undergoing transplant surgery soon after the placement of DES are at increased risk of stent thrombosis (ST) in the perioperative period. Dual antiplatelet therapy (DAPT) with aspirin and a P2Y12 inhibitor such as clopidogrel, prasugrel and ticagrelor is instated post stenting to decrease the incident of ST. Cangrelor has recently been approved by Food and Drug Administration and can be used as a bridging antiplatelet drug. The risk of ischemia *vs* bleeding must be considered when discontinuing or continuing DAPT for surgery. Though living donor transplant surgery is an elective procedure and can be optimally timed, cadaveric organ availability is unpredictable, therefore, discontinuation of antiplatelet medication cannot be optimally timed. The type of stent and timing of transplant surgery can be of utmost importance. Many platelet function point of care tests such as Light Transmittance Aggregrometry, Thromboelastography Platelet Mapping, VerifyNow, Multiple Electrode Aggregrometry are used to assess bleeding risk and guide perioperative platelet transfusion. Response to allogenic platelet transfusion to control severe intraoperative bleeding may differ with the antiplatelet drug. In stent thrombosis is an emergency where management with either a drug eluting balloon or a DES has shown superior outcomes. Post-transplant complications often involved stenosis of an important vessel that may need revascularization. DES are now used for endovascular interventions for transplant orthotropic heart CAD, hepatic artery stenosis post liver transplantation, transplant renal artery stenosis following kidney transplantation, *etc*. Several antiproliferative drugs used in the DES are inhibitors of mammalian target of rapamycin. Thus they are used for post-transplant immunosuppression to prevent acute rejection in recipients with heart, liver, lung and kidney transplantation. This article describes in detail the various perioperative challenges encountered in organ transplantation surgery and patients with drug eluting stents.

Key words: Drug eluting stents; Cangrelor; Stent thro-

mbosis; Organ transplant; Antiplatelet medication; Platelet function assays; Mammalian target of rapamycin inhibitors; Post-transplant immunosuppression; Post-transplant endovascular inhibition; Ticagrelor; Thromboelastograms platelet mapping; Novolimus; Biolimus A9

Core tip: Patients undergoing transplant surgery soon after the placement of drug eluting stents (DES) are at increased risk of stent thrombosis (ST) in the perioperative period. Dual antiplatelet therapy with aspirin and a P2Y12 inhibitor is instated post stenting to decrease the incident of ST. Cadaveric organ availability is unpredictable, therefore, discontinuation of antiplatelet medication cannot be optimally timed. Many platelet function point of care tests are used to assess bleeding risk and guide perioperative platelet transfusion. Response to allogenic platelet transfusion to control severe intraoperative bleeding may differ with the antiplatelet drug. DES are now used for endovascular interventions for post-transplant orthotropic heart coronary artery disease, hepatic artery stenosis post liver transplantation, *etc*. Antiproliferative drugs used in DES are also used for post-transplant immunosuppression.

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INTRODUCTION

Percutaneous coronary intervention (PCI) is presently the most frequent revascularization procedure used for treating coronary artery disease (CAD). It surpasses coronary artery bypass grafting. Balloon angioplasty and coronary stenting are the most common percutaneous coronary interventions.

Angioplasty is complicated by vessel spasm, recoil, and abrupt closure. Coronary stenting with bare metal stents (BMS) may prevent these complications, however, they are associated with restenosis rates of 25%-30%[1]. Studies on stent thrombosis (ST) with BMS show that clinical consequences of angiographic ST includes a 64.4% incidence of death or myocardial infarction at the time of ST and a six-month mortality of 8.9%[2]. For clinically defined ST events, the associated six-month mortality is as high as 20.8%. Due to such high risk of death following ST, it must be prevented at all costs. The angiographic outcome yielded by primary percutaneous intervention (PPCI) by drug eluting balloons (DEB)-only in selected patients was comparable to those stented by BMS alone and when DEB insertion was followed by stenting with BMS. If the patient has potential contraindications to DES, then DEB-only is a good alternative[3].

When the stented coronary artery is narrowed due to the development of neo-intimal hyperplasia within the stent, it is termed as restenosis. An inflammatory reaction, both acute and chronic, results when there is arterial trauma and a foreign body response. Smooth muscle migration and proliferation result in scar tissue formation within the stent, thus narrowing the vessel lumen. This process generally begins to occur in first six to eight weeks after stenting, but can be seen beyond one year after stent placement.

DES was introduced to reduce the rate of restenosis. The antiproliferative drug eluted inhibits smooth muscle and endothelial cell proliferation[4], thus delaying the inflammatory response. The layering of endothelial cells over the stent is slower paced than with BMS. When the stent is endothelialized, it becomes incorporated into the artery. Complete healing of first generation DES may take upto two years[5]. The drug is held and released by a biocompatible polymer coating[6]. However, endothelialization of the stent may also be delayed. This increases the risk of subacute ST. Risk of after DES implantation is related to stent length, stenting across branch ostia, disruption of adjacent vulnerable plaques, and plaque prolapse[7]. Failure to form a complete neo-intimal layer over stent struts or impaired healing makes the stent more susceptible to thrombosis[8]. Premature interruption of DAPT, renal failure, cardiac compromise with low ejection fraction (EF), bifurcation stenting and diabetes contribute to the risk of thrombotic events in DES[9].

DES

The type of stent can have significant implications on the perioperative management of a transplant recipient (Table 1).

First generation DES

Coronary first generation drug eluting stents were coated with antiproliferative drugs sirolimus and paclitaxel. First generation stents used were Paclitaxel eluting TAXUS (Boston Scientific, Natick, MA) stent (PES) and sirolimus eluting CYPHER (Cordis, Miami, FL) stent (SES). Paclitaxel, which is derived from a Pacific Yew Tree (Taxus Brevifolia), is a cytotoxic anti-neoplastic drug which causes cell-cycle arrest in the G2/M phase transition[10,11]. PES, have a bimodal release that is completed in approximately two weeks[12]. Sirolimus is a macrolide antibiotic with potent antifungal, immunosuppressive, and anti-mitotic activities, and is produced by the fungus *Streptomyces hygroscopicus*[11]. Sirolimus is cytostatic, and produces cell-cycle arrest in the G1/S phase transition. Sirolimus eluting stents (SES) slowly elute over a time frame of four to six weeks.

Second generation stents

Everolimus and zotarolimus are drugs used in second generation durable polymer stents. Second generation stents commonly used are zotarolimus eluting stent

Table 1 Types of stents

Generation of DES	Drug eluted	Some commercially available products	Features
First generation	Sirolimus, Paclitaxel	TAXUS, CYPHER	High Incidence of stent thrombosis, subacute as well as late thrombosis
Second generation	Zotarolimus, Everolimus	ENDEAVOR,XIENCE V	Safer and more efficacious as compared to first generation stents
Third generation	Novolimus, Biolimus A9	SYNERGY, BIOMATRIX, NOBORI, DESyne	Newer generation biodegradable stents which have shown superior outcomes

DES: Drug eluting stents.

(ZES) ENDEAVOR (Medtronic Inc. NJ) and everolimus eluting stent (EES), XIENCE V (Guidant Corporation, IN). Everolimus is a derived from sirolimus. Everolimus has a shorter half-life, and a greater bioavailability. It also has different blood metabolite patterns, as compared to sirolimus[13].

Third generation stents

Newer generation biodegradable drug-eluting stents are designed to manage the longer side effects of residual durable polymer which persist after the drug has been completely eluted. The biodegradable polymer is applied to the abluminal side or outside surface only. Thus the inner or luminal side is free from the drug. After 3-4 mo of implantation, this stent loses most of its coating, acquiring a profile which is similar to that of a BMS[14,15]. Novolimus and Biolimus A9 have been used in the third generation biodegradable stents. Biolimus A9 is a highly lipophilic analogue of sirolimus. The uptake by the coronary vessel wall is much better, thus the risk of systemic immunosuppression and toxicity is reduced[16]. Novolimus is an active metabolite of sirolimus. It provides efficacy at lower dose (85 mcg of novolimus *vs* 140 mcg of sirolimus) and a lower polymer load[17]. Recent ones introduced are the SYNERGY, BioMatrix, Nobori and DESyne stents[18]. The NOBORI is a biodegradable biolimus eluting stent. Third generation stents with bioreabsorbable scaffolds such as the Abbott's BVS®, an everolimus-eluting device with a poly-L-lactic acid (PLLA)-base, is now seeing increasing clinical use. Elixir's DESolve®, a PLLA-based novolimus-eluting device is another device used clinically. Biotronik's DREAMS®, a metallic magnesium- based paclitaxel-eluting device, is a third device that has been deployed[19]. The drug attaches directly, without polymer to the textured stent surfaces, in stents such as the BioFreedom stents and Yukon Choice stents[18]. Coatings which are non-pharmacological, such as carbon, silicon carbide and titanium-nitride-oxide provide better outcomes than BMS. Gene eluting stents such as the Genous stent, function by promoting the attachment of endothelial progenitor cells[18].

A meta-analysis of 51 trials that included a total of 52158 randomized patients concluded that all DES have demonstrated superior efficacy when compared with BMS[20]. First generation stents have a high incidence of stent thrombosis, both subacute as well as late thrombosis[9]. Among DES, second-generation devices are substantially safer and more efficacious when compared with first-generation devices[20]. These second generation stents are now being used to revascularize blocked left main coronary artery and are clearly superior to CABG. RESOLUTE all-comers (Randomized Comparison of a Zotarolimus-Eluting Stent with an Everolimus-Eluting Stent for Percutaneous Coronary Intervention) trial showed that ZES was noninferior to EES at 12-mo for the primary end point of target lesion failure[21]. The NOBLE (Coronary Artery Bypass Grafting *vs* Drug Eluting Stent Percutaneous Coronary Angioplasty in the Treatment of Unprotected Left Main Stenosis) and EXCEL (Evaluation of XIENCE Everolimus Eluting Stent *vs* Coronary Artery Bypass Surgery for Effectiveness of Left Main Revascularization) trials were conducted to compare PCI *vs* CABG. The EXCEL trial concluded that there was a equipoise for long-term mortality between CABG and PCI in subjects with unprotected left main coronary artery (ULMCA) disease up to an intermediate anatomical complexity. The anatomical and clinical characteristics impacted the decision making between CABG and PCI, and also in prediction of the long term mortality[22]. Clinical characteristics which shifted long-term mortality predictions in favor of PCI was COPD, male gender and old age. Reduced left ventricular ejection fraction, lower creatinine clearance, younger age and female gender favored CABG[22]. Thus PCI of the ULMCA with drug-eluting stents is safe and effective when performed in high volume centers with expertise[23]. The SYNERGY bioabsorbable polymer everolimus-eluting stent was noninferior to the PROMUS Element Plus everolimus-eluting stent with respect to 1-year target lesion failure[24]. In a large meta-analysis, bioabsorbable polymer based biolimus eluting stents (BP-DES) were associated with superior clinical outcomes compared with BMS and first generation DES and similar rates of death/MI, MI and target vessel revascularization (TVR) compared with second generation durable polymer DES. However, there were higher rates of ST compared with cobalt chromium EES[25]. The novolimus eluting coronary stent DeSyne was found to be superior to ZES at a five year follow up[26].

Various strategies have been employed to reduce the adverse effects associated with the drug eluting stents. A novel curcumin loaded nanoparticles (Cur-NP) preparation administered intravenously after stent implantation recovered endothelium function by accelerating endothelial cells restoration[27].

Combretastatin CA4 inhibits the SMC cycle more

effectively than paclitaxel and sirolimus. It may be a newer antiproliferative drug which can be used for drug-eluting stents[28]. Another drug called MiR-21 modulates the post stenting inflammatory response. This may have a therapeutic potential to clinical efficacy of stenting[29].

ANTIPLATELET MEDICATION

Antiplatelet medications prevent thrombus formation till the stent is completely endothelialized. Intraluminal thrombus formation may lead to vascular occlusion, transient ischemia, or infarction[27]. Antiplatelet dugs interfere with platelet adhesion, release and/or aggregation[30].

Aspirin

Aspirin binds to enzyme cyclo-oxygenase preventing conversion of arachidonic acid to thromboxane, thus interfering with platelet action. Aspirin alone has little or no effect on angiographic or clinical restenosis. Lower doses of aspirin, 75-100 mg, are used in combination with other antiplatelet agents. Higher dose of aspirin is associated with increased risk of bleeding when used along with clopidogrel without any added benefit[31].

Aspirin irreversibly inhibits platelets. Therefore, its action lasts until a significant number of platelets have ben synthesized. By day 3, complete recovery of platelet aggregation may occur in 50% of cases. By day 4, complete recovery occurs in approximately 80% of cases[32]. Reduced aspirin responsiveness can be measured by impedance platelet aggregometry[33]. Some of the potential causes of reduced aspirin responsiveness include non-compliant intake, genetic polymorphisms of COX-1, increased platelet turnover and drug interactions[34].

Clopidogrel

Clopidogrel has an active metabolite which irreversibly inhibits the acts on the ADP P2Y12 receptor. The P2Y12 receptor plays a vital role in the formation of a thrombus since it amplifies and completes the ADP response to thromboxane, thrombin and collagen[35], and completes the activation of GP Ⅱb/Ⅲa and GP Ⅰa/Ⅱa for further stabilization of platelet aggregates[36,37]. At steady state, the average inhibition level observed with a dose of 75 mg of clopidogrel per day is between 40%-60%. The prevalence of reduced clopidogrel response in patients is evaluated between 5% and 44%[38] and is termed as high on treatment platelet reactivity (HTPR). Some of the causes of clopidogrel HTPR include genetic polymorphisms of the P2Y12 receptor and of CYP3As, accrued release of adenosine phosphate, and up-regulation of other platelet activation pathways[35].

Ticagrelor

It is a direct-acting, oral, newer reversible P2Y12 receptor antagonist, and has a faster onset, and is more predictable and potent than clopidogrel. It binds allosterically to the platelet ADP P2Y12 receptor, thus, the binding does not cause a conformational change in the P2Y12 receptor. It has a short offset time. It does need metabolic activation. It has a superior safety profile as compared to clopidogrel or prasugrel as seen in the PLATO (Platelet Inhibition and Patient Outcomes) study[39]. It has been proven superior than clopidogrel in patients with chronic kidney disease. However, it should be avoided in patients with moderate-to-severe hepatic impairment and high bleeding risk[40]. Complications include lung injury and dyspnea due to endogenous adenosine release[41].

Prasugrel

Prasugrel is an oral irreversible inhibitor of the P2Y12 receptor. Current European Society of Cardiology guidelines recommend prasugrel or ticagrelor over clopidogrel in patients with acute coronary syndromes (ACS) after PCI[42]. If clopidogrel is used as a first line antiplatelet agent, then a platelet function assay should be performed, and a switch to prasugrel or ticagrelor is recommended for those with HTPR[43]. The advantage of prasugrel is that it has a 5%-6% or low percentage of non-responders[43].

Cangrelor

Cangrelor is an intravenous short-acting (half-life 3-6 min) P2Y12 inhibitor, which is directly reversible. It does not require metabolic conversion. Intravenous cangrelor can produce rapid platelet aggregation with almost full recovery of platelet activity within 60-90 min of withdrawal[44]. When cangrelor is administered intravenously to patients with CAD, the risk of MACE and stent thrombosis is reduced. There are however, increased events of minor bleeding[44]. Additionally, cangrelor plays an important role in cases where cardiologist is not comfortable preloading a patient with antiplatelet therapy before an angiography, when it is uncertain that the patient may need urgent surgery. It has been recently approved by the FDA in June 2015[45].

It is useful as a "bridging therapy" in patients with stents or acute coronary syndrome who need surgery, since they are increased risk for stent thrombosis when oral P2Y12 therapy is temporarily stopped[46].

The optimal duration of dual antiplatelet therapy has been a topic of debate. Most trials which compare antiplatelet strategies after PCI in a population state that the risk of bleeding and ischemia are average. Unfortunately, the information to recommend choices based on individual patient risks is scarce, especially beyond 1 year of DES placement and DAPT. There are many common risk factors associated with individual patient risks of ischemia and bleeding[47].

A trial compared 6 wk of clopidogrel, aspirin and oral anticoagulation medications with 6 mo of clopidogrel therapy. However, there were no superior outcomes with the 6 wk triple therapy[48]. Another study determined when permanent DAPT is discontinued before 30 d post cobalt chromium everolimus-eluting stent implantation, there was a strong association with ST. If the DAPT was discontinued after 90 d, it was safer[49]. A large multicenter

study determined that the safety and efficacy of a 6-mo DAPT post implantation of new-generation DES was noninferior to that of a 12-mo DAPT[50].

There is a lot of debate regarding short term dual antiplatelet therapy *vs* extended dual antiplatelet therapy. A study concluded that extended DAPT is associated with 8 fewer myocardial infarctions per 1000 treated patients per year. But unfortunately, there were 6 more major bleeding events than shorter-duration DAPT. Thus the duration of the DAPT should ideally be optimized taking into account the patient's values and preferences[51] A meta-analyses concluded that among selected patients undergoing DES implantation, a short duration (3-6 mo) of DAPT appears as the safest strategy. An extended duration (24-36 mo) of DAPT reduces thrombotic complications but with an excess in major bleeding complications[52-54]. The duration of DAPT is challenging to adjust in those patients with an increased bleeding or thrombotic risk. These patients need a personalized DAPT duration, which is tailored to patients's, not stent's, characteristics[55].

Two large studies, the Patient Related Outcomes With Endeavor vs Cypher Stenting Trial (PROTECT), and PROTECT US, determined that at a median follow-up of 4.1 years, major bleeding occurred in 2.8% subjects and ischemic events in 6.3%[47]. There was no difference in mortality or stroke[56].

The SECURITY trial which studied 6 mo *vs* 12 mo dual antiplatelet therapy following second generation DES implantation concluded that in a low-risk population, the 6 mo of DAPT following second-generation DES implantation was acceptable for the incidence of death, MI and stroke[57]. The OPTIMIZE trial results stated that in patients with stable coronary artery disease or low-risk ACS treated with zotarolimus-eluting stents, 3 mo of DAPT was noninferior to 12 mo for NACCE, (NACCE; a composite of all-cause death, myocardial infarction (MI), stroke, or major bleeding) without significantly increasing the risk of stent thrombosis[58].

The 2014 ACC/AHA current guidelines[59] recommend 12 mo of DAPT post DES implantation. As the result of several randomized clinical trials showing the safety of a shorter duration of DAPT, the European Heart Society altered their recommendations to 6-12 mo of DAPT post DES implantation[42].

PERIOPERATIVE MANAGEMENT

Transplant organ recipients usually have end stage organ disease and other comorbidities, and can be assigned the American Society of Anesthesiologists Grade 4 status. Furthermore, all transplant surgery can be classified as high risk. Thus, potential transplant recipients with drug eluting stents require extensive workup and evaluation. It is essential that the transplant anesthesiologist, surgeon and cardiologist be a part of the multidisciplinary team to help determine the optimal management for surgery in these patients. Such patients also need to be screened carefully by the Transplant Center's Selection Committee prior to UNOS listing as a potential organ recipient. Major considerations would be whether the recipient would tolerate such a high risk associated with the transplant surgery and whether the organ is being optimally allocated (Table 2).

Living donor transplant surgery is an elective procedure and can be optimally timed so that the risk of intraoperative bleeding and ischemia is minimized in a drug eluting stent recipient. On the other hand, cadaveric organ availability is unpredictable, therefore, the discontinuation of antiplatelet therapy cannot be optimally planned. Discontinuation of anti-platelet medication for transplant surgery can pose a significant challenge for perioperative management. Patients undergoing transplant surgery soon after the placement of coronary stents are at increased risk of ST in the perioperative period. The risk of perioperative ischemia is higher if the stent were originally inserted for ACS rather than stable coronary artery disease (SCAD). When antiplatelet therapy is discontinued due to risk of bleeding, the risk of ST is clearly elevated, especially during surgery, which is generally a hypercoagulable state due to increased fibrin formation. If the antiplatelet therapy is continued, there may be bleeding, which in turn leads to hypotension. Hypotension may slow the blood through the stent resulting in ST. Thus risk of ST will be elevated in the perioperative period regardless of whether the antiplatelet therapy is continued or not. If the patient is on top of the Transplant Center's recipient list, one may discontinue oral antiplatelet medication and use a bridging therapy till a cadaveric organ is obtained. However, such a strategy may have inherent risks and would need meticulous monitoring.

ACC/AHA guidelines state in patients undergoing urgent noncardiac surgery during the first 4 to 6 wk after BMS or DES implantation, dual antiplatelet therapy should be continued unless the relative risk of bleeding outweighs the benefit of the prevention of stent thrombosis. In patients who have received coronary stents and must undergo surgical procedures that mandate the discontinuation of P2Y12 platelet receptor-inhibitor therapy, it is recommended that aspirin be continued if possible and the P2Y12 platelet receptor-inhibitor be restarted as soon as possible after surgery. Perioperative management of antiplatelet therapy should be formulated by a team of the surgeon, anesthesiologist, cardiologist, and patient, who should weigh the relative risk of bleeding with that of stent thrombosis[59].

Aspirin is usually continued throughout the surgical procedure. The 2014 European Society of Cardiology and European Association for Cardiothoracic Surgery guidelines on myocardial revascularization support the 5 d clopidogrel withdrawal period before CABG. These guidelines also add that platelet function testing should be used to guide antiplatelet therapy interruption rather than a specified arbitrary time period[42]. Recent studies state that patients on aspirin and clopidogrel < 5 d before CABG who had preoperative ADP-induced platelet aggregation ⩾

Table 2 Antiplatelet drugs

Drug	Mechanism of action	Duration of action	Platelet responsiveness	Features
Aspirin	Aspirin binds to enzyme cyclo-oxygenase preventing conversion of arachidonic acid to thromboxane	Effect of aspirin lasts until a significant pool of new platelets is synthesized	Reduced aspirin responsiveness can be measured by impedance platelet aggregometry	Aspirin alone has little or no effect on angiographic or clinical restenosis
Clopidogrel	Irreversibly inhibits the ADP P2Y12 receptor	At steady state, the average inhibition level observed with a dose of 75 mg of clopidogrel per day is between 40%-60%	The prevalence of reduced clopidogrel response in patients is evaluated between 5% and 44% and is termed as HTPR	Some of the causes of clopidogrel HTPR include genetic polymorphisms of the P2Y12 receptor and of CYP3As, accrued release of adenosine phosphate, and up-regulation of other platelet activation pathways
Ticagrelor	Direct-acting, oral, newer reversible P2Y12 receptor antagonist	It binds allosterically to the platelet ADP P2Y12 receptor, thus, the binding does not cause a conformational change in the P2Y12 receptor. It has a short offset time	More predictable and potent than clopidogrel	Should be avoided in patients with moderate-to-severe hepatic impairment and high bleeding risk. Complications include lung injury and dyspnea due to endogenous adenosine release
Prasugrel	Oral irreversible inhibitor of the P2Y12 receptor	Effect of prasugrel lasts until a significant pool of new platelets is synthesized	Better inhibition for those with high HTPR	A 5%-6% or low percentage of non-responders
Cangrelor	Intravenous directly reversible P2Y12 inhibitor	Half-life 3-6 min	Rapid platelet aggregation with almost full recovery of platelet activity within 60-90 min of withdrawal	Useful to preload with antiplatelet therapy before the angiography should the patient's anatomy require urgent surgery

HTPR: High on treatment platelet reactivity.

50% have bleeding risk similar to those receiving aspirin monotherapy, thus a 5 d clopidogrel discontinuation period may not always be necessary[60]. Guidelines also recommend the discontinuation of ticagrelor 5 d prior to surgery and recommencing therapy as soon as it is safe to do so. Since prasugrel has more prolonged and effective platelet inhibition than clopidogrel, it should be stopped 7 d prior to surgery[42].

The risk of stent thrombosis is associated with stent type and time from stenting to surgery. It will be highest if BMS or DES is inserted within 30 d of the transplant surgery. The risk is high when the surgery is carried out < 1 mo after BMS and < 6 mo after DES, is intermediate if performed between 1-6 mo after BMS and 6-12 mo after DES, and low if performed > 6 mo after BMS and > 12 mo after DES[61].

A study involving over 12000 patients with previous coronary stenting who underwent over 17000 surgical procedures stated that cardiac death occurred in 2.5%, myocardial infarction in 1.5%, and serious bleeding event in 6.4%. Surgery increased 1.58 × the risk of cardiac death during follow-up. Older generation stents were associated with higher risk of adverse events as compared to BMS > 12 mo before surgery. Newer DES showed similar safety as BMS > 12 mo and between 6 and 12 mo. They also trended to be safer between 0 and 6 mo[61].

European Guidelines state that most surgical procedures can be performed on DAPT or ASA alone with acceptable rates of bleeding[42]. The timing of surgery mattered most during the first 6 mo after PCI, with respect to MACE events. There was no association of the stent type (BMS *vs* DES) with MACE after surgery. The guidelines further state that whenever possible, the elective non cardiac surgery should be postponed till the completion of the full course of DAPT ideally, 6 mo in SCAD and 1 year in acute coronary artery syndrome (ACS) patients, and that surgery be performed without discontinuation of aspirin[42]. Shorter duration of DAPT may be justifiable if surgery cannot be delayed. In very high risk patients, 5 d prior to surgery, patient maybe switched from clopidogrel to a reversible antiplatelet agent with a short half-life such as Ⅳ tirofiban or eptifibatide, and stop the infusion 4 h prior to surgery[42]. The substitution of DAPT with LMWH or UFH is ineffective. In surgical procedures with low-to-moderate bleeding risk, surgeons should be encouraged to operate while maintaining DAPT[42].

Various Platelet Function Assays for P2Y12 Receptor Antagonisms are Light Transmittance Aggregometry, (LTA), vasodilator stimulated phosphoprotein (VASP), VerifyNow, TEG Plateletmapping and Multiple Electrode Aggregometry (MEA)[62]. The LTA uses plasma and optically measures platelet aggregation, and is considered the gold standard. The VASP uses whole blood and flow cytometry to specifically measure P2Y12 activity, as it is the only assay which is not affected by the ADP's effect on the P2Y1 receptor, and thus is specific for P2Y12 inhibition. The VerifyNow P2Y12 assay uses whole blood, and optically measures platelet aggregation. Advantages of VerifyNow is that it is readily available in clinical settings and is a point of care assay[62]. The Assessment

of Dual AntiPlatelet Therapy with Drug-Eluting Stents (ADAPT-DES) trial is a very large observational platelet function study. It stated that upto 50% of 30-d post-PCI ST could be attributed to HTPR, which was defined as a P2Y12 reaction unit value of > 208 with VerifyNow® test[63]. Point of care platelet function testing can also be done with TEG Plateletmapping (TEG-PM). It measures the degree of platelet inhibition resulting from aspirin or ADP receptor antagonists and correlates well with light transmission aggregrometry[64]. TEG-PM can measure the percentage adenosine 5'-diphosphate platelet receptor inhibition (ADP-PRI) by clopidogrel prior to urgent transplant surgery. An ADP PRI of 30% or more can be classified as high bleeding risk. Another study was conducted to predicted risk of bleeding and adverse outcomes by TEM-PM in patients taking clopidogrel within 7 d of non-cardiac surgery. Interestingly, there was no correlation between duration of clopidogrel omission and percentage ADP-PRI[65].

Excessive bleeding can be treated by allogenic platelet transfusions (PT) in patients on P2Y12 receptor inhibitors. Though the American Association of Blood Banks 2015 clinical practice guidelines suggests prophylactic platelet transfusion for patients having major elective nonneuraxial surgery with a platelet count less than 50 × 10^9 cells/L, there is no recommendation for platelet transfusions for patients on dual antiplatelet therapy[66]. In the APTITUDE-Coronary Artery Bypass Graft (APTITUDE- CABG) study, VASP reactivity index, was assessed before and after *in vivo* PT administered for excessive bleeding in patients undergoing cardiac surgery while on a maintenance dose of aspirin and clopidogrel (n = 45), prasugrel (n = 6), or ticagrelor (n = 3). When compared with baseline, there was a significant relative increase of 23.1% in platelet activation after PT transfusion. PT restores platelet reactivity in patients with ACS/PCI and in patients undergoing cardiac surgery on P2Y12 RI while bleeding with a less effect with increasing potency of P2Y12 inhibition[67]. A recent study stated that clopidogrel had no effect on donor PLT function. Prasugrel has mild effect on donor platelet function. Ticagrelor completely abolished ADP mediated PLT activation in all assays tested. The observed effects were due to Ticagrelor and not elevated adenosine concentrations in the patient's plasma. A modified multiple electrode aggregometry (MEA) assay can be used to determine whether the patient would be likely to benefit from platelet (PLT) transfusions[68].

The BRIDGE trial was a pharmacodynamic study evaluating platelet reactivity of cangrelor *vs* placebo in ACS and/or patients with a stent who were at increased risk of thrombotic events because of discontinuation of an oral P2Y12 inhibitor before cardiac surgery[46]. The primary efficacy end point [percentage of patients with all samples during the infusion achieving platelet reactivity unit (PRU) < 240 as determined by VerifyNow P2Y12 assay, Accumetrics, San Diego, CA] was met in 98.8% of cangrelor- treated patients compared to 19.0% of placebo-treated patients. After discontinuation of cangrelor, platelet reactivity was similar for both cangrelor and placebo groups[55]. Cangrelor has been approved by the FDA in June 2015[45]. When cangrelor occupies the P2 Y12 receptor, the active metabolite of clopidogrel is unable to bind to it. However, this reaction is avoided when clopidogrel is given at the end of the cangrelor infusion. Earlier administration increases the recovery of platelet function. Antiplatelet effects of prasugrel were apparent when prasugrel was administered 0.5 h before cangrelor was stopped[69,70].

In the Drug Eluting Stent Event Registry of Thrombosis (DESERT)[71], the largest case- control registry of late/very late thrombosis after DES, 75% of ST events occurred after 1 year, similar to the 60% rate observed in a study[71]. Furthermore, the clinical presentation of late/very late ST events in DESERT was mainly ST-segment-elevation myocardial infarction (67%). More than half of all ST-related MIs were Q-wave MIs, and subsequent mortality was increased 8-fold after an ST-related MI, the greatest hazard of any MI type[71].

In stent restenosis can be managed with BMS, brachytherapy, rotational atherectomy and cutting balloons, DEB and DES. A meta-analysis concludes that for treatment of any type of coronary in-stent restenosis (ISR), PCI with everolimus-eluting stents is optimal, because of the best angiographic and clinical outcomes. Use of drug coated balloons (DCB) is also favored, because of its ability to provide favorable results without adding a new stent layer[73]. Additionally, when DES are implanted to treat BMS restenosis, at 6 mo, struts coverage is more complete when compared with DES implanted in atherosclerotic lesions[74]. In patients with DES-ISR, EES were superior, both clinically, as well as angiographically, when compared with DEB[75].

POST TRANSPLANT IMMUNOSUPPRESSION

The drugs sirolimus, everolimus, biolimus and novolimus are inhibitors of the mammalian target of rapamycin (mTOR). After organ organ transplantation, the mTORs are used along with calcineurin inhibitors (CNIs) to provide immunosuppression. They are also used as proliferation signal inhibitors coated on DES. Their use in cancer therapy bears the same mechanism. Everolimus antagonizes the negative effects of CNIs kidney cell and neuronal metabolism and stimulates mitochondrial oxidation, thus reducing the vascular inflammation[13]. In transplantation, everolimus has been used post-transplant in heart, liver, lung and kidney transplant recipients to prevent acute rejection. In kidney transplant patients, everolimus may minimize or remove calcineurin inhibitors[76]. Interestingly, renal transplant patients with DES had a low rate of ST, probably related to the immunosuppressants given to prevent kidney rejection[77]. Everolimus has also been approved by the FDA for use in liver transplantation (LT), and is safe for use with tacrolimus within the first month

after LT[78].

POST TRANSPLANT ENDOVASCULAR INTERVENTION WITH DES

DES has been successfully used to stent stenotic lesions post-transplant surgery. Transplant coronary artery disease (TCAD) is a major cause of morbidity and mortality after the first year after orthotropic heart transplantation (OHT). OHT patients with ISR have poor long-term prognosis[79]. EES used on OHT patients with TCAD is associated with a low incidence of target vessel revascularization (TVR) and target lesion revascularization (TLR)[80]. Unfortunately, long-term mortality remains high in orthotropic heart transplantation (OHT) recipients after PCI with either DES or BMS[81].

Transplant renal artery stenosis (TRAS) following kidney transplantation has an incidence rate ranging from 6% to 23%. Endovascular intervention with DES improves blood pressure control and allograft function[82]. ISR occurs in as many as 13% of patients after PTA and stent insertion. A case report describes three such patients, of which, in two patients, the transplant renal artery remained patent after insertion of PES, and one patient required balloon angioplasty 7 mo after the DES was inserted[83]. BMS have been used to treat lung transplant related pulmonary artery stenosis[84]. DES have been placed into the pulmonary veins as a bridge to heart lung transplantation in a patient with extensive and recurrent congenital pulmonary vein stenosis[85]. DES have been safely used and may prevent ISR in patients who undergo intracoronary bone marrow mononuclear cell transplantation post coronary stenting[86]. Orthotropic liver transplantation (OLT) is commonly complicated by hepatic artery stenosis (HAS). It can lead to hepatic artery thrombosis, with subsequent liver failure in 30% of the patients. Though traditionally this was managed with either surgical revascularization or retransplantation, use of DES has resulted in high technical success and provided for excellent patency. Avoidance of hepatic artery thrombosis is possible in > 95% of patients with endovascular treatment and close follow-up[87]. Paclitaxel eluting balloon has been employed successfully to treat biliary anastomotic strictures after liver transplantation[88]. Stents have also been used to manage stenosis in the hepatic veins and/or inferior vena cava above hepatic venous anastomosis to relieve an outflow venous block following living donor liver transplantation[89].

CONCLUSION

Though several perioperative challenges encountered in organ transplantation surgery and patients with drug eluting stents, these can be optimally managed with proper planning and teamwork, ensuring patient safety.

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REVIEW

Complement related kidney diseases: Recurrence after transplantation

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Abstract

The recurrence of renal disease after renal transplantation is becoming one of the main causes of graft loss after kidney transplantation. This principally concerns some of the original diseases as the atypical hemolytic uremic syndrome (HUS), the membranoproliferative glomerulonephritis (MPGN), in particular the MPGN now called C3 glomerulopathy. Both this groups of renal diseases are characterized by congenital (genetic) or acquired (autoantibodies) modifications of the alternative pathway of complement. These abnormalities often remain after transplantation because they are constitutional and poorly influenced by the immunosuppression. This fact justifies the high recurrence rate of these diseases. Early diagnosis of recurrence is essential for an optimal therapeutically approach, whenever possible. Patients affected by end stage renal disease due to C3 glomerulopathies or to atypical HUS, may be transplanted with extreme caution. Living donor donation from relatives is not recommended because members of the same family may be affected by the same gene mutation. Different therapeutically approaches have been attempted either for recurrence prevention and treatment. The most promising approach is represented by complement inhibitors. Eculizumab, a monoclonal antibody against C5 convertase is the most promising drug, even if to date is not known how long the therapy should be continued and which are the best dosing. These facts face the high costs of the treatment. Eculizumab resistant patients have been described. They could benefit by a C3 convertase inhibitor, but this class of drugs is by now the object of randomized controlled trials.

Key words: Kidney disease recurrence; Complement dysregulation; Atypical hemolytic uremic syndrome; C3 glomerulopathies; Dense deposit disease; Plasma therapy; Eculizumab; C3 glomerulonephritis

Core tip: Complement cascade is an important pathway of several kidney diseases. A distinction should be made between kidney diseases with complement overactivation

and those with complement dysregulation. The latter are related to congenital or acquired abnormalities of complement factors. These diseases are linked to constitutional abnormalities of the patients, have high recurrence rate after renal transplantation and represent an important cause of graft loss. Diagnosis and treatment are not easy to be made. Just in the last decade a growing knowledge in the field of genetic and biology allowed the complement inhibitors to be the first class drug in the treatment.

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INTRODUCTION

The aim of this review is to highlight the relevance of recurrent diseases after kidney transplantation and, in particular, to discuss the frequency and severity of recurrence of two groups of renal diseases strictly related to each other: C3 glomerulopathy (C3G) and thrombotic microangiopathy (TMA).

Along with the improved control of acute rejections and infections, the recurrence of primary nephropathy has become the most important cause of graft loss principally for patients who have glomerulonephritis (GN) as the primary disease[1,2]. In some series, recurrence of the original disease was reported to be the principal cause of graft loss more than one year after transplantation[2] (Table 1). Some renal diseases have a higher risk of recurrence and recurrence-related graft loss. Hariharan *et al*[3] observed, in a total of 4913 renal transplants, that the greatest relative risk (RR) for graft failure was related to the recurrence of hemolytic uremic syndrome/thrombotic thrombocytopenic purpura (HUS/TTP) (5.36), membranoproliferative glomerulonephritis (MPGN) (2.77) and focal segmental glomerulosclerosis (FSGS) (2.25). Interestingly, HUS/TTP and many cases of MPGN were related to complement cascade dysregulation and were ascribed to genetic abnormalities or to acquired abnormalities of components of the complement system[4].

ROLE OF COMPLEMENT IN KIDNEY DISEASES

The complement proteins may be seen in the biopsies of all forms of GN, and all three activation pathways have been documented in different kidney diseases and may be activated by different triggers[5] (Figure 1). Indeed, the complement system is activated by three pathways (the alternative, classical and lectin pathways) that generate a common terminal pathway. The classical (CP) and lectin pathways (LP) are triggered by the recognition of pathogens or damaged cell surfaces by antibodies and recognition molecules[6]. Many glomerular diseases, such as membranous nephropathy, IgA nephropathy and lupus nephropathy, involve these pathways. The activation of the alternative pathway (AP) is relatively complex. The AP undergoes continuous low-grade activation in the fluid phase by spontaneous C3 hydrolysis that is responsible for the deposition of a low amount of C3b onto cell surfaces (Figure 2). Self-surfaces are protected from complement damage by several regulators that are either membrane-anchored or in the fluid phase. Perturbation of the balance between complement activators and regulators provides the basis for aHUS and MPGN/C3G[7].

As mentioned above, the complement system is involved in the vast majority of kidney diseases. Two broad categories of kidney diseases should be distinguished. The first category is associated with complement over-activation and characterizes diseases such as lupus nephritis, membranous nephropathy, immune complex-associated MPGN and IgA nephropathy. The second category is related to complement dysregulation and characterizes diseases such as aHUS and C3G. In the former category, complement is activated by other factors, including immune-complex formation and deposition. After transplantation, the original disease may recur but is also more easily controlled by the immunosuppression needed to support the transplanted kidney. In the latter disease, complement activation may occur spontaneously and is often related to abnormalities of complement regulating factors. These nephropathies often recur after renal transplantation because the diseases are related to a constitutional and often genetically determined abnormality of the complement proteins. These abnormalities are not corrected either by the transplant itself or by the immunosuppressive therapy.

Tremendous advances are being made in our understanding of both aHUS/TMA and C3G. With the improvement of our understanding of genetics and biology, it has become increasingly clear that different disease mechanisms may cause the disease formerly called TTP/HUS. Furthermore, these mechanisms may deeply influence the recurrence rate after transplantation[8].

Similarly, the role of complement in C3G has been better defined[9], thus allowing us to move from a histologically based classification of the MPGNs to a new classification based on pathophysiology[10,11].

To date, the term aHUS applies to a heterogeneous group of diseases that have in common a TMA associated with some degree of renal failure. Frequently, aHUS patients have a complement abnormality (a genetic mutation or an autoantibody to complement factors) as the primary etiology. As a consequence, they are affected by a complement mediated TMA (Figure 3)[8].

Similarly, after reclassification, the MPGNs are distinguished into immune-complex- mediated MPGNs and C3Gs. The latter have clear signs of C3 staining with little

Table 1 Causes of graft loss (living kidney transplantation)

	< 1 yr		> 1 yr	
	Non identical	Identical	Non identical	Identical
Acute rejection	5 (41.7%)	0 (0%)		
CAN with/without CR	2 (16.7%)	0 (0%)	16 (31.4%)	5 (23.8%)
CNI nephrotoxicity	0 (0%)	0 (0%)	2 (3.9%)	1 (4.8%)
Recurrence of original disease	1 (8.3%)	0 (0%)	10 (19.6%)	6 (28.6%)
Death with functioning graft	2 (16.7%)	1 (50%)	19 (37.3%)	7 (33.3%)
Discontinuation of immunosuppressant	0 (0%)	1 (50%)	4 (7.8%)	1 (4.8%)
Non-compliance	1 (8.3%)	0 (0%)	0 (0%)	1 (4.8%)
Others	1 (8.3%)	0 (0%)	0 (0%)	0 (0%)
P	0.2002		0.6158	

CAN: Chronic allograft nephropathy; CR: Chronic rejection; CNI: Calcineurin inhibitor.

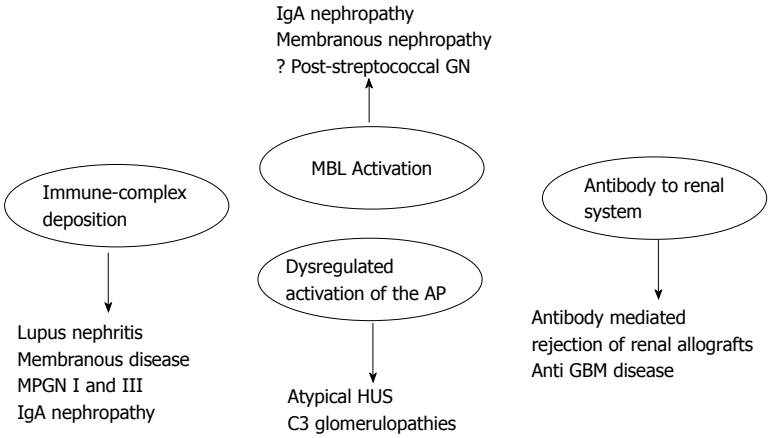

Figure 1 Mechanisms of complement activation in kidney disease. HUS: Hemolytic uremic syndrome; MBL: Mannose binding lectin; IgA: Immunoglobulin A; MPGN: Membranoproliferative glomerulonephritis; GBM: Glomerular basement membrane; AP: Alternative pathway.

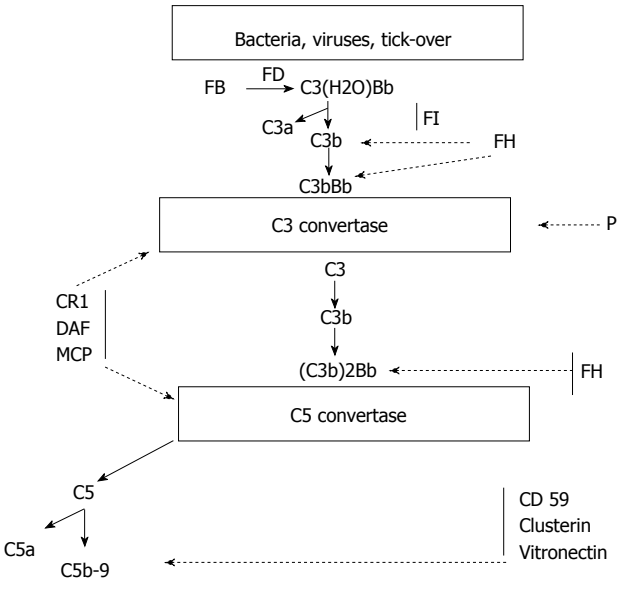

Figure 2 The C3 complement alternative pathway. C3(H2O) Bb: Alternative pathway initiation convertase; FB: Complement factor B; FD: Complement factor D; FH: Complement factor H; FI: Complement factor I; CR1: Complement receptor 1; DAF: Decay accelerating factor; MCP: Membrane cofactor protein; P: Properdin.

or no immunoglobulin deposition evident on renal biopsy. C3Gs are further divided into dense deposit diseases (DDD) and the recently recognized entity C3GN[11].

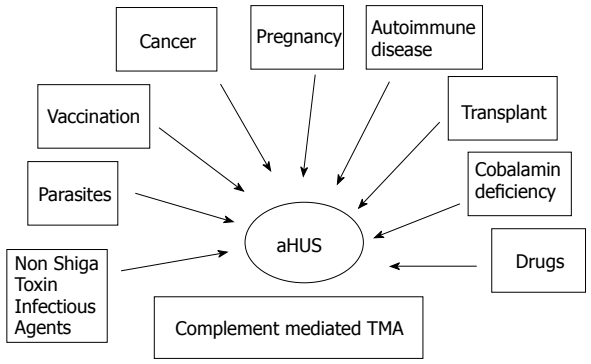

Figure 3 Heterogeneity of atypical hemolytic uremic syndrome. TMA: Thrombotic microangiopathy; HUS: Hemolytic uremic syndrome.

EPIDEMIOLOGY

GNs that occur in the transplanted kidney may be caused either by recurrent or *de novo* disease. In clinical and in the epidemiological studies is necessary to distinguish between these conditions. True recurrence occurs when: (1) post-transplant proteinuria or hematuria or elevated serum creatinine is found after transplantation; (2) biopsy-proven kidney disease is diagnosed in the native kidneys; or (3) the same disease is proven by biopsy in the transplanted kidney[12]. Challenges to the diagnosis of recurrent diseases are manifold. They include: (1)

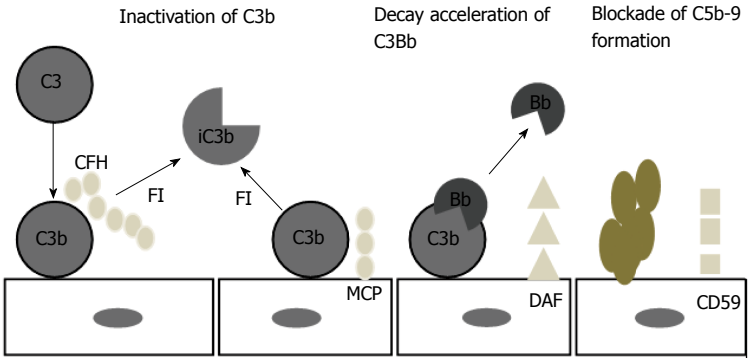

Figure 4 Regulation of complement on surfaces. CFH: Complement factor H; FI: Complement factor I; iC3b: Inactivated C3b; MCP: Membrane cofactor protein; DAF: Decay accelerating factor; CD59: MAC inhibitor protein.

misdiagnosis or mislabeling of native kidney disease; (2) lack of a unified approach to using diagnostic tools for the diagnosis of recurrent disease; and (3) difficulties in differentiating recurrent disease from other causes of renal damage such as drug toxicity and chronic rejection[3,13].

There are still other potential biases occurring among registries dealing with recurrences of renal diseases mediated by complement dysregulation. For example Shiga toxin-related HUS combined with aHUS in many registries. Additionally, the vast majority of registries or networks report data by using the classification of MPGNs, which precedes the results of the consensus report on C3G[14] and the recent consensus report and reclassification of GNs[10,11].

Because of the above-mentioned factors, the data reported by different registries as the North American Pediatric Renal Transplant Collaborative Study (NAPRTCS), the Australia New Zealand Dialysis Transplant Data System (ANZDATA), the Renal Allograft Disease Registry (RADR) and the United States Renal Data System (USRDS) differ significantly in reporting the prevalence of recurrent GNs after transplantation[3,12,15-18]. A study by Shimmura *et al*[2] on 266 living kidney transplants clearly documents that recurrence of the original disease is the third leading cause of graft loss after one year from transplantation (Table 1). The aforementioned study by Hariharan[3] documents the highest RR for graft failure for HUS/TTP and MPGN.

Two other studies on pediatric patients[19,20] report high rates of recurrence for aHUS and type Ⅰ and Ⅱ MPGN according to the old classification, although there is a wide range of rates among the studies.

Series related to the early 2000s indicated that the risk of post-transplant recurrence for aHUS was 20% in pediatric patients and 50% in adult patients[21]. Recently, in 280 patients with aHUS screened for CFH, IF or MCP mutations, post-transplant aHUS recurrence was reported in 33%[22], 37%[23] and 60%[24], respectively.

Fewer data are available regarding the epidemiology of MPGN recurrence according to the new classification. Indeed, many registries are still using the old classification. According to these data, MPGN type Ⅰ recurs in 20%-30% of patients, whereas MPGN type Ⅱ recurs in 80%-100% of patients[25].

More recently, Kasiske *et al*[26], observing 1574 MP GNs in 140109 transplant patients recorded in the USRDS an observation that the true recurrence rate of MPGN increased over time, with the most frequent recurrences of GN between 1995 and 2003.

After the reclassification[10,11], the most interesting and recent data on C3G recurrence are those reported by Zand *et al*[27]. According to these data, the recurrence rate of C3GN is 66.7%, and graft failure occurs in 50% of patients with recurrence.

PATHOPHYSIOLOGY OF TMA AND ITS RECURRENCE

As mentioned above, the complement AP is constitutively active. After the generation of C3b, it binds either to either pathogens or the host cells. This necessitates the prompt and tight control of its activity. In turn, C3b may generate new C3 convertases (C3bBb) that act as an auto-amplifier by creating new C3b molecules. The same enzymes may also generate the C5 convertases that activate C5, the anaphylatoxins C5a and C5b and activate the membrane attack complex (MAC) C5b-C9[28]. In normal conditions, the AP may be spontaneously activated by the process called tick over. Cell surfaces are protected from auto-activation by several factors both in the fluid phase and anchored to the cell membranes.

The principal inhibiting factor is complement factor H (CFH), which acts both in the fluid phase and on cell surfaces. Factor H also act as a co-factor to complement factor I (CFI)[29-31]. Cell surfaces are also protected by at least 4 specific membrane regulators: (1) complement receptor 1 (CR1/CD 35); (2) membrane cofactor protein (MCP/CD46); (3) decay accelerating factor (DAF/CD55); and (4) protectin (CD59), which blocks MAC formation (Figure 4)[32,33].

The loss of this complex regulation results in complement activation, with consequent cell damage[34,35]. The role of complement dysregulation is increasingly recognized as the principal cause of TMAs. It may be caused by genetic mutations or by autoantibodies. Additionally, a triggering factor, often from the environment, is needed.

Figure 5 represents the whole spectrum of TMAs. In this figure, three different conditions are possible: (1) complement-driven TMA (*i.e.*, aHUS), where there is an underlying complement defect; (2) complement-

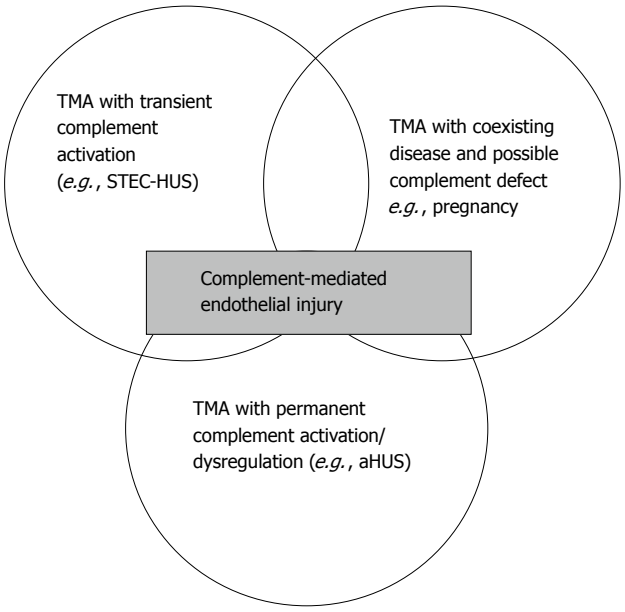

Figure 5 Spectrum of thrombotic microangiopathies. TMA: Thrombotic microangiopathies; STEC-HUS: Shiga toxin-producing *Escherichia coli*-hemolytic uremic syndrome.

enhanced TMA, as in Shiga-toxin-producing *Escherichia coli*-HUS (STEC-HUS); and (3) TMA with coexisting disease (*e.g.*, pregnancy, lupus). The most frequently mutated gene in aHUS is *CFH*. Mutations have been identified in 25% of sporadic cases and 40% of familial cases[6,36,37]. In addition to *CFH,* the *CFH* gene family includes five other complement factor H-related genes (*CFHR1-5*). Deletion, duplications and hybrid proteins occur[38]. As a result, the loss of cell surface protection may occur and aHUS may develop[36,39].

The *CFHR3-CFHR1* deletion is the most frequent deletion associated with aHUS. Recently a new role for the FHR protein was identified by Goicochea de Jorge *et al*[40]. According to these findings, FHRs could act as competitive antagonists to FH. *MCP* (CD46) mutations are found in 5%-9% of aHUS patients[36]. The protein is membrane-anchored and serves as a co-factor of FI. *FI mutations* lead to a lack of complement control accounting for 4%-8% of aHUS[36,37].

In addition to mutations in regulatory genes, gain of function mutations of effector proteins (C3 and factor B) has been reported. Factor B mutations are reported in < 4% cases of aHUS[41-43]. Factor B mutations enhance the C3bB formation. Gain of function mutations in C3 are reported in 2%-8% of aHUS[36,37]. These mutations impair MCP and confer resistance to cleavage by CFI[44,45].

Mutations in the thrombomodulin gene (*THBD*) have been reported in up to 5% of aHUS patients[36]. Few data have been reported on this mutation. It is probable that the *THBD* mutation may influence disease severity in association with other mutations[46].

Autoantibodies against different complement factors, independent of genetic mutations or in association with genetic abnormalities, may cause aHUS. Antibodies to anti-complement factor H (FHAA) account for 3%-8% of aHUS and are often found in association with *FHR1* deficiency or deletion[36]. Recently, in India, an association of FHAA and aHUS was reported in approximately 60% of the pediatric population[47]. Auto antibodies to anti-factor I have been reported only in three patients[48]. Their role is not yet clear.

In many cases, the development of aHUS in patients who are affected by complement abnormalities needs a triggering factor. Among these well-known factors are pregnancy, drugs, and autoimmune disorders. Complement abnormalities were identified in 83% of patients with pregnancy associated aHUS[49]. *CFH* mutations have been reported in 4 patients with aHUS associated with ticlopidine[50]. Further 4% of patients affected by lupus disease documented an aHUS related to complement abnormalities[51,52]. Complement abnormalities strongly influence aHUS recurrence after renal transplantation. Indeed, aHUS recurrence was reported at rates from 20% to 50% in the era when genetic analysis of complement proteins was not available[53-56]. Recently, several studies have highlighted the risk of aHUS recurrence according to different genes abnormalities[53] (Table 2). Complement abnormalities have also been found in conditions that should not be affected by recurrence.

STEC-HUS of the native kidneys should be protected from recurrence. However, two patients with a history of STEC-HUS were recently diagnosed with post-transplant recurrence[57]. Both patients were recognized to be affected by complement abnormalities; one had a heterozygous *CFI* mutation, and the other had a heterozygous *MCP* mutation.

Patients affected by *MCP* mutations rarely have aHUS recurrence after transplantation[58]. Recently, however, transplant failures due to aHUS recurrence have been observed in patients affected by *MCP* mutations[59]. Almost all of these patients were affected by combined *MCP* and *CFH* or *CFI* mutations.

Patients affected by *CFH* mutations are at a high risk of recurrence. In two French case series, recurrences were observed in 80% of children[22] and 75% of adults[60]. The graft failure rates in the case of recurrence are approximately 86%.

Interestingly, the location of CFH mutation impacts the recurrence risk[61]. Indeed, mutations involving the C-terminal domain of *CFH* confer higher risk and have a worse prognosis[49]. This finding is consistent with the critical role of the CFH C-terminal domain in binding to the endothelium and exerting the protective role of the endothelial cells[62].

The majority of FHAA is directed against the C-terminal domain of factor H. As a consequence, a higher risk of recurrence should be expected due to FHAA. However, this does not seem to be the case, because aHUS recurrence due to FHAA is uncommon[63].

Nonetheless, the recurrence risk due to FHAA is not easily understood because 40% of patients with FHAA are also affected by mutations in the complement genes[64]. Additionally, a reduction of FHAA is achievable with the immunosuppressant therapy, thereby reducing

Table 2 Risk of atypical hemolytic uremic syndrome recurrence according to the implicated genetic abnormality

Gene	Protein location	Functional impact	Mutation frequency in aHUS (%)	Recurrence frequency after transplantation (%)
Mutation				
CFH	Plasma	Loss	20-30	75-90
CFI	Plasma	Loss	2-12	45-80
CFB	Plasma	Gain	1-2	100
C3	Plasma	Gain	5-10	40-70
MCP	Membrane	Loss	10-15	15-20
THBD	Membrane	Loss	5	1 case
Genetic polymorphism (frequency in control population)				
Homozygous *CFHR1del* (3%-8%)	Circulating	Undetermined	14-23 (> 90% in patients with anti-CFH antibodies	NA

aHUS: Atypical hemolytic uremic syndrome; C3: Complement C3; CFH: Complement factor H; CFI: Complement factor I; CFB: Complement factor B; MCP: Membrane cofactor protein; THBD: Thrombomodulin; CFHR1: Complement factor H receptor 1; NA: Not available.

the risk of aHUS recurrence.

In summary, the risk of recurrence is 4 times higher in patients with mutations in the *CFH* gene or carriers of the hybrid gene between *CFH*/*CFHR1*. In a recent study by Le Quintrec[65], patients with the hybrid gene lost their grafts due to early recurrence.

The relevance of *CFI* mutations on aHUS recurrence has discordant results and interpretations. The first studies to *CFI* mutations reported a high recurrence rate and graft loss[10,31,53,58,66,67]. A study by Bienaimé *et al*[68] in 2010 reported that patients with *CFI* mutations do not seem to carry a higher risk of recurrence. These data were more recently confirmed by the study mentioned above by Le Quintrec *et al*[65].

MCP mutations rarely affect aHUS recurrence because the endothelial cell surfaces of the transplanted kidney normally express MCP. Only three recurrences have been reported in the literature[69,70]. In these patients, recurrence might be ascribed to combined complement gene mutations[59] or microchimerism from the recipient endothelial cells[70].

Data on the role of THBD are scarce. aHUS recurrence due to *THBD* mutations should not occur because the molecule is membrane-anchored as MCP. Additionally, a small proportion of THBD is present in soluble form. Nonetheless, sporadic cases of recurrence due to THBD have been reported[71,72]. In one patient the recurrence occurred early post-transplantation during the ischemia-reperfusion phase. During this phase, the soluble form of THBD might be not adequate to protect from recurrence.

Patients affected by gain of function mutations (*CFB, C3*) are also exposed to the risk of recurrence. To date, four patient carriers of the *CFB* mutation have been reported to have aHUS recurrences and consequent graft loss[73,74]. Data on recurrence in patients affected by *C3* mutations are discordant. Le Quintrec[65] reported a high recurrence rate, with 4 recurrences in 5 grafts. Previously, Noris *et al*[75] reported only two recurrences in 7 transplanted patients. In an attempt to explain the difference, Zuber *et al*[53] speculated that for some patients, the intra-graft production of normal C3 might occur and might be protective.

Several environmental triggers might act to damage the graft endothelium and to facilitate aHUS recurrence on already damaged cells in patients with genetic abnormalities.

Anti-HLA antibodies[76], ischemia-reperfusion events[77], immunosuppressant drugs[78] and viral infections[79], either isolated or in association, might play a relevant role and favor aHUS recurrence in genetically predisposed patients.

Le Quintrec *et al*[65] attempted to identify the risk factors for aHUS recurrence. Low C3 levels and the presence of a mutation were significant in the univariate analysis. In a multivariate analysis of mutations, a mammalian target of rapamycin (mTOR) inhibitor regimen and recipient age were significantly associated with increased aHUS recurrence rates.

PATHOPHYSIOLOGY AND RECURRENCE OF C3G

After the reclassification of MPGNs, as mentioned above[10,14], C3Gs included the MPGNs caused by complement dysregulation rather than the MPGN immune-complex-related disorders[80]. As a consequence, C3Gs include the GNs for which immunofluorescence microscopy is C3-positive and immunoglobulin-negative.

C3Gs may be sub-divided into DDD and C3GN based on electron microscopy, even if, in some cases the distinction is challenging[14,81]. Recently, advances toward an improved understanding of the characteristics of C3 deposits have been made through proteomic analysis and laser microdissection (LMD)[82]. Laser dissection and mass spectrometry of glomeruli from patients with C3G documented an accumulation of the AP and the terminal complement complex proteins, thus confirming that C3G results from abnormalities of the AP, which lead to glomerular damage[81].

The pathophysiology of AP pathway activation in C3GN and DDD is very similar, with fluid phase dysregulation due to gene mutations or autoantibodies occurring in both disorders. Indeed, as for aHUS, the complement abnormalities in C3Gs may occur on a genetic basis or as acquired factors as autoantibodies.

The most common acquired complement defect is represented by the presence of an antibody called the C3 nephritic factor (C3NeF), which blocks CFH-mediated decay and stabilizes C3 convertase[81,83]. In particular, C3NeF binds to C3 convertase and inhibits the action of factor H, CR1 and DAF, blocking the dissociation of the convertase. C3NeF enhances C3 convertase activity 10 fold[9,84,85]. The frequency of C3NeF is high in C3G, ranging from 50% to 80% of patients[83]. C3NeF may also be associated with genetic mutations. Recently, other auto antibodies have been found in C3Gs. These auto antibodies are directed against C3 convertase, factor B[86] or anti-factor H[87,88]. AP dysregulation in DDD is more frequently autoantibody-induced with respect to TMA. Genetic abnormalities also have been encountered. Few patients have been identified with genetic mutations of factor I, MCP, C3, factor B and factor H[83,89]. In an extensive study by Servais *et al*[83], only 5.3% of the patients affected by C3GN had *CFI* mutations, and 1.8% had *MCP* mutations.

In 2010, Martinez-Barricarte *et al*[90] identified a mutant C3 protein resistant to factor H inactivation in a patient affected by DDD. More recently, a different C3 mutation has been identified.

Mutations in factor H have been reported more frequently among patients affected by C3Gs. Mutations may result in a defective protein or a complete lack of protein H. Mutations may occur in a homozygous or heterozygous manner[91,92] and may be associated with C3NeF, thus documenting the association of different risk factors.

In recent years genetic mutations of the CFHR gene cluster have been reported among patients with C3G[93]. CFHR family gene mutations[94], deletions[95], duplication[96] and hybrid genes[97] have been reported in patients with C3Gs either in isolated patients or family groups.

For example, Gale *et al*[96] reported two Cypriot families whose members were affected by a *CFHR5* mutation. The protein produced by the mutated gene was poorly effective in binding to C3b on cell surfaces and thus led to the deregulation of the fluid phase of the AP. The disease was called CFHR5 nephropathy.

Recently, Malik *et al*[98] reported patients from the same family affected by C3G due to abnormal copies in the *CFHR*3 and *CFHR1* loci. The finding of familial cases of C3G highlights the genetic origin of several C3Gs and the related complement AP dysregulation.

In summary the specific cause of C3G is inadequate regulation of the complement system. The causes of complement dysregulation may be divided into genetic and acquired factors. Among the former are changes in many of the complement genes: Among the latter are specific antibodies called C3 nephritic factors or C3NeFs that impair normal regulation of the complement system. It appears that patients with DDD are more likely to have C3NeFs, while patients with C3GN are more likely to have abnormalities in a group of proteins called the "Complement Factor H-Related" proteins.

Additionally, genetic defects may represent the basis of either C3G or aHUS (Table 3). Indeed, in recent years, a large number of genetic studies have established a strong association between the factor H-related proteins and different diseases involving complement dysregulation. This association, together with the recent functional data on factor H-related proteins such as FH competitors and complement deregulators, has gained the attention of the complement scientific community[99].

From the pathophysiological point of view, many cases of C3Gs and TMA are associated with defective control of the AP. The inevitable questions are whether C3G and TMA are the other sides of the same coin and which factors determine whether a patient develops one disease instead of the other[5].

Animal models highlight that C3G may be the consequence of prevalent dysregulation of fluid phase complement activation, whereas TMA is principally related to complement activation on the capillary wall. The same studies determined that an absolute deficiency of factor H favors fluid phase complement activation and C3G, whereas the absence or abnormality of the binding region of factor H favors TMA[100]. It has also been hypothesized that *CFH* and *CFH/CFHR* mutations induce aHUS to inhibit the CFH binding to most cell surfaces, whereas C3G-associated mutant CFHRs do not inhibit CFH binding to endothelial cell surfaces[6].

Concerning C3G recurrence after transplantation, the finding of familial cases of C3GN highlights the genetic origin and the related complement AP dysregulation of the vast majority of C3GN. These data form the basis of its recurrence after transplantation. However, fewer data are available on C3G recurrence compared to TMA. Indeed, C3G is a rare disease and principally, its pathogenesis and its complement-dependent nature have been recognized only recently. More data are available on DDD recurrence. Indeed, this disease was identified a long time ago based on its characteristic microscopic aspects. This finding occurred long before our understanding of its pathogenesis. In a retrospective analysis of 75 children, the 5-year graft survival rate was only 50%[101]. Almost all adult patients had recurrences after transplantation and up to 25% lost their graft[19].

In a large, retrospective cohort study of 80 adults and children affected by C3G, Medjeral-Thomas *et al*[102] reported a histological recurrence following renal transplantation in all 6 DDD patients. Recurrence was associated with graft loss in 50% of patients. Similarly, four of seven C3GN patients transplanted had histological recurrences. Graft loss occurred in 3 patients. A UNOS review reported a 10-year graft survival rate of 57.5% for patients affected by DDD recurrence[103]. In different studies, the reported rate of DDD recurrence is variable ranging from 18% to 100%[104,105].

Considering only those patients whose diagnosis was made by renal biopsy, the recurrence rate was over 70%[106,107]. Disease recurrence may occur suddenly after transplantation. However, cases of recurrence many years later are also described[107]. The risk factors for

Table 3 Overview of mutations in complement factor H related protein genes

Genetic defect	Phenotypical expression
Duplication in the *CFHR5* gene	C3 glomerulopathy (CFHR5 nephropathy)
Duplication in the *CFHR1* gene	C3 glomerulopathy
Hybrid *CFHR3/CFHR1* gene	C3 glomerulopathy
Hybrid *CFHR2/CFHR5* gene	C3 glomerulopathy
Hybrid *CFH/CFHR1* gene	aHUS
Hybrid *CFH/CFHR3* gene	aHUS

CFHR: Complement factor H related; aHUS: Atypical hemolytic uremic syndrome.

recurrence and graft loss for DDD are not well defined. No relationship with preTx disease presentation or C3 serum levels has been found. Additionally, the C3NeF levels do not correlate with the risk of recurrence[108]. The presence of heavy proteinuria seems to be the only risk factor related to recurrence.

The different genetic variants responsible of C3GN have been already described. Overall, C3GN recurs in two-thirds of transplanted patients and graft loss is common[27,81,83]. Histologically, it recurs with a membranoproliferative pattern. Risk factors for recurrence are still now debated. According to some studies[25], they include the severity of histological lesions in the native kidneys, HLA-B8 DR3, living related donors and previous graft loss for recurrence[109]. To date, our understanding of C3GN recurrence is only based on case reports. Furthermore, the broadest study on C3GN outcomes after recurrence by Zand *et al*[27] was unable to find any risk factor for recurrence. The multiple defects in complement regulatory proteins causing C3GN likely impair the establishment of any well-defined recurrence risk.

Eleven patients affected by CFHR5 nephropathy were successfully transplanted[110]; however protocol biopsies have documented recurrence[111]. The recurrence may be early after transplantation and demonstrates that renal-derived CFHR5 protein cannot prevent the development of CFHR5 graft nephropathy. Very recently Wong *et al*[112] described a high recurrence rate in 5 patients affected by hybrid *CFHR3 1* gene-associated C3GN.

DIAGNOSIS OF RECURRENCE

Diagnosis of recurrence may be easy if the clinical history of the recipient is known and the diagnosis of C3G/aHUS of the native kidneys has been made after an etiological workup and a kidney biopsy. Unfortunately, the clinical history of the recipient and a renal biopsy of the native kidneys are often not available.

In such patients, if the graft is not doing well, a renal biopsy should be promptly performed and examined by light microscopy, immunofluorescence and electron microscopy. When the diagnosis of C3G/aHUS is suspected, a complete workup should be undertaken. The diagnostic approach should include a comprehensive biochemical, genetic and pathologic analysis of the complement AP. This approach should include complement factors and complement regulatory protein levels, measurement of MCP on peripheral blood leukocytes as well as screening for anti-CFH antibodies and C3NeFs. Additionally, the genetic investigation should include mutation screening of CFH, CFI, MCP, C3 and CFB. The screening requires an extensive sequencing of all coding exons. Additionally, a study of recombination in the CFHR region should be made[113]. The genetic studies are not easy to perform because the spectrum of genes currently known to be involved is rapidly expanding[114]. Nonetheless, such studies are vital because the importance of genetic mutation screening to determine the outcome of retransplantation following a failed kidney allograft from a patient with recurrent aHUS has recently been documented[115]. In other words, not all mutations have the similar detrimental effects. The absence of a more severe genotype could facilitate the successful treatment of the recurrence.

RECCOMENDATIONS, PREVENTION ND TREATMENT OF POST-TRANSPLANT aHUS AND C3Gs RECURRENCE

The vast majority of data are available for aHUS because C3G has been only recently defined and data on prevention and treatment rely more on case reports than on evidence-based medicine.

Recommendations

Patients with aHUS as a primary disease and patients with suspected aHUS and with STEC-HUS should be screened for all complement factors and regulating proteins. Additionally, a genotyping for *CFH, CFHR, CFI, MCP CFB* and *C3* should be performed[114].

Patients with a suspected diagnosis of C3G should also be screened for C3NeF and for other autoantibodies that are known to be involved in this disease.

Living donor renal transplantation, even in the eculizumab era, is not indicated for patients with mutations in *CFH, CFI, C3* and *CFB*. In patients with aHUS due to a mutation in *MCP,* donation may be safe after exclusion of other mutations often associated with *MCP* mutation. However, increased evidence for a polygenic pattern for aHUS and C3G and the still-unknown polymorphisms should always consider a living donation with extreme caution[53].

Patients affected by aHUS but with no identified mutations should be recommended to proceed with transplantation combined with intensive plasma exchange (PE)[21].

Prevention

To date, there is limited evidence for preventing C3G recurrence after transplantation. The more validated experience refers to the use of eculizumab to prevent aHUS recurrence[80]. Whether these strategies may be

recommended to prevent C3G will be subject to future research.

More data are available concerning aHUS prevention. The avoidance of any possible endothelial insult has been highlighted[113]. Post-transplant conditions that may cause endothelial insult include ischemia-reperfusion injury, infections, and immunosuppressive drugs. All of these factors could act as triggers to activate the AP in predisposed patients.

An association between calcineurin inhibitors (CNIs) and aHUS recurrence has been hypothesized[7]. Other studies do not confirm this association and note that mTOR inhibitors are frequently used to avoid CNIs and may, *per se*, induce aHUS[116,117]. PE has been used to prevent aHUS recurrence[53]. However, PE has several drawbacks.

First, in some cases, PE fails to prevent aHUS[118]. Second, there is a risk of recurrence when PE is interrupted. Third, the evidence of subclinical recurrent aHUS in patients still under treatment indicates that in some cases, PE does not control complement activation[118].

Pre-transplant rituximab administration has been effective for patients with anti-CFH antibodies[40,119,120]. In these patients, the association of PE may improve the treatment efficacy. The anti-C5 monoclonal antibody (eculizumab) has been used to prevent post-transplant aHUS in several patients. Among the reported patients, nine had either *CFH* mutations or a *CFH/CFHR1* hybrid gene. Another patient had a *C3* mutation[118,121-124]. All of these patients had a complement genetic abnormality with a risk of aHUS recurrence greater than 80%. Only one patient lost the graft due to an arterial thrombosis. All other patients had a successful recurrence-free post-transplant course, even if, to our knowledge, they are still undergoing eculizumab treatment[116].

Treatment

In a retrospective study, Zand *et al*[27] reviewed the outcomes of 14 patients diagnosed with a C3G recurrence after transplantation. Ten patients did not receive any additional treatment. Three patients received rituximab treatment, but the overall outcome was poor.

Another study reported the beneficial effect of plasma infusions (PI) in patients with a genetic mutation in factor H[125]. Case reports documented the efficacy of eculizumab in patients with DDD recurrence[106] and patients with C3GN recurrence[126], although the patient with C3GN repeat allograft biopsies showed progression of the disease. Other studies[127,128] reported eculizumab efficacy for the treatment of recurrent DDD and C3GN. A randomized clinical trial to evaluate the efficacy of eculizumab in patients with C3G is ongoing[129].

An exciting new approach to C3G treatment is the soluble complement receptor 1 (CR1), which promotes the breakdown of active C3b. The infusion of soluble CR1 was reported to improve C3 and serum MAC levels in a patient with DDD recurrence[97].

Before the eculizumab era, patients affected by aHUS recurrence were extensively treated with PE. In the French survey, the outcomes of aHUS recurrence were not different among patients, regardless of treatment with PE[116]. PE combined with belatacept was effective for one patient, as reported by Midvedt *et al*[130]. Eculizumab has been reported to be effective in a recent study by Matar *et al*[131], regardless of concomitant PE treatment. The largest experience in treating recurrent aHUS with eculizumab was reported by Zuber *et al*[118].

According to their findings, eculizumab was efficient in treating aHUS recurrence after transplantation. The treatment should be started as early as possible, and the treatment tolerance is excellent. Interestingly, two patients who received a single dose regimen experienced a delayed relapse[132]. Two attempts of eculizumab discontinuation were followed by new relapses[133].

Overall, these experiences suggest that a high risk of relapse may persist after a first recurrence. This fact suggests caution in withdrawing eculizumab in this setting.

Additionally, active HUS lesions have been observed in patients with a documented C5 blockade receiving eculizumab regularly[118]. Whether a C3 convertase blocker could more efficiently treat these patients is currently unknown.

Two additional studies have documented eculizumab efficacy in plasma therapy resistant or dependent patients with recurrent aHUS[134,135]. More than 80% of the patients achieved TMA-free status.

The efficacy of eculizumab has changed our approach to aHUS and C3G recurrence after transplantation. However several questions remain to be answered, including: (1) Do complement investigations impact therapeutic decisions? (2) For how long should patients with recurrent aHUS or C3G be given eculizumab? and (3) Does eculizumab change our indications for renal transplant for patients on dialysis for aHUS or C3G?[136].

It is crucial to explore the most appropriate dose, dosing intervals and duration of treatment to reduce the enormous financial burden of eculizumab therapy[137].

CONCLUSION

Recurrence of primary disease after renal transplantation is currently one of the most important causes of graft loss.

Recurrence is principally common for those diseases, often glomerulonephritis, caused by constitutional abnormalities of the patient, not kidney related. Among these abnormalities are diseases caused by complement dysregulation such as aHUS and C3Gs. To date, aHUS and C3Gs often represent a contraindication to renal transplantation due to the frequency and severity of recurrent disease. The clinical use of the anti-C5 inhibitor, eculizumab, seems to overcome the limitations to kidney transplantation for selected patients. However, we have highlighted the drawbacks of this therapy, principally represented by the high costs of lifelong therapy. The main perspectives in the field of renal transplantation

of avoiding or treating recurrences are either diagnostic and therapeutic. An improved understanding of genetics and biology will allow an improved knowledge of gene mutations and the possibility of opening new methods in the field of living donor transplantation; Future therapeutic approaches are represented by the availability of purified deficient gene products and the availability of C3 convertase inhibitors. In addition to CR1 as mentioned above, the current targets of research include the compstatin analog Cp40, which can block C3b[138]. Similarly, another research target is a monoclonal antibody able to inhibit the C3 convertase induced by C3NeF[139].

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MINIREVIEWS

Face transplantation: Anesthetic challenges

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Abstract

Face transplantation is a complex vascular composite allotransplantation (VCA) surgery. It involves multiple types of tissue, such as bone, muscles, blood vessels, nerves to be transferred from the donor to the recipient as one unit. VCAs were added to the definition of organs covered by the Organ Procurement and Transplantation Network Final Rule and National Organ Transplant Act. Prior to harvest of the face from the donor, a tracheostomy is usually performed. The osteotomies and dissection of the midface bony skeleton may involve severe hemorrhagic blood loss often requiring transfusion of blood products. A silicon face mask created from the facial impression is used to reconstruct the face and preserve the donor's dignity. The recipient airway management most commonly used is primary intubation of an existing tracheostoma with a flexometallic endotracheal tube. The recipient surgery usually averages to 19-20 h. Since the face is a very vascular organ, there is usually massive bleeding, both in the dissection phase as well as in the reperfusion phase. Prior to reperfusion, often, after one sided anastomosis of the graft, the contralateral side is allowed to bleed to get rid of the preservation solution and other additives. Intraoperative product replacement should be guided by laboratory values and point of care testing for coagulation and hemostasis. In face transplantation, bolus doses of pressors or pressor infusions have been used intraoperatively in several patients to manage hypotension. This article reviews the anesthetic considerations for management for face transplantation, and some of the perioperative challenges faced.

Key words: Face transplantation; Vascular composite allotransplantation; Organ harvest; Facial reconstruction

Core tip: Face transplantation is a complex vascular composite allotransplantation surgery. During donor harvest, osteotomies and dissection of the midface bony skeleton may involve severe hemorrhagic blood loss often requiring transfusion of blood products. A silicon face mask created from the facial impression is used to reconstruct the face and preserve the donor's dignity. The recipient surgery usually averages to 19-20 h. Since the face is a very vascular organ, there is usually massive bleeding, both in the dissection phase as well as in the reperfusion phase, requiring use of pressors. This article reviews the anesthetic considerations for management

for face transplantation, and some of the perioperative challenges faced.

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INTRODUCTION

Face transplantation is a complex vascular composite allotransplantation (VCA) surgery. VCA involves multiple types of tissue, such as bone, muscles, blood vessels, nerves to be transferred from the donor to the recipient as one unit[1]. It is a rapidly evolving field which has benefited tremendously from the advances in microsurgery, transplantation, and immunologic techniques. Complex facial defects can be corrected, both functionally and cosmetically. Restoration involves availability of sufficient blood supply, esthetic unit match, nerve function, and integration into the recipients surrounding structures.

The first face transplant was performed in France in 2005[1] and the first near total face transplant was performed in the United States by the Cleveland Clinic Foundation in 2008[2]. Till date (Jan 2016) there have been 37 (20 partial and 17 full face) transplants done in the world. In Europe, face transplants have been done in France, Spain, Belgium, Turkey and Poland. China has been the only Asian Country to venture in this field[3-5]. There have been five patient deaths reported so far[5].

VCAs were added to the definition of organs covered by federal regulation [the Organ Procurement and Transplantation Network (OPTN) Final Rule] and legislation (the National Organ Transplant Act). The designation went into effect on July 3, 2014[6]. The United Network of Organ Sharing (UNOS) was assigned to oversee all face and hand transplants and take responsibility for developing all relevant policies and byelaws in this field. Thus a special VCA Transplantation committee was formed by UNOS in 2014, to develop aspects of VCA policies such as refining allocation policy, defining criteria for VCAs to be covered in OPTN policy, OPTN membership requirement for VCA transplant programs, data requirements, data collection procedures, *etc.*[7].

Face transplantation is a relatively new and rapidly developing field, and experience and expertise in this field is still limited. The American Society of Anesthesiologists (ASA) has not yet developed any guidelines to manage face transplantation procedures. This article reviews the anesthetic considerations for management for face transplantation, and some of the perioperative challenges faced.

ANESTHETIC CONSIDERATIONS

Donor

The ASA Physical Status Classification System typically classifies a declared brain dead patient whose organs are being removed for donor purposes as ASA Ⅵ. Prior to harvest of the face, a tracheostomy is usually performed because endotracheal intubation may hamper the surgical procedure[8]. The donor operation involves removal of the facial segment which varies as per the recipient's requirements[9]. The donor graft may contain skin, multiple vessels, nerves, muscles, and facial bones. The dissection can be very prolonged and may take 12-15 h, even up to 22 h[9,10]. The osteotomies and dissection of the midface bony skeleton may involve severe hemorrhagic blood loss needing transfusion of blood products. Explantation is done after systemic heparinization. The vascular pedicle consisting of carotid and internal jugular vessels is also dissected and used to flush the graft with cold preservative solution such as University of Wisconsin solution[9]. Though the total ischemia time tolerated by facial grafts is unknown, approximately 4 h should be well tolerated[11-13]. A silicon face mask created from the facial impression is used to reconstruct the face and preserve the donor's dignity[8,14].

If the donor is a multiorgan donor, co-ordination with other solid organ teams is vital. If there is elevated blood loss and hemodynamic instability, then the solid organ team should ideally be prepared to harvest the other organs immediately. Otherwise, solid organ retrieval could be delayed till just prior to the face explantation. The solid organs should ideally be given priority over the VCAs[8].

Recipient

The common indications for face transplantation have been devastating facial injuries which not only produce subsequent disfigurement but also compromise key facial functions, such as breathing, eating, facial expressions, vision *etc.*[3]. Though face allotransplantation may not be life saving, it certainly has a significant impact on an individual whose face has been severely injured, and constitutes a major reconstructive procedure[15,16]. It is essential for both, physical and social survival, and optimal social survival makes physical life worth living[15].

The ASA Physical Status Classification System typically classifies patient with end organ stage disease undergoing a transplant surgery as ASA IV, *i.e.,* a patient with systemic disease that is a constant threat to life. However, since a face transplant is not theoretically life saving, the patient may fall into category ASA Ⅲ, *i.e.,* a patient with a severe systemic disease, with substantive functional limitations. However, the patient may have several other comorbidities which may increase the ASA Grade. Reports published so far have cited damage to other organs as well, due to thermal burns, animal attacks, radiation injury, ballistic trauma, electrical burns, lye burns *etc.*[1,3,16].

The airway management most commonly used in facial transplantations has been *via* a primary intubation of an existing tracheostoma with a flexometallic endotracheal tube[17,18]. Primary orotracheal intubation may be challenging in cases of restricted mouth opening,

with facial skin contractures as commonly seen in burns, chemical trauma, *etc*. In such cases, fiberoptic intubation, awake or asleep, depending on the patient airway and the risks of aspiration, can be performed. Prior to commencement of surgery, a tracheostomy is done and a soft flexometallic endotracheal tube is inserted into the trachea. This is then sutured rather than tied, inorder to prevent compression to venous outflow from the face by pressure exerted by the circumferential tie[17,18].

Face transplantation surgery has a very long duration, usually averaging to approximately 19-20 h[17,18]. One case has been reported to have a surgical time of 36 h[19]. Venous access and hemodynamic monitoring would depend on the patient and existing comorbidities. An arterial line allows accurate monitoring of hypotension especially during massive blood loss, and also sampling for hematocrits, blood gases and coagulation profiles. Radial or femoral arterial lines can be placed, depending on accessibility.

A central line is usually preferred to administer fluids and pressors. The internal jugular and subclavian veins may be at risk of thrombosis, or maybe inaccessible. Though femoral venous access is associated with a higher degree of infection[20], it has been used in several cases[17,18]. Whenever feasible, a subclavian central venous line is preferable, to reduce risk of infections in this group of patients receiving immunosuppressive therapy postoperatively. A slight reverse trendenlenberg position (15 degrees) can be used to facilitate venous drainage and reduce blood loss.

Patients are usually induced using an induction agent such as propofol or etomidate, an opioid such as fentanyl or sufentanil, and a muscle relaxant. Muscle relaxants are usually avoided during the course of the procedure during dissection and reconstruction phases involving neural repair. Anesthesia is usually maintained using propofol, opioid, *e.g.,* remifentanil and inhalationals eg. sevoflurane. No particular anesthetic technique has been proven more superior than the other in face transplantation or free flap surgery. Normothermia is usually maintained by appropriate surface warming and by warming intravenous fluids and blood products administered to the patient. A mean arterial pressure of 65 mmHg ensures adequate perfusion and oxygen deliver to the vital organs including the graft. Urine output of 0.5-1 mL/kg per hour is usually adequate. In cases of severe hypotension, apical and subcostal views in transthoracic echocardiography maybe useful in assessing cardiac function. Antibiotics, timely redosing of antibiotics and immunosuppresssants are crucial to the success of this surgery.

Since the face is a very vascular organ, there is usually massive bleeding, both in the dissection phase as well as in the reperfusion phase. Moreover, osteotomy sites can bleed excessively. Anesthesiologists involved in this surgery have reported that quantification of the bleeding is often difficult due to diffuse bleeding into the drapes and poor visualization of surgical site. Prior to reperfusion, often, after one sided anastomosis of the graft, the contralateral side is allowed to bleed to get rid of the preservation solution and other additives which maybe used for allograft preservation, such as heparin or tissue plasminogen activator[18].

A median of 20 U of packed red blood cells, 13 U of FFP, 2 platelet units, and 13 L of crystalloid administration has been reported[18]. Though usually, massive transfusion protocols advocate 1:1:1 replacement of red blood cells, FFP and platelets[21], the amount of plasma and platelets transfused have been on the lower side due to fear of risk of thrombosis of the facial vessels. Intraoperative product replacement should be guided by laboratory values and point of care testing for coagulation and hemostasis such as thromboelastography. Use of colloids such as dextrans[22] are not preferred, and there is no data currently available on use of albumin for this surgery.

Many surgeons usually discourage use of pressors in microsurgical procedures, and though it is not typically a first line strategy, intraoperative use of pressors should be discussed in advance with the surgical team. It has been observed that there has been no difference in the outcomes when pressors were used or not used, and there is no reliable evidence to support contraindication of pressor use[23]. Frequency of flap necrosis and postoperative complications and adverse events were similar with or without use of intraoperative pressors[24]. Norepinephrine has been analysed as the most potential suitable agent for free flap transfer when compared to epinephrine, dobutamine and doxepine. This is because with norepinephrine, control of blood flow depends mostly on low frequency vasomotion or average blood pressure[22,25]. Though vasoconstriction increased, the blood pressure increased too, resulting in overall increased lap blood flow[26]. Dobutamine increases flap skin conductance, thereby benefiting flap blood flow[26]. Epinephrine decreased flap blood flow[26]. In face transplantation, bolus doses of pressors or pressor infusions have been used intraoperatively in several patients to manage hypotension[17,18].

Post procedure, the regular flexometallic endotracheal tube maybe replaced by a regular tracheostomy tube, prior to transfer of the patient to the intensive care unit.

CONCLUSION

Face transplantation is a long procedure and involves complex planning for airway management, vascular access, fluid and pressor management. Teamwork between the surgeon, anesthesiologist and intensivist is essential for a successful outcome.

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Older candidates for kidney transplantation: Who to refer and what to expect?

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Abstract

The number of older end-stage renal disease patients being referred for kidney transplantation continues to increase. This rise is occurring alongside the continually increasing prevalence of older end-stage renal disease patients. Although older kidney transplant recipients have decreased patient and graft survival compared to younger patients, transplantation in this patient population is pursued due to the survival advantage that it confers over remaining on the deceased donor waiting list. The upper limit of age and the extent of comorbidity and frailty at which transplantation ceases to be advantageous is not known. Transplant physicians are therefore faced with the challenge of determining who among older patients are appropriate candidates for kidney transplantation. This is usually achieved by means of an organ systems-based medical evaluation with particular focus given to cardiovascular health. More recently, global measures of health such as functional status and frailty are increasingly being recognized as potential tools in risk stratifying kidney transplant candidates. For those candidates who are deemed eligible, living donor transplantation should be pursued. This may mean accepting a kidney from an older living donor. In the absence of any living donor, the choice to accept lesser quality kidneys should be made while taking into account the organ shortage and expected waiting times on the deceased donor list. Appropriate counseling of patients should be a cornerstone in the evaluation process and includes a discussion regarding expected outcomes, expected waiting times in the setting of the new Kidney Allocation System, benefits of living donor transplantation and the acceptance of lesser quality kidneys.

Key words: Kidney transplant outcomes; Frailty; Elderly; Expanded criteria donor; Quality of life

Core tip: Transplant physicians must be well-versed in the intricacies of evaluating older kidney transplant candidates. This includes the appropriate selection of candidates which can be challenging due to the extent of comorbidity and frailty in this patient population. For patients who are deemed appropriate for transplant, physicians must be able to counsel them regarding expected outcomes and explain the expected benefit that transplantation confers over remaining on the deceased donor waiting list. Living donor kidney transplantation, even from older donors, should be encouraged. If no living donor is available, the rationale for accepting lesser quality kidneys should be discussed.

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INTRODUCTION

Although the incidence of end stage renal disease (ESRD) in the United States for patients ⩾ 65 years old is declining, prevalence continues to increase due to increasing patient survival. Older patients (⩾ 65 years) now constitute over 40% of the ESRD population and with an aging general population, this is likely to grow further. Among 116990 incident ESRD patients in 2013, 56977 (48.7%) were ⩾ 65 years and the mean age was 62.5 years[1]. Due to the above trends, the number of older patients referred for kidney transplantation will likely continue to increase as well. As such, transplant physicians must be well versed in the unique issues that arise in the evaluation of older candidates. In this review, we answer key questions that confront both physicians and patients during the evaluation process.

WHO IS AN APPROPRIATE CANDIDATE?

An appropriate candidate is a patient whose survival and quality of life are expected to improve with transplantation as compared to remaining on dialysis. Unfortunately, there are no clinical criteria that accurately and reliably predict this. Older age alone is not a contraindication to transplantation[2]. Transplant centers, however, may arbitrarily set their own age cut-offs. For candidates who do not have a living donor, this may be influenced by the expected waiting time in an individual center. In choosing the appropriate candidate, it is logical to only consider patients with reasonable long-term prognosis. However, determining who these patients are can be quite complex and there may be an inherent bias to exclude older patients due to perceived poor outcomes. Grams *et al*[3] developed a prediction model specific to older patients (⩾ 65 years) using United States Renal Data System (USRDS) data of 128850 incident Medicare-primary older adults with ESRD and United Network for Organ Sharing (UNOS) data of 6988 Medicare-primary first kidney transplant recipients aged ⩾ 65 years. They identified 19 variables (15 comorbidities, age, dialysis vintage, sex and transplantation year) that predicted post-transplant outcomes. Based on the model, 11756 (9.1%) were found to be excellent kidney transplant candidates with a predicted 3-year post kidney transplant survival of 87.6% or higher. Of note, 76.3% of these patients were never placed on the waiting list or referred for living donor kidney transplantation. The authors concluded that using a simple risk prediction model may help identify suitable candidates and ultimately improve older candidates' access to transplantation. In another more recent study, Dusseuz *et al*[4] developed a simple clinical scoring system using data from the French national prospective registry. By applying this scoring system on incident dialysis patients aged 70 or above, they identified a subgroup of patients that had a 70% probability of survival within 3 years, representing about 20% of the entire cohort. They suggested that this subgroup of patients, despite their older age, were worthy of being referred for kidney transplant evaluation.

Medical evaluation

The primary reason for graft loss in the older patient population is death with a functioning graft hence a great deal of emphasis is usually placed on the medical evaluation to determine suitability for transplant. Transplant centers may have variable selection criteria especially in older patients. Although several guidelines[2,5,6] exist with regards to the medical evaluation of a kidney transplant candidate, these are not specific for the older population. In general, however, individual organ systems are evaluated by means of history taking, physical examination and ancillary testing. If there is end-stage or severe disease, for example multi-vessel coronary artery disease not amenable to revascularization, then this usually becomes a reason to exclude patients from transplantation. Screening for infection and malignancy is also inherent to the evaluation especially in older patients due to their heightened susceptibility for both[7].

Particular focus is given to the cardiovascular work-up because cardiovascular causes comprise the leading cause of death among transplant recipients[1]. Unfortunately, the optimal method of screening for cardiovascular disease, in particular coronary artery disease, is not known[8,9]. Transplant centers may have variable approaches, usually ranging from cardiac stress testing to more invasive testing such as coronary angiography. Stress testing is relatively easy and inexpensive to perform, but has suboptimal sensitivity and specificity especially in diabetics[10]. As such, some centers may opt to go straight to a coronary angiogram. For example, at our center patients who are older than 70 years of age are required to undergo coronary angiography and if there is a significant burden of coronary artery disease, then a patient is deemed to be "too high risk" and therefore unsuitable for kidney transplantation. As part of the

cardiovascular work-up, additional attention is also given to imaging the iliac vessels to assess for patency and calcification. The imaging modality of choice at our center is computed tomography ± angiogram but a non-contrast magnetic resonance angiogram may also be a reasonable alternative if calcific burden is the main concern. At our center, not surprisingly, the primary reasons for excluding patients aged 60 years old or above are coronary artery disease, peripheral vascular disease (PVD), or both. It must be noted, however, that there are no studies that specifically compare the survival of these "very high risk" patients with transplantation as opposed to remaining on dialysis. Therefore, the decision to exclude these patients from transplantation remains rather subjective.

Measures of global health

Although a medical evaluation is able to closely scrutinize individual medical conditions, measures of global health and overall burden of disease may be more predictive of an older patient's prognosis post-transplant. Measures of global health that are increasingly being recognized as important predictors of outcome in kidney transplantation include comorbidity indices and measures of functional status, physical performance, and frailty.

Comorbidity refers to the presence of two or more chronic diseases or conditions. The Charlson Comorbidity Index (CCI) is the most widely used tool to quantify comorbidity. In the kidney transplant population, high CCI scores, indicating increased comorbidity, have been shown to correlate with an increased risk of death[11,12]. However, in a study by Heldal *et al*[13], although increasing CCI scores predicted mortality in younger patients (ages 45-54 and 60-69 years), these were not predictive in those aged 70 years or older. Additionally, the applicability of the CCI, however, has been questioned in kidney transplant candidates[14]. In a recently published Dutch study, Laging *et al*[14] developed the Rotterdam Comorbidity in Kidney Transplantation (RoCKeT) score as an alternative to the CCI. The RoCKeT score is determined by the presence of cardiovascular disease (3 points), cerebrovascular accident (2 points), PVD (2 points), diabetes mellitus (2 points), liver disease (2 points), lung disease (2 points), malignancy (2 points) and human immunodeficiency virus (1 point). Not surprisingly, comorbidity was highest in the oldest age group in that 75% of patients aged 70 to 79 had comorbidity (at least 1 point). When RoCKeT scores were categorized and analyzed for the influence on patient survival, the group with the highest scores (5-9) had a significantly lower survival than those without comorbidity (score of 0). After multivariate analysis, patients with a score of 5-9 had a 2.7 increased risk of death compared to patients with a score of 0. Despite this, 50% of patients in the highest comorbidity category survived more than 10 years. The authors concluded that patients with severe comorbidity should not be excluded from transplantation due to superior patient survival compared with published survival data of hemodialysis patients. Moreover, meticulous selection of high-risk patients for kidney transplantation can lead to successful outcomes.

Functional status is measured by a patient's self-report of his or her ability to perform certain tasks. These tasks may include the ability to walk a certain distance, climb stairs, or perform activities of daily living. Functional status measurements are subjective and are obtained *via* questionnaires such as the short form-36 (SF-36) Physical Function (PF) scale, Vulnerable Elderly Survey-13, or Physical Activity Scale for the Elderly. A number of studies have reported an association between functional status and patient survival[15-19]. In the largest study to date, Reese *et al*[19] analyzed 19242 *Fresenius* dialysis patients who had answered the SF-36 PF scale pre-transplant and had linked post-transplant data *via* the UNOS registry. Patient PF scores were divided into PF quartiles and these were correlated with time to kidney transplantation and the net survival benefit of kidney transplantation *vs* remaining on the waiting list. Patients in the lowest quartile were significantly older than those in the highest quartile (median age 54 years *vs* 46 years). In terms of survival, patients who were in the lowest PF quartile had the worst 3-year survival rates (84% compared to 92% for the highest quartile). When compared to remaining on the waiting list, patients across all PF quartiles had a survival benefit with transplantation. The lowest PF quartile had a survival benefit evident by 6 mo after transplantation. Another important finding in this study is that patients in the lowest PF quartile were more likely to be inactivated on the waiting list (adjusted hazard ratio *vs* highest quartile, 1.3) and less likely to be transplanted (adjusted hazard ratio *vs* higher quartile, 0.64). The authors concluded that functional status measures may be more useful in counseling patients regarding their probability of transplantation. It must be noted however that this study did not examine patients who were excluded from kidney transplant listing and who presumably had poorer baseline functional status, *i.e.*, the study only examined the best patients referred for transplant. Also, only 12% of the cohort were 65 years or older. Therefore, for patients referred for transplant who are older or with potentially worse baseline functional status, the applicability of this study's findings in regards to the survival benefit of transplant *vs* remaining on the waiting list remains to be determined.

Physical performance is the measured ability to perform tasks or exercise. Examples include measurements of gait speed or grip strength. The short physical performance battery (SBBP) is a combination of tests with a sub-score assigned. Measures of physical performance are objective and may be superior to reports of functional status in that these avoid reporting bias and overestimation of patients of their health status. Hartman *et al*[20] in a study of 26 patients aged ≥ 60 years and referred for kidney transplantation, found that these patients with renal failure had lower SBBP scores, gait speed and grip strength compared to patients with diastolic heart failure (71 patients), chronic obstructive pulmonary disease (176 patients) or those with high cardiovascular risk (294 patients). Interestingly, despite

their inferior physical performance, renal failure patients were less likely to report functional impairment on disability questionnaires. We are not aware of any studies to date that have measured physical performance and correlated these with outcomes in kidney transplant patients. In other solid organ transplant candidates, particularly in lung transplant, the six-minute walk test (6MWT)[21] has been used routinely in pre-transplant evaluations and has been shown to be a predictor of morbidity and mortality[22,23]. The 6MWT measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 min. It would certainly be interesting to see if the 6MWT can be used similarly in older kidney transplant candidates to predict outcomes.

Frailty is a state of decreased physiologic reserve and is defined by the presence of 5 features, namely unintentional weight loss, weakness as measured by decreased grip strength, slow walking speed, low physical activity and self-reported exhaustion. It has been shown by McAdams-DeMarco *et al*[24-26] in successive papers that frailty is associated with increased hospital readmission post-transplant, graft loss and mortality. In a prospective study of 537 kidney transplant recipients in a single center[26], frailty was measured at time of transplantation. Those who were frail, defined as having at least 3 out of the 5 features, were found to have a hazard ratio for death post-transplant of 2.22 (1.03-4.81, $P = 0.042$) compared to patients who were non-frail. In the subgroup of patients who were 65 years or older, 1-year survival was 85.8% in the frail group as opposed to 97.4% and 97.5% in the intermediately frail and non-frail groups, respectively. The authors suggested that frail patients should be identified pre-transplant and that patient survival may improve with appropriate management and closer monitoring of these patients.

A common theme to all the global measures of health discussed above is that it is not clear as to who is "too sick", "too debilitated", "too weak" or "too frail" to undergo kidney transplantation. Although these tools may help risk stratify patients, each candidate should be assessed on an individual basis and all data considered as a whole in determining a patient's suitability for transplant.

WHAT OUTCOMES CAN BE EXPECTED POST-TRANSPLANT?

Older recipients have decreased patient and graft survival compared to younger patients[27]. Graft loss is commonly due to patient death, the top 3 causes being cardiovascular disease, infection and malignancy[28]. There is less acute rejection in older patients and if graft loss is censored for death, graft survival actually improves with increasing age[7,29].

Despite inferior patient survival in older compared to younger patients, kidney transplantation is pursued due to the survival benefit that it confers when compared to remaining on the deceased donor waiting list. In a study by Rao *et al*[30], 5667 patients aged ⩾ 70 years who were waitlisted for kidney transplantation were analyzed based on scientific registry of transplant recipients (SRTR) data. Of these patients, 2438 ultimately underwent kidney transplantation and when compared to those who remained on the waiting list, the transplanted patients had a 41% reduction in risk of death (0.59 relative risk of death). The time to equal risk was 125 d and the time to equal survival was 1.8 years from transplant. Of note, the mortality benefit that was seen in this study extended to the subgroup of patients aged ⩾ 75 years, those with diabetes and those who received an expanded criteria donor. This study confirmed the findings of an earlier study by Wolfe *et al*[31] wherein the subgroup of patients aged 60-74 years was found to have a 61% lower mortality (0.39 relative risk of death 18 mo after transplantation) compared to similar patients on the waiting list. This survival advantage was calculated to translate into a 4-year increase in life expectancy (from 6 to 10 years).

In addition to superior patient survival compared to remaining on the waiting list, kidney transplantation is pursued due to the improvement in quality of life (QOL) that it confers[32,33]. Transplant patients have superior QOL compared to dialysis patients[34], though this may not be a fair comparison given that transplanted patients are a highly selected group. Age may have an effect on post-transplant QOL[35-37]. In a single center study by Weber *et al*[36], they compared the post-transplant health-related QOL of patients ⩾ 65 years with younger patients and with the general population. They found that physical QOL in older patients was significantly lower compared to younger patients and the general population. However, mental QOL was better than younger patients and similar to the general population. Humar *et al*[37] compared QOL of patients ⩾ 65 years to younger patients and with national norms for this age group. They found that older transplanted patients scored higher in their general health perception, social functioning and mental health compared to national norms and also scored higher on social functioning and mental health compared to younger transplanted patients. Both these studies, however, did not look at pre-transplant QOL data to determine if there was an actual improvement in QOL before and after transplant. In a study by Laupacis *et al*[38] of 166 patients, 22 of whom were ⩾ 60 years, they found that mean health-related QOL scores of almost all measures improved from pre-transplant to 6 mo after transplantation.

WHICH TYPE OF KIDNEY IS BEST?

Clearly, living donor (LD) transplantation confers the best outcomes in terms of patient and graft survival[39]. This eliminates time on the waiting list, reduces dialysis vintage and allows for preemptive transplantation, affords patients better quality kidneys, and reduces the incidence of delayed graft function and a potentially tumultuous immediate post-transplant course. Moreover, due to the

elective and scheduled nature of LD transplant surgery, recipient issues can be addressed in a controlled manner prior to surgery thereby reducing perioperative risk. This was shown in a study by Gill *et al*[40] of 25468 patients aged ≥ 65 years based on USRDS data who were listed for kidney transplantation, of which 11072 received a kidney transplant either from a LD, standard criteria deceased donor (SCD), or expanded criteria deceased donor (ECD). All patients were categorized based on cardiovascular (CV) risk as either being high, intermediate, or low CV risk. Among patients transplanted and across all CV risk categories, the death rate was lowest for patients who received a LD transplant and highest for recipients of an ECD kidney. Compared to patients who remained on the waiting list, a survival advantage was obtained, but importantly, times to equal risk and equal survival differed depending on the type of kidney transplanted and a patient's risk category. For patients who received a LD transplant, those who were low or intermediate CV risk had an immediately lower risk and higher survival post-transplant, and those who were high CV risk had a time to equal risk of only 43 d compared to similar patients who received an SCD (110 d) or ECD (180 d).

Despite the known advantage that living donor transplantation confers, older patients may have more limited living donor options as they may be hesitant to accept kidneys from younger donors such as their children or grandchildren. An alternative would be to pursue living donor transplantation from older donors such as their spouses or peers. Several studies have shown that recipients of kidneys from older living donors have reasonable outcomes[41-43]. Englum *et al*[41] studied 250827 patients based on UNOS data who received a kidney transplant, of which 92646 were LD kidneys and 4186 from donors aged ≥ 60 years. Not surprisingly, graft and patient survivals of patients who received a kidney from an older LD were worse compared to those who received a kidney from a younger LD. However, patients who received a kidney from an older LD aged 60-64 years and 65-69 years had similar graft survivals to patients who received a SCD kidney, superior graft survivals to ECD recipients and superior patient survivals to both SCD and ECD recipients. Patients who received a kidney from a LD aged ≥ 70 years had graft survivals similar to ECD recipients but significantly better patient survival. Given the organ shortage and current waiting times for a deceased donor kidney, it would make sense for an older patient who has an available older LD to pursue transplantation from an older LD rather than wait for an SCD or ECD kidney.

For those without living donor options, patients are faced with an increasing waiting time on the deceased donor list. The median number of years to deceased donor transplant was 5.5 years in 2003 and 7.6 years in 2007[39]. Waiting time could be shorter or longer depending on where a patient is listed and his or her sensitization status and blood type. As older patients' time on the waiting list increases, the less likely they are to be transplanted as their health deteriorates and they are either removed from the waiting list or they die[44]. Compared to younger patients, the risk of death while waiting for a transplant is higher for older patients[39]. It is therefore of paramount importance for older patients to get transplanted sooner rather than later. Kidneys that are thought to be of lesser quality should be considered for older candidates as waiting times for these kidneys are usually shorter. Rao *et al*[30] and Merion *et al*[45] demonstrated that recipients of ECD kidneys had superior survival compared to similar patients who remained on the waiting list or those who received standard therapy (waiting list and non-ECD transplantation). Massie *et al*[46] examined the outcomes of patients who received high kidney donor profile index (KDPI) kidneys and compared these to outcomes of patients who remained on the waiting list until receipt of a KDPI < 70% kidney. The times to equal risk and equal survival post-transplant with the comparison group were 6 and 18 mo, respectively for the KDPI 81%-90% group and 7.2 and 19.8 mo, respectively for the KDPI 91%-100% group. At 4 years post-transplant, the KDPI 81%-90% group and 91%-100% group had a 17% and 10% lower mortality, respectively, than the comparison group. However, after 4 years the mortality rate was not statistically significantly different. The study found that the benefit of the high KDPI kidneys was greatest in patients ≥ 50 years who were listed at centers with a median wait time of ≥ 33 mo. In another study, Rose *et al*[47] found that among 5257 patients that received a kidney from a deceased donor aged ≥ 65 years (defined in this study as an ECD kidney) in the United States, 10-year mean death-censored graft survival exceeded patient survival in patients aged ≥ 60 years. Among those aged ≥ 70 years, the difference was over 20 mo. Of note, there was a 7-8 mo difference in the 10-year mean patient survival between those who received an ECD kidney and similar patients who received a kidney from a deceased donor aged < 65 with a KDPI of 60%-69%. The authors concluded that for patients aged ≥ 60 years, kidneys from older donors can provide a lifetime of allograft function and that ECD transplantation should be encouraged in this age group. In a study from Spain, Pérez-Sáez *et al*[48] looked at outcomes of 2040 patients waitlisted for transplant, of whom 389 (mean age 68.9 ± 5.8 years) received a kidney from a deceased donor aged ≥ 75 years. They found that there was a 56% lower risk of death in patients who received a transplant compared to those who remained on the waiting list. However, patients ≥ 70 years, diabetics and those with chronic obstructive pulmonary disease did not derive any statistically significant benefit.

HOW DOES THE NEW KIDNEY ALLOCATION SYSTEM (KAS) AFFECT OLDER PATIENTS?

In an attempt to balance equity with utility, kidney allocation in the United States was changed in December

2014[49]. One of the goals of the new KAS is to increase unrealized graft years by matching high quality kidneys with recipients who have longer life expectancy[50,51]. As a result, transplant rates among older candidates are expected to decrease[51]. In an analysis of the early impact of the new KAS a year after its implementation, Stewart *et al*[52] noted a significant reduction in transplants where donor and recipient age differed by more than 30 years (21.1% pre-KAS *vs* 16.3% post-KAS). Among recipients aged 65 years or older, transplant rates significantly decreased from 22.9% of all kidney transplants pre-KAS to 18.1% post-KAS across all donor KDPI's, with the most prominent reduction in transplants from donor kidneys with a KDPI of 0-20%. This occurred despite an increase in the number of waitlisted patients aged ⩾ 65 years (21.3% pre-KAS to 24.9% pre-KAS).

Another important feature of the new KAS is that kidneys from donors with a KDPI > 85% are now being allocated nationally. Whether this would lead to increased utilization of these organs and subsequent shorter waiting times remains to be seen. Broader sharing of these kidneys may lead to increased cold ischemic times and increased discard rates of marginal kidneys. In early analysis[52], there was a significant reduction in transplant rates of kidneys from donors aged ⩾ 65 years (3.1% pre-KAS *vs* 2.5% post-KAS, $P = 0.0085$) and a non-significant reduction in transplanted kidneys with a KDPI of 86%-100% (8.6 pre-KAS *vs* 7.9% post-KAS, $P = 0.0645$). The kidney discard rate 1-year post KAS was slightly higher (19.4% post-KAS *vs* 18.5% pre-KAS, $P = 0.05$).

With these changes in the new KAS, we believe that older recipients should be motivated further to look for living donors including older living donors. If no living donor is available, then listing for kidneys with a KDPI > 85% should be highly considered. Consenting for KDPI > 85% kidneys should include a discussion regarding expected outcomes and rationale for accepting these kidneys.

CONCLUSION

Determining who among older kidney transplant candidates is appropriate for transplantation can be challenging and complex. A thorough medical evaluation with particular focus on cardiovascular health must be employed. Additional tools such as measures of comorbidity, functional status, physical performance, and frailty may be helpful. Those older patients who ultimately undergo transplantation have decreased patient and graft survival compared to younger counterparts, but have superior patient survival compared to those who remain on the deceased donor waiting list. Living donor transplantation confers the best outcomes for older recipients with reasonable outcomes from older living donors. If no living donor is available, most older patients will likely benefit from accepting lesser quality kidneys such as those that have a KDPI > 85%. In the era of the new KAS where transplant rates among older patients are expected to decrease, appropriate counseling of older recipients regarding their options is of paramount importance.

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More than skin deep? Potential nicotinamide treatment applications in chronic kidney transplant recipients

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Abstract

Non-melanoma cutaneous carcinomas, or skin cancers, predominantly squamous cell carcinomas (SCCs), are the most common malignancies occurring in kidney transplant recipients (KTRs). Squamous cell carcinoma risk is dramatically elevated in KTRs, occurring at rates of up 45-250 times those reported in general populations. New non-melanoma skin cancers in KTRs with a prior non-melanoma skin cancer also develop at 3-times the rate reported in non-KTRs with the same clinical history. The unique aggressiveness of SCCs in KTRs increases patient morbidity, due to the high rate of new lesions requiring treatment, frequently surgical excision. Oral nicotinamide shows promise in the chemoprevention of the especially aggressive non-melanoma skin cancers which occur in KTRs. This benefit might be conferred *via* its inhibition of sirtuin enzymatic pathways. Nicotinamide's concurrent hypophosphatemic effect may also partially ameliorate the disturbed calcium-phosphorus homeostasis in these patients-a putative risk factor for mortality, and graft failure. Conceivably, a phase 3 trial of nicotinamide for the prevention of non-melanoma skin cancers in KTRs, lasting at least 12-mo, could also incorporate imaging and laboratory measures which assess nicotinamide's impact on subclinical cardiovascular and chronic kidney disease risk, and progression.

Key words: Kidney transplantation; Skin neoplasms; Niacinamide; Phosphorus

Core tip: Our unique review describes promising evidence

that oral nicotinamide may have dual therapeutic clinical applications in chronic kidney transplant recipients (KTRs). First, nicotinamide, *via* its inhibition of sirtuin-mediated enzymatic pathways, may reduce the rate of KTR non-melanoma skin cancers. Second, nicotinamide's hypophosphatemic effect could lower the rate of cardio-renal outcomes in KTRs. These hypothesized benefits warrant further study in randomized, placebo-controlled trials of nicotinamide treatment with both intermediate, and eventually, hard clinical outcomes.

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NON-MELANOMA SKIN CANCER IN GENERAL AND KIDNEY TRANSPLANT RECIPIENT POPULATIONS

Updated United States incidence estimates for non-melanoma cutaneous carcinoma, or skin cancer (NMSC) in the Medicare fee-for-service population indicate a pronounced rise of 100% for the entire two decade period from 1992 to 2012, and a sustained 6-year 2006 to 2012 elevation in NMSC rates of 35%[1]. Data recorded from kidney transplant recipient (KTR) populations spanning over four decades now, consistently yield 45- to 250-fold general population standardized incident rates for squamous cell carcinomas (SCCs), accompanied by 10-fold greater rates for basal cell carcinomas (BCCs)[2,3]. Analyzing data from 24088 United States KTRs who underwent an initial kidney transplant between 1995 and 2001, Kasiske *et al*[3] reported 3-year age-adjusted NMSC rate ratios of 90.0 for male KTRs, and 92.3 for female KTRs, relative to general population controls. Comparison of KTR and non-solid organ transplant recipient (SOTR) populations with a prior history of NMSC, undergoing surveillance for new NMSCs, also demonstrates a persistently greater risk for additional skin cancers in KTRs. While non-KTRs experience a 3-year cumulative 18% risk of a subsequent SCC after a first SCC[4], 3-year rates of 52%, and 59%, for a (at least one) new SCC in KTRs after an initial SCC, have been reported[5].

Pre-cancerous actinic keratoses (AKs), Bowen's disease (SCC *in situ*), and keratoacanthomas are commonly associated with SCCs in KTRs[2]. Most SCCs among KTRs, as in non-SOTRs, develop, typically, on sun-exposed areas[2]. However, KTR SCCs have an increased tendency to be multiple, and aggressive, compared to SCCs which develop in non-SOTRs not exposed to chronic immunosuppression[2] (Figure 1). The characteristic aggressiveness of KTR SCCs exacerbates patient morbidity, because of the disproportionate rate of new lesions requiring, cryotherapy, electro-dessication and curettage, or surgical excision[2]. This increased morbidity, although non-fatal, also results in significant medical costs, reflecting the national United States economic burden of NMSC and AK care, tabulated, as of 2007-2011, at $4.8 billion, annually[6]. KTR SCCs, additionally, have a greater potential for metastasis, and death[2,5].

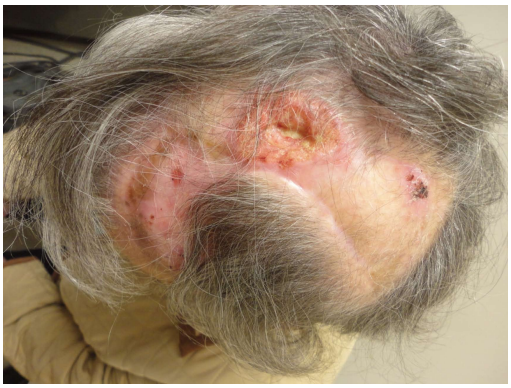

Figure 1 The unique aggressiveness of squamous cell carcinoma in kidney transplant recipients. Deeply invasive, recurrent, poorly differentiated and ulcerated squamous cell carcinoma in a 73-year-old female kidney transplant recipient with background alopecia from prior radiation therapy for this squamous cell carcinoma.

Successful interventions to reduce the incidence and complications associated with all NMSCs, SCCs, in particular, as well as AKs, would represent a significant advance in the management of KTRs. Despite heroic "conversion" protocols from calcineurin inhibitor-based to mechanistic/mammalian target of rapamycin (mTOR) inhibitor-based (primarily, sirolimus) immunosuppressive regimens, KTRs with a predilection for NMSCs, especially SCCs, continue to develop new malignant skin lesions at grossly elevated rates[7,8]. The sirolimus converted group reported by Euvrard *et al*[7], for example, still experienced a 2-year incidence of 22% for SCC, and 47.6% for total NMSCs (71 new lesions in 20 patients). NMSC-prone KTRs converted to sirolimus also appear to increase their relative risk for death after mTOR conversion. A recent meta-analysis of such "conversion trials" underscored the lingering therapeutic dilemma: While sirolimus use significantly lowered SCC risk, it conferred an overall mortality penalty-a 1.59-fold excess risk of death[8].

Ultraviolet (UV) radiation, immunosuppressive therapy, and human papillomaviruses (the latter with an ostensible link to SCC, specifically), are all believed to contribute to the development of NMSC among KTRs, while their precise etiologic pathomechanisms, alone, or in concert, require elucidation[2]. Although topical sunscreens are an effective prophylactic against sunburn, their use may not afford protection from UV radiation-induced immunosuppression of the skin[9], and the incidence of skin carcinomas, especially SCCs, continues to climb, steeply[1]. The ideal chemopreventive treatment, as an adjunct to barrier sun protection, would

be an oral agent that is safe, well-tolerated, inexpensive, and readily available. A potential candidate emerging to fill that therapeutic niche is oral nicotinamide (NAM).

Nicotinamide as a potential non-melanoma skin cancer chemopreventive agent

Overt, pellagrous nicotinic acid (NA; niacin) deficiency has long been recognized as a cause of severe sunlight sensitivity in exposed skin[10]. Recent clinical trial reports from an Australian investigative group suggest that oral NAM can reduce the occurrence of both AKs and NMSCs in non-SOTRs who have a history of AKs, and/or NMSCs[11,12]. The initial 4-mo, placebo-controlled phase 2 studies (n = 35, and n = 41 participants, respectively) reported that oral NAM lowered the rate of appearance of new AKs (*i.e.*, total AK counts) by 29% to 35%[11]. In a subsequent 12-mo phase 3, placebo-controlled trial of 386 patients with prior NMSCs, NAM treatment (n = 193 active; n = 193 placebo) reduced the average unadjusted new NMSC rate (total lesions per patient) by 27%. NAM treatment resulted in comparable rate reductions for SCCs (-30%), and BCCs (-20%), as separate outcomes[12]. Chen *et al*[13], from the same Australian investigative group which conducted these non-SOTR NAM chemoprevention studies[11,12], recently reported consistent results, in terms of effect sizes, from a phase 2 study of 22 KTRs. Patients at least 12 mo post-transplant, with stable renal function, and a history of $\geqslant$ 2 histologically-confirmed NMSCs in the previous 12 mo, were randomized 1:1 (n = 11 per group) to receive NAM 500 mg, or placebo, twice daily, for 6 mo. Skin exams and AK counts were performed at 2-mo intervals. The 6-mo NMSC rate (mean lesions per patient) was non-significantly lower for the NAM group (mean = 2.7; 95%CI: 1.4 to 5.3; total = 30 cancers), compared to placebo (mean = 4.2; 95%CI: 2.2 to 7.8; total = 45 cancers), although the numeric trend was dominated by one patient in the placebo group with 20 NMSCs (8 BCC and 12 SCC). The estimated relative rate difference was 0.35 (95%CI: -0.62 to 0.74, P = 0.36). Baseline AK counts (reported as means of 61.6 in the placebo, and 60.1 in the NAM groups, respectively), were also non-significantly lower in KTRs receiving NAM compared to placebo by 16% at 6 mo (95%CI: 7% to 34%; P = 0.15)[13]. Also of importance, there were no between groups differences for adverse clinical events, or changes in complete blood counts, liver function studies, eGFR, or urinary microalbumin/creatinine ratios[13]. Additional, if limited, independent confirmatory data have been provided in a research letter published by Drago *et al*[14], in *The New England Journal of Medicine*. These Genoa, Italy investigators studied NAM given at a dose of 250 mg thrice daily, relative to matched placebo, in 24 KTRs (n = 12 per group), also followed for 6-mo. The following is a verbatim description of their findings[14].

At baseline, no significant differences were observed between the sizes of light-damaged areas in patients (identified visually, by touch, and by means of polarized light dermoscopy), in the two groups. At 6 mo, 88% of the patients who received nicotinamide had partial regression of some or all actinic keratoses and surrounding light-damaged areas; in 44% of the patients who received nicotinamide, there was complete resolution in some of these areas (no lesions were detected on biopsy). In 91% of the patients who received placebo, the size of light-damaged areas increased, new light damaged areas developed, or both.

Two plausible anti-cancer biological effects of nicotinamide have been described by the Australian investigators which provide some independent validation of their clinical trial observations: Nicotinamide has been shown to promote DNA repair after UV exposure, and lessen local UV immunosuppression, in the skin[15]. NAM's reported effects on sirtuin enzymes and mediated pathways might also confer anti-cancer properties. Briefly, sirtuin (Sir2) enzymes, are an ancient class of nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylases which have been conserved from bacteria to humans (for example, SIRT1, *etc.*). They perform a myriad of biological functions, including transcriptional silencing, DNA recombination and repair, apoptosis, axonal protection, fat mobilization, and lifespan regulation[16-18]. Sirtuins deacetylate various proteins, notably p53, as well as histones, acetyl-coA synthetase, alpha-tubulin, Foxo3, Ku70, and NF kappa-beta. Lysine residues are the specific targets of sirtuin deacetylation reactions, allowing for tight cellular regulation. Sirtuin-catalyzed deacetylation is linked to the cleavage of NAD^+, producing NAM, and O-acetyl ADP-ribose (OAADPr), in conjunction with deacetylated ribose. NAM produced from this reaction is a non-competitive inhibitor of sirtuins, which provides a mechanism for modulation of these enzymes by cellular nicotinamide concentrations[16-18]. Importantly, the inhibitory activity of NAM on sirtuin-mediated deacetylation is not conferred by nicotinic acid (NA)[18] (Figure 2). *SIRT1* overexpression has been observed in human prostate cancer, adult T-cell leukemia, primary colon cancer, and, significantly, in skin tissue biopsies from patients with AK, Bowen's disease, SCC, or BCC[16,17]. One mechanism by which elevated *SIRT1* concentrations are believed to enhance malignant cell growth is *via* deacetylation of "tumor suppressor" p53 protein, inhibiting p53's apoptotic activity[16,17]. *SIRT1* interacts directly with p53 and deacetylates the protein's C-terminal (Lysine)382 residue, which prevents p53 from trans-activating apoptotic genes, and promoting apoptosis[17]. NAM may also affect another of *SIRT1's* downstream target proteins, the retinoblastoma tumor suppressor protein (Rb), reducing Rb phosphorylation (or "hyper"-phosphorylation)[19]. This has already been demonstrated, for example, in a mouse model of polycystic kidney disease, where NAM treatment reduced sirtuin-enhanced cyst formation[19]. Anti-cancer chemopreventive agents such as curcumin (a natural phenol responsible for the yellow color of turmeric), which suppresses Rb phosphorylation in prostate cancer cells, are under investigation[20].

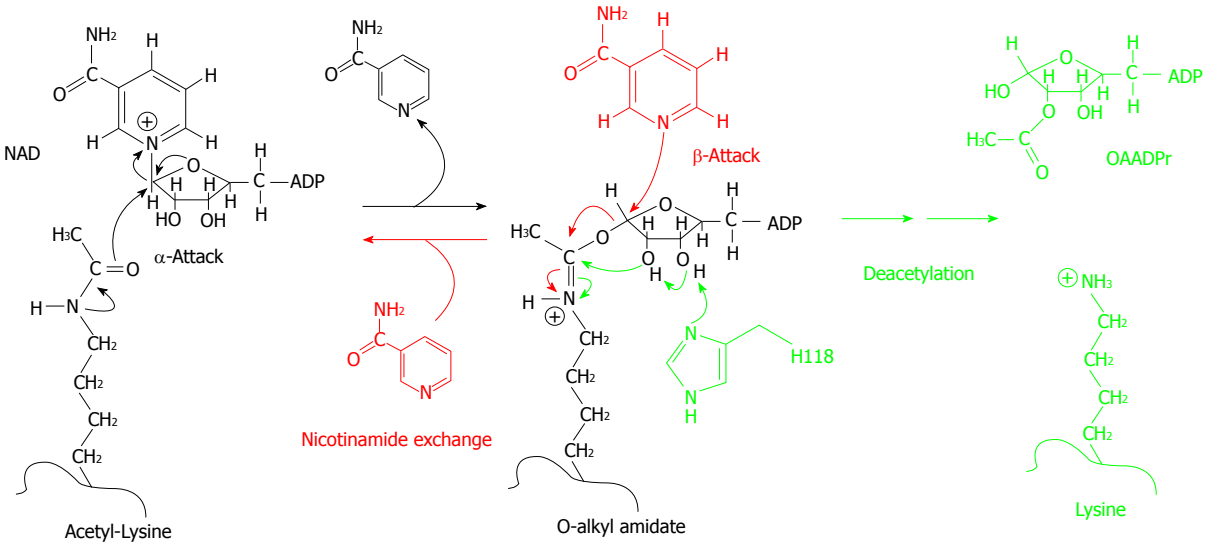

Figure 2 Nicotinamide-SIRT binding. At high concentrations, NAM can bind to SIRT when it contains the O-alkyl-amidate intermediate, resulting in "NAM exchange", reforming NAD^+ and acetyl-lysine, and decreasing deacetylation activity - a process unique to NAM, not NA. Reproduced from[18]. NA: Nicotinic acid; NAM: Nicotinamide.

Comparable NAM-induced p53 and Rb alterations could mediate the potential benefits of NAM treatment for the chemoprevention of AKs and NMSCs, in general, and KTR patient populations[11-14]. NAM's putative *in vivo* role as an inhibitor of sirtuins, and sirtuin-catalyzed "pro-oncogenic" de-acetylation, and hyper-phosphorylation reactions[16-19] warrants investigation, as another pathophysiological correlate, in NAM-treated patients.

NICOTINAMIDE AND HYPOPHOSPHATEMIA: FROM TOXICITY MONITORING TO THERAPEUTIC INTERVENTION

Extensive analyses by others[21], and our group[22-26] (Figure 3), demonstrate that both NAM and NA lower serum phosphorus in chronic kidney disease (CKD) patients, and indeed across the entire spectrum of renal function. Mediated *via* an elegant mechanism, *i.e.*, direct inhibition of sodium-dependent transport of phosphorus in the small intestine[21], this consistent phosphorus-lowering effect mandates surveillance of serum phosphorus levels in patients on chronic oral NAM/NA to avoid the theoretical development of clinical hypophosphatemia[21]. But such potential toxicity might be counterbalanced by a distinctly positive clinical phenomenon: Since baseline serum phosphorus concentrations appear to predict total and/or cardiovascular disease (CVD) mortality in CKD and KTR populations[27-31], as well as native kidney, or kidney graft failure[27,30-32], conceivably, NAM-induced phosphorus-lowering could reduce such hard outcomes in these patients. Moreover, as NAM does not induce prostaglandin-mediated flushing, cause hyperuricemia, or adversely affect glucose tolerance, we believe it will have a better tolerability and safety profile relative to NA, confirming a substantive, decades old body of clinical evidence[33,34] from non-SOTR patient populations-now updated to include those studied on oral NAM therapy in the recent phase 2 and 3 Australian AK/NMSC prevention trials[11-13]. The sporadic occurrence of mild thrombocytopenia in end-stage renal disease (ESRD; stage 5 CKD) patients treated with NAM[35], has not been confirmed from either the recent placebo-controlled studies conducted among patients with normal renal function[11,12], or KTRs[13], for NMSC or AK prevention, nor was it reported in the large multicenter ENDIT trial[34], or a 30-year toxicity review of NAM trials which preceded ENDIT[33]. COMBINE, an ongoing, randomized, placebo-controlled trial (NCT0 2258074) in patients with an estimated glomerular filtration rate (eGFR) of 20-45 mL/min per 1.73 m^2, which includes a NAM treatment arm (1.5 g/d, given as 0.75 g twice daily), will provide more definitive data on the incidence of this toxicity in stage 3b-4 CKD[35,36].

OVERVIEW OF DYSREGULATED CALCIUM-PHOSPHORUS HOMEOSTASIS AND ITS CLINICO-PATHOLOGIC IMPLICATIONS IN CKD AND KIDNEY TRANSPLANTATION

Deranged calcium-phosphorus metabolism often complicates CKD[36], worsens with the progressive development of ESRD[21,36], and is not fully reversed after kidney transplantation[28-30,37,38]. Moreover, notwithstanding abnormalities, particularly hyperparathyroidism (with resultant increased fractional excretion of urinary phosphorus/decreased tubular reabsorption of phosphorus)[37], which may persist long term despite successful transplantation, and excellent kidney graft function, chronic

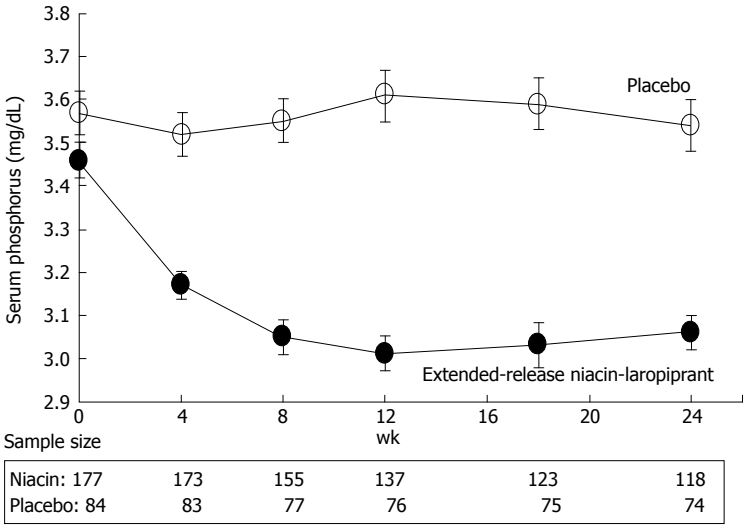

Figure 3 Phosphorus-lowering effect of nicotinic acid in chronic kidney disease. Mean serum phosphorus concentrations in dyslipidemic patients with stage 3 chronic kidney disease receiving extended release niacin-laropiprant, or placebo. Error bars are standard errors. Reproduced from Ref. [24].

KTRs whose GFR subsequently declines to stage 4 (15-29 mL/min per 1.73 m^2) to 5 (< 15 mL/min per 1.73 m^2), or even 3b (30-44 mL/min per 1.73 m^2) CKD, are prone to the hyperphosphatemia, inadequate vitamin D status (*i.e.*, deficiencies of 25-hydoxy vitamin D3, and/or 1,25-dihydroxy vitamin D3), elevated concentrations of parathyroid hormone (PTH), and increased levels of the phosphatonin fibroblast growth factor 23 (FGF23), characteristic of stage 3b to 5 CKD in the native kidneys of non-KTRs[28-31,37,38].

Chronic upregulation of the hormonal mechanisms evolved to maintain calcium-phosphorus homeostasis, including normal serum phosphorus concentrations, may have adverse clinical sequelae. For example, higher serum concentrations of phosphorus (*via* increased dietary intake, and/or a reduced GFR) apparently stimulate production of the bone-derived hormone FGF23, which induces compensatory renal phosphorus excretion[30,36]. Elevated FGF23, in turn, may directly induce cardiac myocyte hypertrophy, clinically manifest as left ventricular hypertrophy (LVH)[36], and lower endogenous production of calcitriol (1,25-OH vitamin D3), from 25-OH vitamin D3[36]. Significant associations have been reported between FGF23 elevations LVH, CVD events, mortality, and progression to ESRD in patients with CKD stage 3-4[36]. Accordingly, sustained elevations in FGF23 concentrations, despite being a homeostatic adaptation to maintain normal serum phosphorus, may enhance the risk for developing LVH, CVD, and ESRD[36]. Higher phosphorus concentrations also stimulate chronic excess PTH secretion, eventually leading to clinical hyperparathyroidism[36,39]. Inexorable CKD progression, accompanied by further nephron loss, ultimately overwhelms these compensatory hormonal mechanisms, causing a preponderance of advanced CKD patients to manifest concurrent hyperphosphatemia, increased FGF23 and PTH levels, and suppressed 1,25-OH vitamin D3 concentrations[36,39].

The precise *in vivo* molecular mechanisms through which extracellular phosphate exerts its cytotoxic effects are not fully elucidated. However, investigations have demonstrated that extracellular phosphorus can form insoluble nanoparticles with calcium and fetuin-A, commonly dubbed calciprotein particles (CPPs)[39,40]. Highly bioactive ligands, CPPs can have cytotoxic effects such as causing cell death, or inducing osteogenic transformation of vascular endothelium, and renal tubular epithelium. CPPs, furthermore, are detectable in the circulation of both animal models, and humans, notably in patients with CKD, implicating their potential role in tissue injuries mediated by phosphatemia[39,40]. Recently, a novel assay of serum calcification propensity, the transformation time ("T50") from primary calciprotein particles to secondary calciprotein particles, has been validated, and appears to reflect the pathophysiological milieu engendered by derangement of calcium-phosphorus metabolism which may predispose to ectopic, including vascular, calcification[29,30]. These interrelated perturbations-hyperphosphatemia, inadequate status of vitamin D, elevations in PTH and FGF23, and more recently, greater calcification propensity (reduced T50)-are of epidemiological, and potentially, clinical relevance, because they have been associated, with fatal CVD[26,35], graft failure[30-32,40] or rapid decline in eGFR[38], and total mortality[28-31,40], among KTRs.

Even after possible "over-adjustment" for these co-variable measures of disturbed calcium-phosphorus homeostasis-which may be in the causal pathway between "phosphorus toxicity"[39], and its clinical sequelae-the relationship between serum phosphorus concentrations, and outcomes, can persist in sizable observational KTR cohort studies. Pihlstrøm *et al*[31], for example, investigated the association between baseline phosphorus concentrations and major CVD events, kidney graft loss, and all-cause mortality by proportional hazard survival analyses in 1840 stable KTRs derived from the Assessment of LEscol in Renal Transplantation (ALERT) trial, a multicenter randomized, double-blind, placebo-controlled study examining the effect of fluvastatin (40-80 mg daily) on CVD (primarily coronary heart disease, CHD), and renal outcomes in 2102 KTRs. Patients were recruited a mean of 5.1 years after transplantation, and followed for 6 to 7 years. During a mean follow-up of 6.7 years, death censored graft loss was recorded in 333 patients,

277 patients experiencing a major CVD event (defined as time to cardiac death, nonfatal myocardial infarction, or undergoing a coronary revascularization procedure), and 342 died, 168 from CVD, including cerebrovascular, or other major (*e.g.*, thoraco-abdominal aortic) vascular disease. Serum phosphorus (per 1 mg/dL increase) was associated with death from all causes, hazards ratio (HR) 1.23 (CI: 1.07-1.43, $P = 0.005$), and graft loss, HR 2.61 (CI: 2.25-3.04, $P < 0.001$), in unadjusted models. The relationship between serum phosphorus and mortality lost significance, HR 1.07 (CI: 0.89-1.28, $P = 0.488$), upon multivariable modeling (with PTH > 65 pg/mL), but persisted for graft loss, HR 1.52 (CI: 1.27-1.82, $P < 0.001$)[31]. Similarly, Wolf *et al*[30] studied a single center cohort of 984 chronic, stable Hungarian KTRs (median transplant vintage, 72 mo, interquartile range 40-114 mo; mean eGFR 51, SD = 21; 57% men). After a median follow-up of 37 mo (interquartile range, 35-39 mo), 87 patients died and 101 patients suffered kidney graft loss. Outcome data were analyzed in full models that adjusted for eGFR, age, gender, systolic blood pressure, body mass index, albumin, calcium, the modified Charlson Comorbidity index, and graft vintage, as well as serum phosphorus, PTH, and FGF23. These investigators reported that a 0.9 mg/dL (*i.e.*, a 1 SD) increase in phosphorus predicted the composite endpoint of death or graft failure when analyzed as a continuous variable per SD increase (0.9 mg/dL), HR 1.23 (CI: 1.08-1.40, $P = 0.002$), in the fully adjusted modeling, which included FGF23[30].

CONCLUSION

NAM shows promise[11-14] as an agent for the chemoprevention of the especially aggressive[2-5,7] NMSCs which occur in KTRs. This beneficial phenomenon may be mediated *via* its inhibition of sirtuin enzymatic pathways[16-19]. Phase 3 trials to test this specific hypothesis merit serious consideration. NAM treatment also confers a consistent reduction in serum phosphorus concentrations[21,35,36], with the accompanying potential to correct disturbed calcium-phosphorus homeostasis-for example, lower FGF23 concentrations, as with NA[26], and possibly improve calcification propensity[40]. These combined ameliorative effects of NAM treatment afford unique trial design opportunities. Conceivably, a phase 3 trial of NAM for NMSC prevention in KTRs, lasting at least 12-mo[12], could also incorporate imaging and laboratory measures which assess NAM's impact on subclinical CVD and CKD risk, and progression, a strategy being employed by the 12-mo COMBINE trial[36] in stage 3b-4 CKD patients.

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MINIREVIEWS

Overview of the progress on haploidentical hematopoietic transplantation

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Abstract

Allogeneic hematopoietic stem cell transplant (HSCT) remains the only potentially curative option for variety of hematologic disorders. Lack of a suitable fully HLA-matched donor limits this option for many patients. Without a suitable related or unrelated HLA-matched donor, umbilical cord blood and haploidentical family members provide a potential source of stem cells. Timely donor availability makes haploidentical donors an attractive alternative donor source. Initial attempts at haploidentical HSCT was associated with significantly increased mortality owing to high rates of graft rejection and severe graft-versus-host disease caused by major donor-recipient HLA-disparity. However, over the past decade, outcomes of haploidentical HSCT have improved significantly. Here, we review the advantages and challenges of haploidentical transplantation. We also discuss new developments to attempt to overcome the challenges to a successful haploidentical transplantation.

Key words: Haploidentical donor; Hematopoietic stem cell transplantation; Hematological malignancies; Transplant related mortality

Core tip: Over the past decade, haploidentical donors have emerged as a viable alternate graft source for patients without a HLA-matched donor. Several strategies including graft manipulation, conditioning regimen optimization and better graft-versus-host disease control have significantly improved the outcomes of haploidentical hematopoietic stem cell transplant (HSCT). Here, we summarize some of the recent advances in the field of haploidentical HSCT in adults.

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INTRODUCTION

Hematopoietic stem-cell transplantation (HSCT) is

considered to be the only potentially curative therapy for several hematologic diseases. Most institutions currently consider a HLA-matched sibling as a preferred donor source, typically followed by either HLA-matched unrelated or an alternative graft source depending on the clinical scenario. The likelihood of having an HLA-matched sibling donor is approximately thirty percent after consideration of factors such as donor consent and health status. The probability of finding a suitable matched unrelated donor is strongly influenced by patient's ethnicity and can range from more than 75% for Caucasians to less than 20% for certain ethnic groups such as African Americans[1]. In absence of related or unrelated HLA-matched donor, umbilical cord blood and haploidentical family members provide a potential source of graft. The use of haploidentical hematopoietic stem cell transplantation as an alternative graft source has been substantially increasing.

Utility of haploidentical related donors has a number of advantages including immediate donor availability for many patients facilitating a shorter interval to transplant. In addition, having a related donor makes post-transplant donor-derived cellular therapy more easily accessible. Challenges include major donor-recipient HLA-disparity which can cause delayed immune reconstitution, graft failure and severe graft *vs* host disease (GVHD) due to T-cell alloreactivity[2,3]. This review highlights the major advances over the past decade to overcome the obstacles to successful haploidentical transplantation.

DONOR SELECTION

In contrast to unrelated donor transplant HSCT where finding the best HLA matched donor is the most important factor in determining transplant outcome, increasing HLA disparity in haploidentical matching does not have the same detrimental impact with dedicated techniques such as modification of post-transplant T cell reconstitution with cyclophosphamide. In 2010, Kasamon *et al*[4] evaluated the impact of donor and recipient HLA in 185 patients who underwent un-manipulated bone marrow haploidentical transplant. Post-transplant cyclophosphamide was used as GVHD prophylaxis. In this study, the number of HLA-mismatches did not influence the rate of acute GVHD or disease free survival.

Donor characteristics that influence the outcome of haploidentical transplant were also investigated in a large study by Wang *et al*[5] involving 1210 patients with hematologic diseases. Grafts consisted of G-CSF mobilized T-cell replete bone marrow and peripheral stem cells. Similar to the prior studies, the degree of HLA disparity did not influence the incidence of acute GVHD and treatment related mortality (TRM). Younger donor age (< 30 years) was associated with a lower incidence of acute GVHD compared to older donor age (> 30 years). Younger donor age and male gender were also associated with less TRM and better overall survival (OS). The benefit of male recipient gender was lost when maternal donors were excluded. There was a higher risk of grade Ⅱ-Ⅳ acute GVHD with maternal donors compared to paternal donors. In a male recipient, a maternal donor also correlated with a higher TRM rate and decreased OS. The impact of non-inherited maternal antigen (NIMA) disparities was evaluated in 264 patients. NIMA mismatched donors conferred a lower incidence of acute GVHD compared to non-inherited paternal antigen (NIPA) mismatched donors. Based on these results, authors concluded younger, male, NIMA-mismatched donor is a preferred donor in setting of T-cell replete haploidentical transplant. This study did not evaluate the influence of natural killer (NK) cell alloreactivity and donor CMV status. In contrast to Wang *et al*[5], several trials demonstrated decreased risk of relapse and survival advantage with using maternal donors[6]. A more potent anti-leukemic effect of maternal donor grafts has been attributed to the maternal immune system exposure to fetal antigens during pregnancy[7].

Another factor influencing haploidentical transplant outcome is donor *vs* recipient NK cell alloreactivity. Tumor cells are able to escape T-cell adoptive immune response by down regulating cell surface MHC class Ⅰ. NK cells are an important component of innate immunity and have MHC-unrestricted ability to target malignant cells. Cytotoxic activity of NK cells are mainly under the negative feedback control from inhibitory killer immunoglobulin-like receptors (KIRs) through binding to self HLA class Ⅰ antigen. This phenomenon is known as "missing self"[8-10]. KIR-KIR ligand mismatched in the donor-recipient direction lead to loss of the inhibitory feedback and activation of donor NK cells targeting recipient hematopoietic cells and leukemic cells. In contrast to allo-reactive T-lymphocytes, NK cells are thought to be capable of inducing graft *vs* leukemia (GVL) effect without promoting GVHD. In 2002, a study by the Perugia group demonstrated therapeutic efficacy of allo-reactive NK cells in 57 patients with acute myeloid leukemia (AML) following haploidentical transplant[11]. Twenty out of 57 patients had KIR-ligand incompatibility in the graft *vs* host direction. The probability of OS at 5 years was markedly improved in patients with AML who had NK allo-reactive donors (60% *vs* 5%, $P = 0.0005$). Similar results were observed in the updated analysis of 112 patients with high risk AML who received T-cell depleted haploidentical transplants[12]. Fifty one of 112 patients had NK cell allo-reactive donors. The conditioning regimen included TBI (8 Gy), fludarabine (40 mg/m^2 per day for 4 d), thiotepa (5 mg/kg per day for 2 d) and rabbit ATG. A significantly lower relapse rate (3% *vs* 47%, $P < 0.003$) and better EFS (67% *vs* 18%, $P = 0.02$) was observed in patients transplanted in any CR with NK allo-reactive donors compared to recipients of non-allo-reactive grafts. Although transplantation from NK allo-reactive donors improved survival in the entire cohort, subset analysis suggested that transplantation from NK allo-reactive donors did not decrease the incidence of relapse in patients transplanted at chemo-resistant relapse. There was no significant difference in incidence of acute GVHD between the two cohorts (10% *vs* 11%). These findings reinforced the theory that GVL activity by allo-reactive NK cells translated into prolonged OS. Subsequently, several

studies revealed a favorable impact of allo-reactive NK cells on transplant outcome in patients undergoing HLA-haploidentical transplant[11,13-15]. An important role of donor-recipient KIR mismatch was also demonstrated after non-myeloablative T cell-replete haploidentical transplantation using post-transplant cyclophosphamide in a retrospective study involving 86 patients with high risk hematologic malignancies[16]. On the contrary, a deleterious effect of KIR mismatches was seen in the earlier studies[17,18]. Due to ongoing controversy, currently the KIR testing is not considered mandatory for donor selection in haploidentical transplant setting.

HAPLOIDENTICAL STEM CELL TRANSPLANT STRATEGIES

T-cell depletion

The first successful haploidentical transplants were done in the 1980s in children with severe combined immuno-deficiency syndrome (SCIDS) using T-cell depleted bone marrow grafts. T-lymphocyte depletion in this setting mitigated GVHD associated with crossing a major HLA-barrier without compromising engraftment[19]. Subsequently, this approach was implemented successfully in several studies of patients with SCIDS. In contrast to SCIDS, haploidentical transplantation was less successful in the setting of acute leukemia owing to a high rate of graft failure. Increased risk of graft failure was attributed to host derived T-lymphocytes that survived the conditioning regimen[20-22]. A decade later, it was shown in preclinical studies (murine models) that infusion of a large number of donor hematopoietic stem cells can overcome the MHC barrier and promote engraftment[23]. In 1993, cell dose escalation approach was tested in 36 patients with acute leukemia following myeloablative total body irradiation (TBI) based preparative regimen. Mega doses of stem cells (on average > 10×10^6 $CD34^+$ cells/kg body weight) were obtained by supplementing T cell-depleted bone marrow transplants with granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells. Using this approach, nearly 80% of patients achieved primary engraftment. The sole GVHD prophylaxis consisted of T-cell depletion of the graft. Only 18% of the patients developed grade Ⅱ-Ⅳ acute GVHD[24,25]. Subsequently, several modifications were introduced to optimize the T-cell depletion of the graft including positive immuno-selection of the $CD34^+$ cells using the Ceprate system in 1995 and Clinimacs device in 1999[13,26]. In addition, to reduce the toxicity associated with the myeloablative TBI based conditioning regimen, fludarabine was substituted for cyclophosphamide in 1995[27]. After optimizing the conditioning regimen and graft processing, Aversa *et al*[28] investigated haploidentical transplantation in 284 patients with acute leukemia. Ninety five percent of the patients achieved engraftment with minimal GVHD. The relapse rate was 17% in acute myeloid leukemia (AML) and 27% in acute lymphoblastic leukemia (ALL) patients transplanted in any CR. Incidence of TRM was 40% mainly due to opportunistic infections. Seventeen year DFS was 30% in ALL and 43% in AML patients transplanted in any CR. Among the long term survivors, chronic GVHD was not observed in any patients[28].

The major disadvantages of using T-cell depleted grafts are the high rate of relapse and life-threatening infections post-transplant[29]. Due to poor thymic function in adults, post- allogeneic transplant T-cell immune recovery depends on peripheral expansion of donor T-lymphocytes. In a T-cell depleted graft, passive transfer of T-lymphocytes is minimal leading to profound delay in immune recovery. To overcome these obstacles several strategies have evolved over the past decade including selective T-cell depletion, adoptive transfer of donor T-cells post-transplant, and T-regulatory cell (T-reg) add backs.

Selective T cell depletion

The principle behind adoptive T-cell therapy is to eliminate donor allo-reactive T cells responsible for GVHD while sparing other immune cells, which facilitate immune reconstitution. To selectively deplete allo-reactive donor T-cells, *ex vivo* T-cells are activated against host antigen presenting cells. Activated T-cells are removed using several methods including immunotoxin, immune-magnetic selection and photodynamic purging[30-32].

Another innovative approach is to selectively remove T-cells responsible for GVHD (TCR alpha-beta) while sparing gamma-delta T-cells (γδ T-cells). Gamma-delta T-cells account for 1% to 10% of peripheral T-cells. Based on *in-vitro* studies, human T lymphocytes which express γδ T-cells receptor have MHC-unrestricted innate cytotoxic activity against tumor cells[33,34]. In a recent study, Lang *et al*[35] retrospectively evaluated the immune recovery after TCRαβ/CD19-depleted haploidentical HSCT in 41 pediatric patients with acute leukemia, myelodysplasia and nonmalignant disease. Primary engraftment was seen in 88% of the patients. The incidence of grade Ⅱ and grade Ⅲ-Ⅳ acute GVHD was 10% and 15% respectively. At one year follow up, the event free survival (EFS) of patients with acute leukemia or myelodysplasia transplanted in CR1-CR3 was 100%. One year EFS of patients with subsequent HSCT (CR2-CR6) or with active disease was 29% and 11%, respectively. The use of TCRαβ/CD19-depleted stem cells substantially accelerated immune recovery. In comparison to $CD34^+$ selected grafts (historic control), patients achieved a higher $CD3^+$ at days +30 and +90, $CD34^+$ at day +30 and $CD56^+$ at day +14. The Italian group also reported similar results in 16 adults with high risk acute leukemia after TCRαβ/CD19-depleted haploidentical HSCT.

A more recent strategy to separate GVHD and the GVL effect involves selectively depleting naïve T cells identified by $CD45RA^+$ expression[36,37]. Naïve T-cells are shown to be the most allo-reactive amongst the T-cell subsets. *Ex vivo* depletion of $CD45RA^+$ T-cells and adoptive transfer of CD45RA-memory T cells hasten the immune reconstitution post-transplant, enhances the GVL effect while abrogating GVHD. This strategy was recently evaluated in a study of

17 adults with high risk hematologic malignancies (16 AML and 1 myelodysplasia) with KIR receptor-ligand mismatched haploidentical donor[38]. The conditioning regimen included total lymphoid irradiation (8 Gy), fludarabine (150 mg/m^2), cyclophosphamide (60 mg/kg), thiotepa (10 mg/kg) and melphalan (140 mg/m^2). Patients received a CD34$^+$ selected stem cell graft on day 0 followed by an infusion of CD45RA-depleted stem cells on day +1. NK cell infusion was given on day +6. Post-transplant GVHD prophylaxis included sirolimus and mycophenolate mofetil (MMF). All patients achieved primary engraftment. Neutrophil and platelet engraftment was rapidly achieved at median day +11 and +17 respectively. Acute GVHD was not seen in any of the patients. There was no infection related mortality. A phase Ⅱ study of selective depletion of CD45RA$^+$ T Cells from allogeneic peripheral blood stem cell grafts from HLA-matched related and unrelated donors for prevention of GVHD is currently under investigation[39].

SELECTIVE T-CELL ADD BACK

Con-infusion of donor-derived regulatory T-cells (Tregs) with conventional T-cells (Tcons) is another method to manipulate the T-cell depleted graft to improve haploidentical transplant outcome. In pre-clinical studies of bone marrow transplantation, infusion of donor-type CD4$^+$CD25$^+$Tregs abrogated GVHD without compromising the cytotoxic ability of T-cons against tumor cells[40,41]. A first in human study by Di Ianni *et al*[42] investigated infusion of Tregs, followed by Tcons in 28 patients with high risk hematologic malignancies who underwent haploidentical transplantation. After TBI containing conditioning regimens, patients received infusion of donor derived T-regs (2 × 10^6 Tregs) on day-4. CD34$^+$ stem cells were infused on day 0 followed by Tcons. Two out of five patients who received 2 × 10^6 Tcons/kg developed acute GVHD which led to decreasing the cell dose of Tcons to 1 × 10^6 cells/kg. Chronic GVHD was not observed in any patients. All patients achieved primary engraftment. Compared to conventional mismatched HSCT, pathogen specific CD4$^+$ and CD8$^+$ were detected earlier in the study cohort (as early as 2 mo *vs* 9-12 mo). CMV-related death, a major cause of mortality in original T-cell depleted HSCT, was not observed. At median 1 year follow up, 46% of the patients were disease free. Subsequently, Martelli *et al*[43] evaluated the impact of Tregs - Tcons infusion in reducing post-transplant relapse risk in 43 adults with acute leukemia. This method significantly reduced the risk of relapse and ameliorated GVHD. Grade 2 or more acute GVHD was seen in 15% of patients. At median follow up of 46 mo, only two patients relapsed resulting in an incidence of relapse that was significantly lower than historical controls. Despite promising results of T-cell depleted haploidentical transplant, this approach is costly, technically demanding and labor intensive which limits its application to highly experienced centers.

T CELL REPLETE GRAFT

Earlier attempts at using un-manipulated haploidentical transplant were associated with an unacceptably high rate of GVHD related mortality due to donor T-cell alloreactivity. To overcome this obstacle, several strategies have evolved over the past decade including G-CSF primed graft[44,45] and more recently post-transplant high dose cyclophosphamide.

HIGH-DOSE POST-TRANSPLANT CYCLOPHOSPHAMIDE

Cyclophosphamide is an alkylating chemotherapeutic agent which has been used for many years as a component of conditioning regimens. Preclinical trials in the early 1970s revealed short course of cyclophosphamide after bone marrow transplantation can target allo-reactive T-cells and reduce the risk of GVHD[46-48]. In contrast to calcineurin inhibitors, cyclophosphamide is capable of inducing T-lymphocyte apoptosis[49]. Hematopoietic stem cells are resistant to high dose cyclophosphamide due to expression of high levels of aldehyde dehydrogenase[50]. Original clinical trials exploring cyclophosphamide efficacy as the post-transplant GVHD prophylactic agent were performed in the haploidentical transplant setting. In 2002, O'Donnell *et al*[51] evaluated the transplant outcome of 13 patients with high risk hematologic malignancies who received T-cell replete haploidentical transplant after a non-myeloablative conditioning regimen with TBI and fludarabine. GVHD prophylaxis included post-transplant cyclophosphamide 50 mg/kg on day +3 in combination with MMF and tacrolimus. Due to high rate of graft failure (2 out of 3 patients) the protocol was amended to add cyclophosphamide 14.5 mg/kg to the conditioning regimen. Subsequently, 8 of 10 patients obtained primary donor cell engraftment. After 99 d follow up, 6 patients (46%) developed acute GVHD. Six months incidence of DFS was 50%. This study demonstrated the feasibility and possibility of rapid engraftment in a non-myeloablative haploidentical transplant setting using post-transplant cyclophosphamide.

Subsequently, Luznik *et al*[52] compared safety and efficacy of administration of cyclophosphamide on day +3 and +4 rather than only on day +3 among 68 patients with hematologic malignancies after non-myeloablative haploidentical bone marrow transplant. Primary engraftment was achieved in 87% of the patients. Notably, a very low incidence of grade Ⅲ acute GVHD (6%) with no grade Ⅳ acute GVHD was observed at one year follow up. The only difference between the two cohorts was a trend toward a lower incidence of chronic GVHD after two doses of post-transplant cyclophosphamide (5% *vs* 25%, P = 0.05). The 2-year OS and EFS rates were 36% and 26%, respectively. A major contributor to the low OS rate was a high incidence of relapse (58% at 2 years).

A similar outcome was observed in a large phase Ⅱ study of high dose post-transplantation cyclophosphamide as GVHD prophylaxis after non-myeloablative HLA-haploidentical bone marrow transplantation in 210 patients with hematologic malignancies[53]. Sustained donor cell

engraftment was obtained in 87% of the patients. The cumulative incidences of grades Ⅱ-Ⅳ acute GVHD was 27%. At 5 year follow up, OS and EFS were 35% and 27%, respectively. As seen in the prior studies, relapse was a major cause of mortality. Five year cumulative incidence of relapse was 55%.

In parallel multicenter phase 2 trials, BMT CTN 0603 and BMT CTN 0604, patients with acute leukemia or lymphoma underwent reduced intensity bone marrow haploidentical transplantation (0603) or double cord blood transplant (0604)[54]. The conditioning regimens contained 200 Gy TBI in addition to fludarabine and cyclophosphamide. In CTN 0603, the GVHD prophylaxis consisted of post-haploidentical transplant cyclophosphamide 50 mg/kg on day +3 and +4 followed by tacrolimus and MMF. In CTN 0604, GVHD prophylaxis included MMF and cyclosporine after double umbilical cord transplant. Among haploidentical transplant recipients, 100-d incidence of grade Ⅱ-Ⅳ acute GVHD and 1-year incidence of chronic GVHD were 32% and 13%, respectively. After double cord transplant 100-d incidence of grade Ⅱ-Ⅳ acute GVHD and 1-year incidence of chronic GVHD were 40% and 24%, respectively. One year cumulative incidence of relapse after haploidentical and double umbilical cord transplant were 45% and 31%, respectively. The OS and EFS rates were 62% and 48% respectively after the haploidentical transplants. Similar OS (54%) and EFS (46%) were seen after double cord transplant. The authors concluded that both RIC haploidentical and double umbilical cord HSCT are valid options in patients with hematologic malignancy. Currently a multicenter randomized phase Ⅲ trial (BMT CTN 1101) is investigating the effectiveness of haploidentical and double umbilical transplant in patients with leukemia or lymphoma[55].

Despite relatively low rates of GVHD with non-myeloablative haploidentical transplant, a high incidence of relapse has remained the main challenge in high risk hematologic malignancies. To address this obstacle, use of more intense (myeloablative) preparative regimens and peripheral blood stem cell graft was explored. In a prospective study by Solomon *et al*[56], 20 adults with high risk (relapsed/refractory) hematologic malignancies were treated with myeloablative conditioning followed by peripheral blood derived haploidentical transplant. The conditioning regimen consisted of fludarabine 30 mg/m^2 for 4 d, intravenous busulfan 130 mg/m^2 per day for 4 d, and Cy 14.5 mg/kg per day for 2 d. GVHD prophylaxis included high dose cyclophosphamide on day +3 and +4 followed by tacrolimus and MMF. All patients achieved primary engraftment. One year cumulative incidence of grade Ⅱ-Ⅳ acute GVHD and chronic GVHD were 10% and 5%, respectively. At median follow up of 20 mo, DFS and OS were 69% and 50%, respectively. The cumulative incidence of relapse was approximately 40%. The major drawback of this trial was high incidence of hemorrhagic cystitis due to BK virus infection. This adverse event was observed in two third of the patients. This was attributed to the combination of high dose busulfan and cyclophosphamide. Association of BK induced hemorrhagic cystitis and high dose busulfan in setting of mismatched HSCT was reported previously in several studies[57]. To alleviate this problem, the conditioning regimen was changed to TBI-based myeloablative regimen in the subsequent study[58]. In this phase Ⅱ prospective trial, 30 patients underwent peripheral stem cell haploidentical transplant using fludarabine 25 mg/m^2 per day for three days and 1200 cGy TBI as the preparative regimen. All patients achieved primary engraftment. Median time to neutrophil and platelet engraftment was 16 d and 25 d, respectively. Incidence of grade Ⅱ-Ⅳ acute GVHD was 23%, whereas moderate to severe chronic GVHD occurred in 22% of patients. In the entire cohort, 2-year NRM and OS were 3% and 78%, respectively. Among patients with low or intermediate risk disease NRM and OS were 0% and 100%, respectively. Relapse rate was significantly reduced in comparison to patients treated at the same center with matched related transplant. Incidence of post-transplant BK virus associated hemorrhagic cystitis was significantly reduced after TBI-based regimen compared to the busulfan-based conditioning regimen (30% *vs* 75%, $P = 0.005$).

Similar results were observed in several other trials of myeloablative haploidentical transplant[59,60]. Raiola *et al*[59] confirmed the low rate of GVHD and encouraging rate of DFS and OS in 50 patients with high risk hematologic disease (23 patients in CR and 27 patients with active disease) after un-manipulated myeloablative haploidentical transplant[59]. GVHD prophylaxis contained post-transplant cyclophosphamide on day +3 and +5 followed by cyclosporine and MMF. In the entire cohort, 12% of the patients developed grade Ⅱ-Ⅲ acute GVHD. Moderate chronic GVHD was seen in 10% of patients. The actuarial 22-mo DFS for patients transplanted in CR and patients with active disease was 68% and 37%, respectively[61]. The overall risk of relapse after myeloablative haploidentical HSCT was approximately 40% which compares favorably with that reported for non-myeloablative haploidentical HSCT. Therefore, despite the lack of randomized trials, myeloablative haploidentical transplant may be a reasonable option in younger patients with high risk hematologic malignancy in absence of timely access to a conventional donor.

Haploidentical related donor vs matched related sibling or matched unrelated donor (Table 1)

Encouraging results of haploidentical transplant compared to matched related or matched unrelated transplant has been suggested by several non-randomized studies. In 2015, a large retrospective study compared the transplant outcome of 868 patients with acute leukemia after haploidentical transplant and 9815 patients with HLA-matched sibling donor (MRD)[62]. However, leukemia free survival was significantly longer after matched sibling donor transplant compared to haploidentical transplant (T-cell depleted or T-cell replete grafts). Haploidentical transplant was associated with higher TRM. The probability of relapse was not significantly different between the two

Table 1 Unmanipulated haploidentical hematopoietic stem cell transplant *vs* matched related and matched unrelated hematopoietic stem cell transplant

Ref.	Disease	Conditioning regimen (*n*)	Graft type (*n*)	GVHD prophylaxis	Neutrophil engraftment	Grade Ⅱ-Ⅳ acute GVHD	Chronic GVHD	Relapse rate	DFS	OS
Bashey *et al*[69] 2013 *n* = 271	Acute leukemia/ CML/ myeloma/ lymphoma/ MDS	RIC (102) MA (169)	MRD (117) MUD (101) Haplo (53)	CNI based CNI based CNI + MMF + PT-Cy	NR	6 mo 27% 39% 30% (*P* = NS)	2 yr 54% 54% 38% (*P* < 0.05)	2 yr 34% 34% 33% (*P* = NS)	2 yr 53% 52% 60% (*P* = NS)	2 yr 76% 67% 64% (*P* = NS)
Di Stasi *et al*[70] 2014 *n* = 227	AML/MDS	RIC (227)	MRD (81) MUD (108) Haplo (32)	CNI + MTX CNI + MTX + ATG CNI + MMF + PT-Cy	30 d 99% 96% 97% (*P* = 0.44)	100 d 24% 19% 26% (*P* = 0.68)	3 yr 46% 42% 24% (*P* = 0.52)	1 yr 28% 23% 33% (*P* = 0.75)	3 yr 36% 27% 30% (*P* = 0.12)	NR
Luo *et al*[71] 2014 *n* = 305	Acute leukemia/ lymphoma/ MDS	MA + ATG (305)	MRD (90) MUD (116) Haplo (99)	CNI + MMF + MTX CNI + MMF + MTX CNI + MMF + MTX	15 d 97% 97% 78% (*P* < 0.001)	3 mo 15.60% 39% 42% (*P* < 0.0001)	2 yr 24% 41% 41% (*P* = NS)	5 yr 34% 21% 14% Haplo *vs* MRD *P* = 0.008 Haplo *vs* MUD *P* = 0.17	5 yr 63% 58% 58% (*P* = 0.57)	5 yr 77% 63% 60.80% Haplo *vs* MRD *P* = 0.026 Haplo *vs* MUD *P* = 0.38
Ciurea *et al*[63] 2015 *n* = 2174	AML	RIC (825) MA (1349)	MUD (737) Haplo (88) MUD (1245) Haplo (104)	CNI + MMF or MTX CNI + MMF + PT-Cy CNI + MMF or MTX CNI + MMF + PT-Cy	30 d 93% 96% (*P* = 0.25) 90% 96% (*P* = 0.02)	3 mo 19% 28% (*P* = 0.05) 16% 33% (*P* = 0.001)	3 yr 34% 52% (*P* = 0.002) 30% 53% (*P* < 0.0001)	3 yr 58% 42% (*P* = 0.006) 44% 39% (*P* = 0.37)	3 yr 9% 23% (*P* = 0.0001) 14% 20% (*P* = 0.14)	3 yr 46% 44% (*P* = 0.71) 45% 50% (*P* = 0.38)
Wang *et al*[64] 2015 *n* = 450	AML in CR1	MA (ATG in haplo cohort)	MRD (219) Haplo (231)	CNI + MMF + MTX CNI + MMF + MTX	NE engraftment 2 d longer after MRD *P* = 0.004	100 d 36% 13% (*P* < 0.001)	1 yr 42% 15% (*P* < 0.001)	3 yr 15% 15% (*P* = 0.98)	3 yr 74% 78% (*P* = 0.34)	3 yr 79% 82% (*P* = 0.36)
Ghosh *et al*[67] 2016 *n* = 987	Lymphoma	RIC (987)	MRD (807) Haplo (180)	CNI based PT-Cy ± CNI	28 d 95% 97% (*P* = 0.31)	100 d 25% 27% -0.84	1 yr 45% 12% (*P* < 0.001)	3 yr 37% 40% (*P* = 0.51)	3 yr 48% 48% (*P* = 0.98)	3 yr 62% 61% (*P* = 0.82)
Kanate *et al*[72] 2016 *n* = 917	Lymphoma	RIC (917)	MUD + ATG (241) MUD (491) Haplo (185)	CNI based CNI based PT-Cy based	28 d 97% 97% 94% (*P* = 0.32)	100 d 17% 12% 8% (*P* = 0.44)	1 yr 33% 51% 13% (*P* < 0.001)	3 yr 36% 28% 36% (*P* = 0.07)	3 yr 38% 49% 47% (*P* = 0.02)	3 yr 50% 62% 60% (*P* = 0.2)

AML: Acute myeloid leukemia; ATG: Anti-thymocyte globulin; CR: Complete remission; CNI: Calcineurin inhibitor; DFS: Disease free survival; GVHD: Graft *vs* host disease; Haplo: Haploidentical; MMF: Mycophenolate mofetil; MTX: Methotrexate; RIC: Reduced intensity conditioning; MA: Myeloablative; MDS: Myelodysplasia; MUD: Matched unrelated donor; MRD: Matched related donor; NE: Neutrophil; NR: Not reported; NS: Not significant; OS: Overall survival.

cohorts. Therefore, the authors concluded haploidentical GVL effect is similar to MRD.

Ciurea *et al*[63] also retrospectively compared the transplant outcome of patients with AML after haploidentical transplant (*n* = 192) using post-transplant cyclophosphamide and MUD (*n* = 1982). In the haploidentical cohort, 104 patients received MA and 88 had reduced intensity conditioning. In MUD cohort, 1245 patients (63%) received MA and 737 (37%) received RIC regimens. Compared to MUD, thirty day neutrophil engraftment was lower after haploidentical transplant in MA setting (97% *vs* 90%, *P* = 0.02). In RIC setting, day 30 neutrophil engraftment rate was similar between the two cohorts (96% and 93%, *P* = 0.25). Acute and chronic GVHD was notably lower after haploidentical transplant. In the MA setting, three month incidence of acute GVHD (16% *vs* 33%, *P* < 0.0001) and 3-year incidence of chronic GVHD (30% *vs* 53%, *P* < 0.0001) were significantly lower with haploidentical in comparison to MUD transplant. Similar results were obtained in RIC setting. A lower rate of GVHD with haploidentical transplant was attributed to the use of bone marrow as a graft source and the use

of post-transplant cyclophosphamide. Among patients receiving myeloablative and RIC regimens, three-year DFS and OS were comparable in haploidentical and MUD transplant.

Transplant results of matched sibling donor (MSD) transplant and T-cell replete haploidentical transplant was also evaluated by Wang *et al*[64]. In this prospective, multicenter, nonrandomized trial, 450 patients with acute leukemia in CR1 underwent MSD (n = 219) or haploidentical (n = 231) transplant. Cyclosporine, MMF, and low dose methotrexate was used as GVHD prophylaxis regimen in both groups. All individuals in both cohorts achieved donor-cell engraftment. The median time to achieve neutrophil engraftment was 2 d longer after MSD transplant. The 100-d cumulative incidence of grade Ⅱ-Ⅳ acute GVHD after haploidentical and MSD transplant was 36% and 13% (P = 0.001), respectively. The incidence of chronic GVHD was significantly higher after haploidentical transplant compared to MSD (42% *vs* 15%, P < 0.001). However, the rate of GVHD related death was similar in both groups. Among haploidentical and MSD recipients, the 3 year probability of DFS (74% *vs* 78%, P = 0.34) and OS (79% *vs* 82%, P = 0.36) were comparable. There was no difference in 3-year cumulative incidence of relapse between the two cohorts (15% *vs* 15%, P = 0.98). Lower incidence of GVHD after MSD was attributed to combination of cyclosporine, methotrexate and MMF for GVHD prophylaxis. Prior studies also reported significantly lower rate of GVHD using this combination in recipients of MSD transplant[65,66].

More recently Ghosh *et al*[67] performed a registry analysis comparing outcomes of 987 patients with lymphoma following reduced intensity haploidentical HSCT (n = 180) with MSD HSCT (n = 807). GVHD prophylaxis for the haploidentical group consisted of post-transplant cyclophosphamide with or without calcineurin inhibitor and MMF. GVHD prophylaxis for the MSD group contained calcineurin inhibitor based approaches. The cumulative incidence of grade Ⅱ-Ⅳ acute GVHD was similar between the two cohorts (27% in haploidentical cohort *vs* 25% in MSD cohort, P = 0.84). Cumulative incidence of chronic GVHD was significantly lower with haploidentical HSCT (12% *vs* 45%, P < 0.001). Chronic GVHD was the main cause of death in 5 patients in MSD cohort. There was no significant difference in the three-year cumulative incidence of relapse (37% in haploidentical *vs* 40% in MSD, P = 0.51), DFS (48% *vs* 48%, P = 0.96) and OS (61% *vs* 62%, P = 0.82). Therefore, based on this retrospective registry study in patients with lymphoma, RIC haploidentical HSCT using post-transplant cyclophosphamide provides comparable survival outcome to MSD HSCT with significantly lower risk of chronic GVHD.

CONCLUSION

HSCT is the only curative option for a large number of hematologic diseases. A minority of patients (30%) have a suitable HLA-identical sibling donor. For patients who lack MSD, MUD HSCT is frequently the preferred graft source. However, the presence of a suitably matched unrelated donor depends on factors such as the ethnicity of the patient, with a likelihood of finding an acceptably matched unrelated donor less than 20% in certain minorities compared to approximately 80% in Caucasians. A major disadvantage of MUD transplant is the prolonged time from patient referral to donor identification and collection of stem cells. Delay in the process of unrelated donor search due to logistical issues may increase the risk of disease progression or relapse[68]. Immediate availability of a haploidentical donor makes this approach an attractive treatment option for patients who lack an HLA-identical MSD or those for whom a MUD cannot be found in a timely manner. The field of haploidentical HSCT has matured significantly over the past two decades. In earlier studies of haploidentical HSCT, HLA-incompatibility barrier resulted in unacceptably high rate of GVHD and graft rejection leading to inferior OS. While effective T-cell depletion followed by infusion of mega doses of highly purified stem cells permitted high engraftment rates and reduced incidence of GVHD, higher risk of relapse and delay in immune reconstitution remained a significant obstacle. Newer methods of graft manipulation including adoptive T-cell immunotherapy and selective T-cell depletion have been shown to hasten immune recovery and reduce the risk of relapse. Despite the promising results, these approaches are costly and labor intensive, hence may not be globally available. In recent years, use of post-transplantation cyclophosphamide for GVHD prophylaxis after T-cell replete haploidentical HSCT has yielded encouraging results in adults. In several non-randomized studies, survival outcomes following haploidentical HSCT with post-transplant cyclophosphamide have been comparable to MSD or MUD transplant. Ultimately, a prospective randomized controlled trial such as BMT CTN 1101 is needed to determine the optimal approach to haploidentical transplant.

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MINIREVIEWS

Review of allogeneic hematopoietic stem cell transplantation with reduced intensity conditioning in solid tumors excluding breast cancer

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Abstract

Solid tumors in adults constitute a heterogeneous group of malignancy originating from various organ systems. Solid tumors are not completely curable by chemotherapy, even though some subgroups are very chemo-sensitive. Recently, oncologists have focused on the use of allogeneic hematopoietic stem cell transplantation (allo-HSCT) with reduced intensity conditioning (RIC) for the treatment of some refractory solid tumors. After the demonstration of allogeneic graft-versus-leukemia effect in patients with hematological malignancies who received allo-HSCT, investigators evaluated this effect in patients with refractory metastatic solid tumors. According to data from experimental animal models and preliminary clinical trials, a graft-versus-tumor (GvT) effect may also be observed in the treatment of some solid tumors (*e.g.*, renal cell cancer, colorectal cancer, *etc*.) after allo-HSCT with RIC. The use of RIC regimens offers an opportunity of achieving full-donor engraftment with GvT effect, as well as, a reduced transplant-related mortality. Current literature suggests that allo-HSCT with RIC might become a choice for elderly and medically fragile patients with refractory metastatic solid tumors.

Key words: Renal cell carcinoma; Allogeneic hematopoietic stem cell transplantation; Colorectal cancer; Ovarian cancer; Sarcoma

Core tip: Some refractory metastatic solid tumors including renal, ovarian and even colon cancers may respond well to allogeneic hematopoietic stem cell transplantation (allo-HSCT) with reduced intensity

conditioning (RIC). Their lower toxicity profiles and lower non-relapse mortality rates constitute the advantages of RIC. The use of allo-HSCT with RIC or non-myeloablative regimens can be a feasible option among fragile patients, such as geriatric patients and patients with comorbidities. Future studies are needed for a clear-cut understanding of the mechanisms of graft-versus-leukemia and graft-versus-tumor effects of donor T-cells and their subsets in order to optimize the efficacy of such treatment modalities in patients with refractory solid tumors.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is primarily used in patients with relapsed or high-risk hematologic malignancies and the efficacy of this treatment has been substantially demonstrated. The first allo-HSCT in the literature in a patient with a solid tumor was published in late 90s[1]. The principles of allo-HSCT consist of maximal tumor cytoreduction with high-dose chemoradiotherapy and adequate immunosuppression in order to provide engraftment of donor stem cells, as well as graft-versus-tumor (GvT) effect[2]. Studies investigating high dose chemotherapy with autologous stem cell rescue in patients with solid tumors yielded controversial and disappointing results[3-7]. This has led to the development of novel approaches, including allo-HSCT with reduced intensity conditioning (RIC) regimens, which aim to create and take advantage of a GvT effect in order to induce more durable responses[1,2,8-10]. Today, types of conditioning regimens that are used prior to allo-HSCT include myeloablative (MA), RIC and non-myeloablative (NMA) regimens. MA regimens lead to irreversible cytopenia and therefore, stem cell support is needed. In contrast, NMA regimens cause minimal cytopenia and can be given without stem cell support. RIC regimens do not completely fit in the criteria for MA and NMA regimens. The marrow aplasia is reversible; however, stem cell support is mandatory.

NMA/RIC regimens for allo-HSCT have introduced a new era for treating elderly and those with comorbidities[11-13]. The RIC regimens are currently being used for as much as 40% of all allo-HSCTs and becoming increasingly popular. The growing knowledge on the immune system and T-cell biology has made allo-HSCT a promising approach for the treatment of some solid tumors. Several phase Ⅰ and Ⅱ studies, which were conducted by the European Society for Blood and Marrow Transplantation Solid Tumors Working Party (EMBT-STWP) documented the presence of a GvT effect in patients with various solid tumors, such as renal, ovarian and colon cancers and soft tissue sarcomas[2].

This novel strategy provides a switch from a chemotherapy-based to an immunotherapy-based approach[14]. Replacing conventional MA regimens with NMA/RIC regimens prior to allo-HSCT has two main goals: (1) to diminish the high transplant-related morbidity and mortality[15-19]; and (2) to induce allo-reactivity against the metastatic solid tumor *via* a GvT effect[1,12].

The successful engraftment rates together with a lower transplant related mortality (TRM) and the presence of GvT effect made allo-HSCT with RIC an attractive option for the treatment of several solid tumors within the last decade[20-24]. The lower toxicity obtained by the reduction of chemoradiotherapy dose also enables allo-HSCT with RIC to become a choice for the elderly and medically fragile patients with metastatic solid tumors[1,12]. This review briefly describes the background, rationale, and clinical results of allo-HSCT with RIC as an immune-based strategy *via* GvT effect for the treatment of some metastatic solid tumors, including renal cell carcinoma (RCC), metastatic colorectal cancer (mCRC) and ovarian cancer.

CYTOTOXIC ADOPTIVE T-CELL THERAPY

Advances in systemic therapy for metastatic cancer have focused on important cellular pathways with critical roles in cancer development and progression[25]. Although a dramatic success is obtained in the minority of patients, this approach provides a relatively short-term benefit in the majority and exposes them to chronic toxicities, including cardiac and dermal toxicities and thus, is not cost-effective[26].

The mechanisms during the evasion of adoptive immune system by tumor cells have been described as growth, angiogenesis and tissue remodeling. During this process, the tumor cells also exploit the innate inflammatory response. Besides these mechanisms, the role of tumor microenvironment is also regarded as a new target for therapy[27]. Advances in understanding of cancer immunology and especially the role of the adoptive immune system, have identified new targets for the treatment of solid tumors[27].

The term, adoptive T-cell therapy (ATCT), involves the expansion of cytotoxic immune effector cells. It may be either specific or non-specific[25]. The GvT effect and tumor response after allo-HSCT with RIC may be regarded as a non-specific ATCT, as it involves leukocyte-activated killer cells (LAKs) and cytokine-induced killer cells (CIKs), which are described and discussed in this paper. ATCT is not yet considered as a standard treatment modality in the medical oncology practice. However, it is considered as the most potent immunotherapeutic approach according to the results of some early phase trials[27].

GVT EFFECT

The effect of immune system in inducing tumor regression is well-described. Graft-versus-host disease (GvHD) that occurs after allo-HSCT contributes to and maintains an anti-leukemic effect[28]. Thus, it is referred as graft-versus-leukemia (GvL) effect. This effect was first demonstrated with the eradication of leukemia in mice receiving non-syngeneic allogeneic transplant after irradiation[29]. Since then, several direct and indirect evidences of GvL effect after allo-HSCT have been reported. The GvL effect is generally associated with GvHD[30]. A stronger GvL effect is observed in chronic GvHD than in acute GvHD[31]. The probability of being in remission is also higher in patients with GvHD when compared to patients without GvHD[32]. Other strong evidences for the presence of an immune-mediated GvL effect are the significantly increased relapse risk in patients receiving T-cell depleted transplants and the lower risk of relapse observed in patients undergoing allo-HSCT rather than autologous HSCT[2,33-36]. The direct evidence of GvL effect comes from the studies reporting that donor lymphocyte infusions (DLI) given after transplant might augment the GvL effect of allo-HSCT and DLI infusion without cytotoxic therapy might induce and maintain remission in patients who relapse after allo-HSCT[37-40].

The GvL effect, which eradicates malignant cells *via* fas-dependent killing and perforin degranulation, is mediated by donor T cells ($CD4^+$, $CD8^+$ and natural killer - NK-cells)[41,42]. The major cytokines that potentiate the GvL effect include interleukin-2 (IL-2), interferon-C and tumor necrosis factor-α[43]. Post-transplant adoptive therapy with cytotoxic T-lymphocytes (CTLs) against human cancer-associated antigens, minor histocompatibility antigens (*e.g.*, HA-1, HA-3, *etc.*) or T-cell receptor genes may be used to induce anti-tumor effects[44]. The development of acute and chronic GvHD has been linked to a better response to therapy in solid tumors[2]. Identification of antigen targets of GvT and development of targeted therapies may further improve the immune effect of allo-HSCT for solid tumors and reduce the treatment toxicity[2].

Allo-HSCT is an immuno-modulatory therapy aiming at exploiting a GvT effect. However, it has to be emphasized that a delicate balance between effective immunosuppression, GvHD and relapse should still be considered.

Allo-HSCT with RIC in renal cell carcinoma

RCC is a common malignancy diagnosed in patients older than 50 years of age and almost one third of cases are metastatic at the time of diagnosis[45]. Despite various treatment strategies including hormonal therapy, chemotherapy and immunotherapy, the prognosis of metastatic RCC is extremely poor with a median survival of 10 mo and a 5-year survival of less than 5%[46,47]. RCC is sensitive to immunotherapy. Interferon-α with or without IL-2 (especially at high doses) have been widely used. However, the rates of response (10%-20%) and long-term progression- free survival (4%-15%) are still unsatisfactory[48-50]. Allo-HSCT with RIC is considered as a promising option in this setting[11,13].

A response rate of 53% has been reported in the first series of allo-HSCT with NMA conditioning for cytokine-refractory RCC[11]. Another trial included 75 metastatic RCC patients and reported a sustained engraftment in 74 out of 75 patients after allo-HSCT with NMA conditioning[51]. In this study, chronic GvHD was observed in 50% and was associated with a significant tumor response.

The largest series of allo-HSCT with NMA conditioning in RCC patients was published by the EBMT-STWP, in which a fludarabine-based conditioning regimen was administered to all 124 patients prior to peripheral blood allo-HSCT[52]. Engraftment failure was observed in 2.4%. TRM at the end of first year was 16% and associated mostly with acute GvHD. A response rate of 22.5% was achieved including complete response in 4 patients at a median of 150 (42-600) dpost-transplant.

Nowadays, patient selection for allo-HSCT has become an important issue, since disease progression after transplantation is more frequent among patients with rapidly progressive tumors. In order to determine which patients benefit most from allo-HSCT, 70 patients who underwent allo-HSCT were evaluated according to pre-transplant characteristics, such as performance status, C-reactive protein and lactate dehydrogenase levels in a study conducted by EBMT. This study suggested that these parameters could be used to stratify patients with advanced RCC who are candidates for allo-HSCT and to assist clinicians in decision-making and selection of an appropriate treatment program. As a result the patients with good prognostic criteria had a longer median survival than those with poor prognostic criteria, 23 mo *vs* 3.5 mo, respectively[45]. Another study reported a higher response rate in the presence of an early transplantation, HLA-mismatched donors, higher Karnofsky score, lower number of metastatic sites and limited chronic GvHD[52]. Currently, some other scoring systems are also developed for predicting survival in previously treated RCC patients[46].

In conclusion, NMA conditioning followed by allo-HSCT in patients with RCC is feasible and it might prolong survival, especially in patients with favorable prognostic characteristics.

Allo-HSCT with RIC in colorectal cancer

Inoperable metastatic colorectal cancer (mCRC) is an incurable disease. Despite advances in therapy, median survival with fluorouracil-leucovorin, irinotecan, and oxaliplatin as first-line therapy is 18 to 22 mo and in case of resistance to these agents, the median survival declines 9 to 12 mo with second-line chemotherapy[53,54]. Combination of chemotherapy with monoclonal antibodies such as cetuximab or bevacizumab improves remission rates and survival; however, long- lasting remission usually cannot be achieved, especially in the presence of resistant disease[55,56].

Allo-HSCT following RIC has emerged as a novel

immunotherapy-based therapeutic strategy for the management of mCRC[15,57,58]. In a study including six advanced mCRC patients, one complete response and one mixed response, including regression of lung and lymph-node metastasis and progression of liver metastasis were obtained[59]. In a multicenter EBMT trial, among 39 patients with progressive mCRC overall disease control was achieved in 18 (46%) and 1 complete (2%), 7 partial (18%), and 10 stable disease responses (26%) were reported after allo-HSCT[60]. Allo-HSCT with RIC might be an alternative to conventional strategies, especially in young patients with refractory mCRC.

Allo-HSCT with RIC in ovarian cancer

Ovarian cancer (OC) is the most fatal gynecologic malignancy and the fifth-leading cause of death among women in the developed countries[61]. Despite extensive surgery and use of new generation drugs such as taxanes (mostly in combination with carboplatin), relapse rates may reach up to 50%. Although sensitive to high-dose chemotherapy (especially based on carboplatin combinations), the median overall survival is about 2 years for relapsing disease[62,63]. The only benefit of high-dose chemotherapy does appear to be delayed relapse[64,65].

In a study, including five refractory OC patients who underwent allo-HSCT with RIC, tumor regression were observed in four patients during acute or chronic GvHD and relapse occurred in one patient treated with methylprednisolone for chronic GvHD[66]. A retrospective study from the EBMT-STWP database included 17 heavily pre-treated OC patients and mortality was reported in 11 patients, 8 of which were due to tumor progression at a median follow-up of 296 d (5-1599)[67]. Grade 2-4 acute GvHD was reported in eight patients, seven (41%) of which had a partial response. Tumor regression was achieved in one out of three patients who received DLI. This data supports the existence of a graft-versus-ovarian cancer effect in correlation with GvHD. In another retrospective multicenter study with 30 allografted OC patients, objective response was observed in 50% and TRM was 20% at the end of first year[68]. The median overall survival was 10.4 mo with a median follow-up of 74.5 mo (16-148). Overall survival was significantly higher among patients with chronic GvHD (17.6 mo *vs* 6.5 mo, $P < 0.05$).

Allo-HSCT with RIC for OC could be a feasible treatment option. However, supporting data are limited.

Allo-HSCT with RIC in soft tissue sarcomas

Soft tissue sarcomas (STS) constitute a rare and heterogeneous group of malignant tumors, which include less than 1 percent of all adult malignancies. Prognosis of STS is poor with a median survival of about 1 year with conventional treatments[69].

In experimental animal models of allogeneic transplantation, immune-mediated effect against sarcoma has been shown[70,71]. However, reports on STS treated with allo-HSCT mostly consist of single case reports and small series of patients from HLA-matched sibling donors. Although some authors have reported the evidence of a graft-*vs*-sarcoma effect, no evidence of cancer regression following allo-HSCT with RIC regimens were reported among patients with various histologic subtypes[72-74]. In a retrospective study, 14 adult patients from EBMT database with advanced STS received allo-HSCT with RIC for chemo-refractory disease, excluding rhabdomyosarcoma (most frequently a pediatric disease with an extremely different natural history) and they were assessed regarding whether a GvT effect could be generated in this setting. TRM was reported in two patients and progressive disease was observed in eight patients. Four patients experienced long-lasting disease stabilization following allo-HSCT. Authors concluded that an immune-mediated effect cannot be excluded in some STS[75].

In conclusion, allo-HSCT with RIC may give rise to some degree of significant responses in some refractory metastatic solid tumors, such as renal, ovarian and even colon cancers. The advantages of RIC regimens are their lower toxicity profiles and lower non-relapse mortality rates. Allo-HSCT with RIC or NMA can be a feasible option for geriatric patients and patients with comorbidities. Future studies are needed for a clear-cut understanding of the mechanisms of GvL and GvT effects of donor T-cells and their subsets in order to optimize the efficacy of such treatment modalities in patients with refractory solid tumors.

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"Contrast nephropathy" in renal transplantation: Is it real?

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Abstract

The risk of contrast-induced nephropathy (CIN) in renal transplant recipients is increased in diabetics, patients with impaired basal kidney function, patients in shock, patients presenting with acute emergency and in old age recipients. Approximately one-third of all hospitalized patients with acute kidney injury is attributed to CIN. In the United States, it is the third leading cause of hospital-acquired renal failure. Therefore, efforts should be directed to minimize CIN-related morbidity and mortality as well as to shorten hospital stay. While the role of peri-procedural prophylactic hydration with saline is unequivocal; the use of acetyl cysteine is not based on robust evidence. The utility of theophylline, aminophylline, calcium channel blockers, natriuretic peptide, and diuretics does not have proven role in attenuating CIN incidence. We aim to analyze the evidence for using various protocols in published literature to limit CIN-associated morbidity and mortality, particularly during surveillance of the renal allograft survival.

Key words: Contrast; Renal; Transplantation; Nephropathy

Core tip: The renal transplant is usually a solitary kidney with diverse hemodynamic changes and exposed to the immunosuppressive agents for a long period. Any superadded stress such as contrast-induced nephropathy (CIN), will definitely affect allograft function. We provide in this article a comprehensive review of the current evidence on the true incidence, the mechanism of damage induced by CIN and available preventive measures to counteract the possible effect induced by CIN.

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INTRODUCTION

Perioperative transplant complications are reported to involve about 15%-20% of the kidney transplant recipients. Diagnostic ultrasound (US) is the most common and first line imaging modality[1], since it is safe, noninvasive, gives a rapid diagnosis and also a portable tool for many surgical emergencies requiring bedside imaging[2-5]. The utility of ultrasonography in management of hydronephrosis, renal masses, renal artery stenosis (RAS) and pyelonephritis in renal allograft is well documented[1].

Computed tomography (CT) scanning and CT-guided interventions play a vital role in investigating post-operative complications. Magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) can be used safely where there is renal dysfunction, since the "Gadolinium-based" contrast media can be safely used with minimal nephrotoxic effects. Post-transplant complications such as vessel thrombosis can also be assessed using these modalities. Allograft "morphology and function" can be effectively assessed by using intravenous Gadopentetate Dimeglumine (DTPA) to the MRA technique[6].

However, the patients with pacemakers, aneurysmal clips, or evident claustrophobia cannot be safely exposed to MRI studies. Gadolinium-based media have been linked to the development of nephrogenic systemic fibrosis (NSF). Another drawback of MRI, is the "layering" of the excreted gadolinium in the urinary bladder causing multiple image artifacts. Alternatively, CT is better for the evaluation of the kidney and urinary bladder for renal stones and ureter and bladder abnormalities. To summarize, MRI is usually dedicated to the evaluation of transplant recipient, whereas CT and CT angiogram are reserved for potential donors[6].

MECHANISM OF CONTRAST-INDUCED NEPHROPATHY

Vasoconstriction induced by the contrast media (CM) can be explained by the direct action of contrast media on vascular smooth muscle and from metabolites such as adenosine and endothelin. Moreover, the osmotic criteria of contrast media, especially in the tubular lumen, affects water reabsorption, leading to a magnifying interstitial pressure. This will be augmented by the increased salt and water load to the distal tubules, will decrease GFR and lead to local compression of the vasa recta. All these factors will aggravate medullary hypoxemia and renal vasoconstriction in an already volume depleted patient.

Finally, contrast media could increase resistance to blood flow by increasing its viscosity and by deranging red blood cells (RBCs) deformability. These manifest as local ischemia leading to activation of reactive oxygen species that result in damage to renal tubules[7].

Up till now, we are sure why renal failure patients are sensitive to contrast utilization. Whether their primary disease is a contributing factor or not, this has to be elucidated by additional future research.

CONTRAST NEPHROPATHY IN RENAL TRANSPLANTATION

Intravenous contrast (Table 1)

Only a relatively handful of studies have looked into the contrast-induced nephropathy (CIN) in the renal transplant recipients. Light *et al*[8], 1975 studied thirty-four renal transplant recipients received drip infusion urograms post-transplantation. Twenty-two patients exhibited a change in renal function within 1-4 d of the urogram that was indistinguishable from allograft rejection that is a tender swollen kidney, a rise in serum creatinine, oliguria, diminished urinary sodium, weight gain and hypertension. Two patients developed acute tubular necrosis (ATN) and required hemodialysis, but renal function in the remaining 20 patients improved after therapy for "graft rejection" with intravenous methylprednisolone sodium succinate. Kidneys from older-age donors that were functioning sub-optimally at transplant and kidneys, which exhibited subsequent clinical allograft rejection, were more at risk for CIN. These suggested occult vascular lesions might have been present in the allograft, which was exacerbated when exposed to the irritant vascular effects of contrast media, producing a mild, reversible toxic nephritis. However, several renal grafts with normal function and also those, which never exhibited rejection activity, were also adversely affected by exposure to contrast media. Therefore these agents should be used cautiously, if at all, in the early post-transplant period[8]. CIN was more common and more severe in those with impaired kidney function. This study also found that kidneys from older donors were at higher risk for CIN. In this study, contrast was used before stable creatinine was achieved, these kidney transplant recipients were not on a CNI, and there is no mention of use of any prophylaxis to prevent CIN. More than half of these patients were thought to have acute rejection and were treated as such without consistent biopsy documentation[8].

The incidence of acute kidney injury (AKI) induced by CIN resulting from direct exposure to contrast media in kidney transplants recipients still controversial. The main insult is the ensuing vasoconstriction of the afferent glomerular arterioles and reduction in renal blood flow and glomerular filtration rate. Renal vasoconstriction, as well as direct tubular epithelial toxicity, is the two major mechanisms by which contrast causes AKI as explained by Haider *et al*[9] in 2015. Immediately after contrast

Table 1 Trials concerned with contrast nephropathy

No.	Trial	Year	No. of KTRs	Need for HDX	CIN	Comments
1	Light *et al*[8]	1975	34	Two	22	20 patients improved after therapy for "graft rejection"
2	Moreau *et al*[12]	1975	231	None	Nil	No increase in risk of CIN in KTRs if contrast studies were performed with normal renal function
3	Peters *et al*[11]	1983	93	None	Very high (84.3%)	No increased risk was found > 120 d post-transplant
4	Ahuja *et al*[10]	2000	35	None	> 21%	Patients received high osmolality contrast, and 94% were on CyA therapy
5	Charnow *et al*[16]	2015	76	None	> 13.2%	CIN did not affect allograft function and survival, according to the researchers
6	Haider *et al*[9]	2015	124	None	5.60%	The largest retrospective study evaluating incidence of CIN in KTRs. CNIs were being used in 95% patients at the time of contrast administration
7	Bostock *et al*[15]	2016	40	One	12.50%	Renal dysfunction is 3 times more frequent in KTR treated with EVAR, though overall survival did not differ between groups. Decreased pre-operative eGFR and higher iodine/eGFR ratio are associated with post-operative renal dysfunction
8	Fananapazir *et al*[14]	2016	104	None	7% and 3%	Incidence of CNI = 7% (7/104) based on a rise of ≥ 0.3 mg/dL and 3% (3/104) based on a rise of ≥ 0.5 mg/dL. With a strict definition (≥ 0.5 mg/dL) had a pre-CT eGFR < 60 mL/min per 1.73 m^2. No patients required DX or had allograft loss 30 d after contrast use

CIN: Contrast-induced nephropathy; HDX: Hemodialysis; KTRs: Kidney transplant recipients; CNIs: Calcineurin inhibitors; EVAR: Endovascular aortic aneurysm repair; eGFR: Estimated glomerular filtration rate.

use, there is a transient increase in renal blood flow followed by a prolonged reduction in flow resulting in renal ischemia. So, there is "clustering" of two risk factors here, as both calcineurin inhibitors and IV contrast cause renal ischemia by the dual mechanism: (1) by increasing the release of the vasoconstrictors such as endothelin; and (2) by blocking the release of vasodilators including prostaglandins and nitric oxide[10].

Ahuja *et al*[10] (2000) also studied 35 kidney transplantation recipients (KTRs) as regard the effect of "volume expansion" as well as the effect of cyclosporine therapy; which documented the presence of CIN in a percentage exceeding 21%, with incidence of CIN was about 15% in patients received volume expansion and exceeds 42% in those who did not. None of these patients had AKI requiring dialysis. In this study, two main insults were reported, first: They received "high osmolality" contrast, and second: 94% were on cyclosporine therapy. The baseline serum creatinine in patients with and without CIN was 1.54 ± 0.17 mg/dL and 1.97 ± 0.20 mg/dL, respectively, $P = 0.15$, but the volume of contrast was not reported here. Another study- demonstrated by Peters *et al*[11] in 1983-reported a very high incidence of CIN (84.3%) in the early post-transplant period, but no increased risk was found > 120 d post-transplant.

Moreau *et al*[12] (1975) demonstrated clear evidence that there was no increase in the risk of CIN in kidney transplant recipients if contrast studies were performed against a background of normal renal function. Data observed from these studies showed that older donor kidney, early post-transplant period, impaired baseline kidney function, and lack of prophylactic volume expansion, appear to be important risk factors for increasing the incidence of CIN in kidney transplant recipients (Figure 1). In fact, a direct comparison between these studies regarding the incidence of CIN among is challenging, as the definition of AKI used was not uniform. There were differences in baseline serum creatinine; use of hyper-, hypo-, or iso-osmolar contrast; volume of contrast given; and the proportion of patients with known risk factors for CIN, including: Diabetes Mellitus, congestive heart failure, and concomitant use of CNI, in these studies which make it difficult to conclude the actual incidence of CIN in kidney transplant recipients.

To date, Haider *et al*[9] (2015) study is considered the largest retrospective study evaluating the incidence of CIN in kidney transplantation. The incidence of CIN in this study was low (5.6%), much lower than reported by Ahuja *et al*[10] (2000). Two fundamental factors were implicated in this low incidence of CIN in this landmark study first: the relatively elevated baseline eGFR (> 70 mL/min per 1.73 m^2) and second: The use of "hypo-osmolar" contrast applied in this procedure[9].

Another possible explanation for the low incidence of CIN in this study is that Diabetes Mellitus and hypertension in these patients may not have damaged the renal allograft to the extent to potentiate CIN. Another important factor is the age of the kidney rather than the age of recipient may affect the susceptibility for CIN. Furthermore, among all procedures utilizing iodine-based contrast, coronary angiography with the percutaneous intervention was responsible for 49% of cases of CIN[13]. However in Haider *et al*[9] (2015) work, only 4.8% of patients have had cardiac catheterization (none of them had CIN), and this might also have leading to low incidence of CIN-AKI in this group of patients. Their inability to identify association with known risk factors for CIN may be explained by the very small number of patients complicated by CIN events.

On the other hand, Fananapazir *et al*[14], 2016, declared in the most recent trial that CIN incidence was very low,

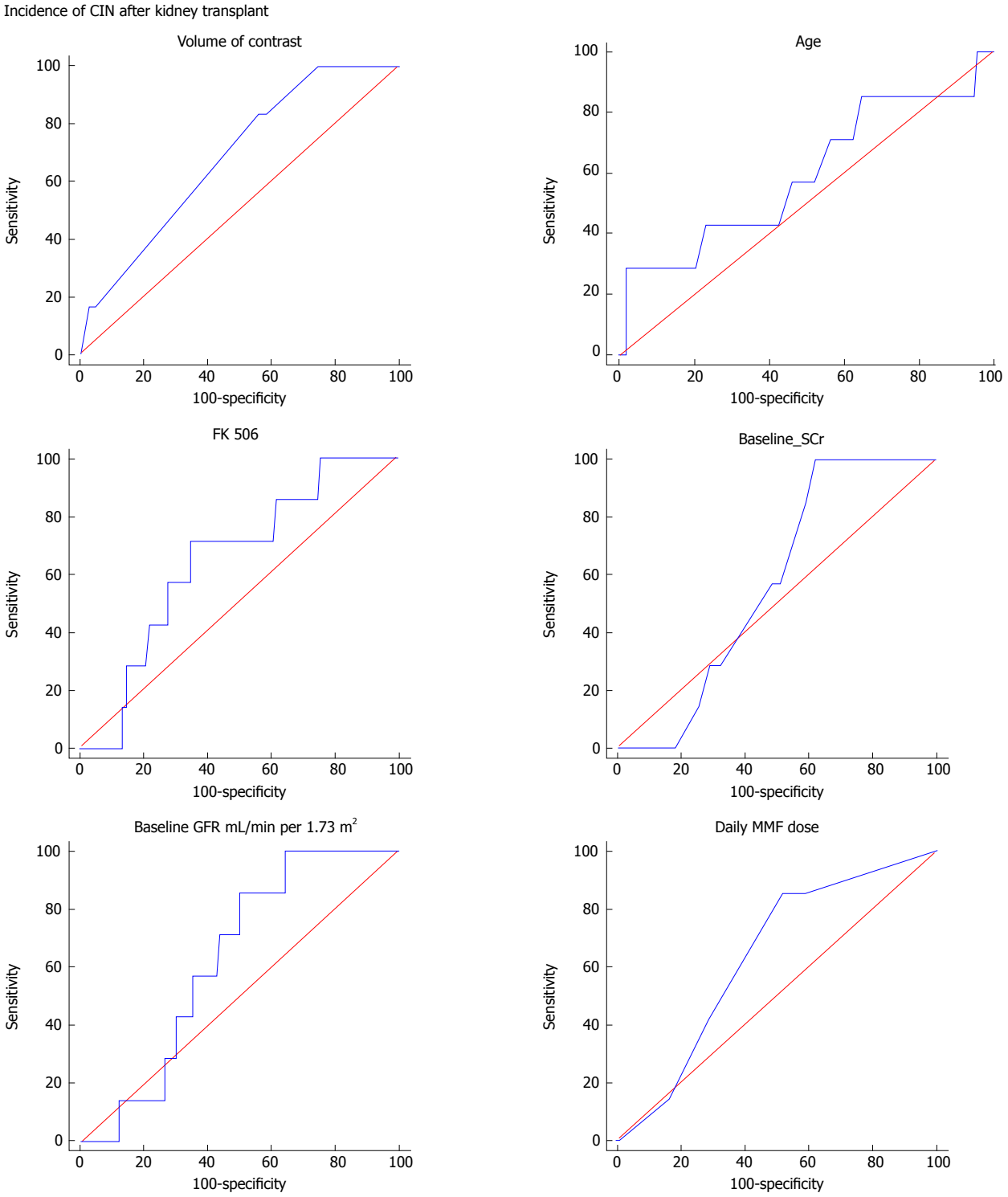

Figure 1 Receiver operating characteristics curves for age, FK506 levels, daily Cellcept dose, baseline Cr., eGFR, and volume of IV contrast. Area under the curve (AUC) for age, FK506 levels, daily Cellcept dose, baseline creatinine, eGFR, and volume of IV contrast were 0.60, 0.64, 0.63, 0.57, 0.63, and 0.68, respectively[9]. Adapted from Haider *et al*[9], Incidence of Contrast-induced Nephropathy in Kidney Transplant Recipients. *Transplantation Proceedings* 2015; **47**: 2379-2383 (with permission). GFR: Glomerular filtration rate.

i.e., 7% and 3% according to an elevation of SCr of > 0.3 and 0.5 respectively, after a low osmolality contrast administration. There was with no need for emergent dialysis or an allograft loss 30 d post-operative[14].

Moreover, Bostock *et al*[15] in 2016, also demonstrated that CIN following endovascular aortic aneurysm repair (EVAR) in kidney transplant recipients could have deranging sequelae. The Vascular Quality Initiative (VQI) database was interrogated to select all kidney transplant recipients who underwent EVAR between 01/2003 and 12/2014. Their primary outcome was renal dysfunction, defined as AKI (rise in serum creatinine concentration

> 0.5 mg/dL above the baseline or new post-operative hemodialysis requirement). Within the EVAR VQI dataset, 40 subjects were kidney transplant recipients (40/17, 213, 0.2%). Renal dysfunction occurred in 5/40 patients in the kidney transplant recipients group in comparison to 779/17173 patients in the non-transplanted group (12.5% *vs* 4.5%, $P < 0.01$). Emergency EVAR was indicated in 2 (5%) patients who required hemodialysis after surgery and died later. One-year survival after EVAR was similar in both groups (92.9% *vs* 93.1%, $P = 0.73$). Kidney transplant recipients who developed renal dysfunction had significantly lower pre-operative eGFR's (29.5 *vs* 54.7, $P = 0.007$) and a significantly higher iodine/eGFR ratio (0.78 *vs* 0.39, $P = 0.02$) despite receiving a similar volume of contrast (70.0 *vs* 68.8, $P = 0.97$). Renal impairment was three times more frequent in kidney transplant recipients treated with EVAR, despite the overall survival did not differ between groups. Diminished pre-operative eGFR and a higher iodine/eGFR ratio were associated with post-operative renal dysfunction[15]. Charnow *et al*[16] 2015, showed an incidence about 13% of CIN in allograft recipients undergoing CT or cardiac catheterization with contrast media. CIN was relatively common in kidney transplant recipients undergoing (CT) or cardiac catheterization with contrast media. Charnow *et al*[16] (2015) at the University of Cincinnati in Ohio studied 76 contrast exposures (45 CT scans and 31 catheterizations) in 50 kidney transplant recipients (50% male) with a mean age of 53.3 years and means. Cr level of 1.46 mg/dL. The investigators reported CIN - defined as a rise in s. Cr by > 0.3 mg/dL or 25% from baseline within 4 d. after the procedure - in 10 of 76 procedures (13.2%). Results demonstrated: 6 (13.3%) of the 45 CT scans and 4 (12.9%) of the 31 catheterizations resulted in CIN[16].

Abu Jawdeh's group (2015)[16] also examined the risk factors for CIN. In a multivariate model, exposure to N-acetylcysteine (NAC) and a lower hemoglobin level was significantly associated with an increased risk of CIN, but not with CNI use. They assumed that NAC might have been used in high-risk subjects for CIN, a bias that could explain the increased risk of CIN associated with NAC use. At the last follow-up, CIN did not affect allograft function and survival, according to the investigator[16].

CIN is accompanied by a significant rise in mortality and morbidity; Abu Jawdeh suggested that extrapolation of knowledge about CIN affecting the native kidneys and applying this to allografts might not necessarily reflect the best practice. Allografts are solitary kidneys that exposed to significant hemodynamic alterations and also under the effect of lifelong immuno-suppressive agents. Both these factors might affect susceptibility to contrast-induced renal injury. They also suggested that CIN is potentially modifiable if risk factors are well identified and the proper preventive precautions are performed. The 13.3% incidence of CIN identified in this study is consistent with previous studies looking at native kidneys[16]. Due to the retrospective nature of this study and the small sample size, this study should be interpreted with caution.

Finally, it appears that the strict "definition of CIN" in various studies was not universal. While Charnow *et al*[16] defined CIN as a rise in s. Cr by > 0.3 mg/dL or 25% rise from baseline within four days of contrast exposure, Bostock *et al*[15] defined CIN as an AKI with elevation of SCr > 0.5 mg/dL from baseline, or new post-operative hemodialysis (HD) requirement%. Haider *et al*[9] (2015) defined CIN as either an absolute rise in serum creatinine of $\geqslant$ 0.5 mg/dL or a $\geqslant$ 25% drop in estimated glomerular filtration rate (eGFR) after contrast administration. On the other hand Fananapazir *et al*[14] (2016) applied two definitions for CIN in the most recent study, they found CIN in 7% based on a rise of $\geqslant$ 0.3 mg/dL and 3% based on a rise of $\geqslant$ 0.5 mg/dL. Patients with the more strict definition ($\geqslant$ 0.5 mg/dL) had a pre-contrast eGFR < 60 mL/min per 1.73 m^2.

"Ultrasound with contrast": Contrast enhanced ultrasound (CEUS) is a promising radiological technique with increased popularity. It has a superiority over the color Doppler ultrasound in evaluation of kidney microvasculature studies. A wide variety of diagnoses can be applied including differentiation of cystic from solid lesion, solid mass assessment, pseudotumor and RAS. Moreover, CEUS can help in elucidating the hemodynamic changes associated with chronic allograft nephropathy (CAN)[17].

US contrasts are gas microbubbles of nearly the same size of RBCs, which enclosed in a protein, lipid or polymer shell[18]. They last intravascular only for few minutes (time of CEUS examination), after that, the gas exhaled through the lungs and the shell metabolized by the liver[19], so renal excretion is not a possibility. As these contrast agents is not excreted through the kidney, allograft integrity cannot be deranged. So, their use in KTRs with impaired renal function is completely safe. Furthermore, CEUS is the sole available technique for dynamic evaluation of kidney perfusion, particularly so, when the use of contrast media is mandatory in CT and MR studies in patients with renal dysfunction. CEUS has a wide safety margin in comparison with other radiological modalities[20,21].

Prevention of CIN-induced AKI in the renal transplant recipient: There are no specific measures dedicated to prevent CIN-induced AKI in the renal allograft, but rather universal recommendations. The optimal recommendations for CIN prevention are still uncertain.

The following precautions are suggested with increased risk of CIN (S. creatinine $\geqslant$ 1.5 mg/dL (132 micromols/L) or an eGFR < 60 m$/1.73 m^2), especially in diabetics: (1) Avoid volume depletion and NSAID[22,23]; (2) Avoid use of high osmolar agents (1400-1800 mosmol/kg)[24,25]; (3) Try to use US and MRI without gadolinium contrast, or CT scanning without contrast media when possible; (4) Choose iodixanol or nonionic low-osmolar

agents, *e.g.*, iopamidol or ioversol rather than iohexol[25]; (5) Apply lower doses of contrast and avoid repetitive, closely spaced studies (< 48 h apart)[12,13,15,16,25]; (6) In an absence of contraindications to volume expansion, start isotonic intravenous fluids before and continued several hours after contrast use. Optimal type and timing are not well documented. "Isotonic bicarbonate" is preferred to isotonic saline as a "volume expander"[23,26-29]. "Isotonic bicarbonate" regimen: A bolus of 3 mL/kg for one hour prior to the procedure, and continued at a rate of 1 mL/kg per hour for "6" h after the procedure[23,26-29]. Suggested regimen for isotonic saline: Isotonic saline (1 mL/kg per hour), starting at least 2-6 h before, and continued for 6-12 h after the procedure. Duration of intravenous fluid should be directly proportional to the degree of renal dysfunction (*i.e.*, longer duration for severe renal impairment); (7) Based upon potential benefit, low toxicity, and cost, Acetylcysteine (AC) can be given: 1200 mg orally twice/day, the day before and the day of the procedure. Intravenous AC is NOT recommended due to lack of evidence of benefit and potential risk of anaphylactoid reactions[30,31]; and (8) Prophylactic use of "mannitol" or other diuretics is NOT recommended[32,33]. Prophylactic HF/HDX after contrast exposure is NOT advised on stage 3 and 4 CKD[34].

Oral contrasts

Two documented contrast media are already in use for oral imaging procedures: First: Barium sulphate, a commonly used oral contrast agent (for GI studies); Second: Gastrografin, which is a substitute agent for the barium in special situations. Generally, barium, as well as gastrografin, is safe, passing through the gastrointestinal tract easily like food and drink[35].

Barium sulphate is by far the most common contrast material used orally. It can also be utilized rectally. Multiple forms are available, including powder, liquid, paste and tablets. They are generally safe. Only mild unpleasant taste can be observed. If given by enema, abdominal fullness, change in bowel habits and whitish discoloration may be observed for only a few days[36].

Nephrostogram

A nephrostogram is a radiological tool performed to check the nephrostomy catheter and to rule out any abnormalities in the kidney and ureters, for example, obstructive uropathy. It is performed by disconnecting the catheter from its drainage bag and injecting the iodinated contrast through its lumen, monitored with fluoroscopy and static X-ray imaging. Nephrostogram is a very safe technique with few documented complications. Only mild pain with the possibility of the introduction of infection can occur. Unfortunately, this procedure has no known alternative technique[37].

CONCLUSION

The risk of CIN affecting renal allograft function is significant especially in diabetics, old age and in volume depleted subjects. This risk can be greatly mitigated through optimizing the hydration status in peri-procedure period, by avoiding nephrotoxic medications, by careful use of safe and widely spaced contrast media with the possible minimal amount of contrast media and possibly by prophylactic peri-procedural administration of isotonic bicarbonate. Some of the questions remain unanswered that require randomized controlled trials involving larger number of renal transplant recipients in order to maximize safety of the renal allograft.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Impact of preformed donor-specific antibodies against HLA class Ⅰ on kidney graft outcomes: Comparative analysis of exclusively anti-Cw *vs* anti-A and/or -B antibodies

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Abstract

AIM

To analyze the clinical impact of preformed antiHLA-Cw *vs* antiHLA-A and/or -B donor-specific antibodies (DSA) in kidney transplantation.

METHODS

Retrospective study, comparing 12 patients transplanted with DSA exclusively antiHLA-Cw with 23 patients with preformed DSA antiHLA-A and/or B.

RESULTS

One year after transplantation there were no differences

in terms of acute rejection between the two groups (3 and 6 cases, respectively in the DSA-Cw and the DSA-A-B groups; *P* = 1). At one year, eGFR was not significantly different between groups (median 59 mL/min in DSA-Cw group, compared to median 51 mL/min in DSA-A-B group, *P* = 0.192). Moreover, kidney graft survival was similar between groups at 5-years (100% in DSA-Cw group *vs* 91% in DSA-A-B group, *P* = 0.528). The sole independent predictor of antibody mediated rejection (AMR) incidence was DSA strength (HR = 1.07 per 1000 increase in MFI, *P* = 0.034). AMR was associated with shortened graft survival at 5-years, with 75% and 100% grafts surviving in patients with or without AMR, respectively (Log-rank *P* = 0.005).

CONCLUSION

Our data indicate that DSA-Cw are associated with an identical risk of AMR and impact on graft function in comparison with "classical" class I DSA.

Key words: Donor-specific antibodies; Antibody-mediated rejection; Anti human leukocyte antigen class Ⅰ; AntiHLA-Cw antibodies; Graft survival; Solid-phase immunoassays

Core tip: The clinical importance of preformed antiHLA-Cw donor-specific antibodies (DSA) in kidney transplant patients remains controversial, so we performed a retrospective study comparing 12 patients with DSA exclusively antiHLA-Cw with 23 patients with preformed DSA antiHLA-A and/or B. Antibody-mediated rejection occurrence and graft survival frequency, respectively, at one and at five years of follow-up, were comparable between groups. Our data support a similar deleterious impact considering DSA-Cw or DSA-A/-B in terms of risk of AMR and impact on graft function.

Santos S, Malheiro J, Tafulo S, Dias L, Carmo R, Sampaio S, Costa M, Campos A, Pedroso S, Almeida M, Martins LS, Henriques C, Cabrita A. Impact of preformed donor-specific antibodies against HLA class Ⅰ on kidney graft outcomes: Comparative analysis of exclusively anti-Cw *vs* anti-A and/or -B antibodies. *World J Transplant* 2016; 6(4): 689-696 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i4/689.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i4.689

INTRODUCTION

In kidney transplantation the presence of preexisting anti human leukocyte antigen (HLA) donor-specific antibodies (DSA) has impact on graft outcomes. Their presence is associated with an augmented risk of antibody-mediated rejection (AMR)[1] and worst graft survival[2].

Classically, antibodies against major HLA Class Ⅰ (A and B) and Class Ⅱ (DR and DQ) antigens are considered to be responsible for most cases of AMR. AntiHLA-Cw are considered less immunogenic when are paralleled to other class I antiHLA antibodies, mainly due to minor HLA-Cw antigen expression on cell surface[3]. Indeed, some studies found that the incidence of antiHLA-Cw antibodies in sensitized patients was lesser than that for HLA-A or HLA-B antibodies[4-6].

However, the progress of additional sensitive assays that identify HLA antibodies, namely solid-phase immunoassays, demonstrated that HLA-C locus may induce an antibody reaction comparable to the other usually tested *loci*[4,5,7,8]. In 2012, Ling *et al*[5] showed that kidney transplantation in patients with isolated antiHLA-Cw antibodies was effective (no rejections occurred) when using induction treatment with anti-thymocyte globulin (ATG) and IVIG. Another study evaluated 22 patients with pretransplant DSA antiHLA-Cw in comparison with 88 patients allosensitized but with no detectable preformed DSA and concluded that they seem to be at superior risk for AMR occurrence[9]. Recently, Bachelet *et al*[10] in their retrospective and multicenter study showed that antiHLA-Cw DSA have the same effect on graft outcome as DSA against "classical" HLA *loci* (A, B, DR, DQ), suggesting that antiHLA-Cw should also be considered in transplant allocation procedures and in immunologic risk stratification of patients.

As this subject remains controversial, we decided to conduct a retrospective study in kidney transplant patients to investigate the clinical impact of preformed antiHLA-Cw DSA comparing them to DSA against the other HLA class I loci, namely antiHLA-A and/or B.

MATERIALS AND METHODS

Patients

From the database of our Histocompatibility Center 35 adults who received a kidney transplant since 2007 were identified as having pretransplant donor specific antibodies (DSA) exclusively antiHLA-A and/or -B or exclusively antiHLA-Cw. Twenty-three patients had DSA antiHLA-A and/or antiHLA-B: 6 with DSA antiHLA-A only; 11 with DSA antiHLA-B only and 6 with DSA antiHLA-A and -B. This group was designated DSA-A-B. Twelve patients had DSA exclusively antiHLA-Cw, and this group was designated DSA-Cw. The patients were all transplanted with a negative T- and B-cell cytotoxic crossmatch (standard NIH technique). The Institutional Review Board at Hospital Santo António, CHP approved this study.

AntiHLA antibody testing

Patients in the waiting list were examined for antiHLA IgG by multiplex microsphere based on Luminex X-map® Technology (LABScreen® Mixed kit, OneLambda, Canoga Park, CA, United States). The cut-off for positive samples was the Normalized Background (NBG) ratio advocated by the manufacturer and executed by the HLA fusion® software (One Lambda Inc.). To determinate the specificity of the HLA antibodies, single-antigen bead (SAB) assays (LabScreen Single Antigen Beads®, OneLambda, Canoga Park, CA) were executed in patients with a positive

screening, using the same pretransplant sera. The mean fluorescence intensity (MFI) was measured using LABScan ™ 100 flow analyzer (Luminex®, Austin, TX, United States). The analysis was performed using HLA fusion® software (One Lambda Inc.) and a cut-off for a positive reaction were set in MFI value of ⩾ 1000.

Donor typing and crossmatch

Samples of all deceased donors were routinely typed before recipient selection in loci HLA-A*, B*, Cw* and DRB1* using polymerase chain reaction (PCR) amplification with specific sequence primers (SSP; Olerup SSP® low resolution HLA typing kits, Stockholm, Sweden). After donor HLA typing, using that information, a virtual crossmatch (virtual XM) was executed. The strength of each single DSA was based on the MFI of one SAB. In the case of several DSA against different HLA-antigens, we considered the cumulative strength of all DSA by adding the individual MFI values.

Immunosuppression

Thirty-three of the total of 35 patients (94.3%) received induction therapy: Ten patients with a monoclonal antibody anti-IL-2 receptor (Basiliximab Novartis®, 20 mg twice at day 0 and 4), and 23 patients with polyclonal ATG Fresenius® (3 mg/kg for 5-7 d). All patients had an equivalent maintenance immunosuppression using three oral drugs: A calcineurin inhibitor [tacrolimus (FK-506) in the majority of patients (32/35 patients) or cyclosporine (CsA) in 3 patients], mycophenolate mofetil (MMF) and a corticosteroid. FK-506 was started at a dose of 0.1-0.15 mg/kg per day, and was adjusted to maintain levels between 8 and 12 ng/mL during the first month post-transplant, between 7 and 10 ng/mL the next 2-3 mo and between 5 and 8 ng/mL thereafter. MMF was started at a dose of 2000 mg/d, and decreased based on white blood cells count. Methylprednisolone was administered intravenously at doses of 500, 250 and 125 mg/d on the day of transplantation, days 1-2 and days 3-4 after the operation, respectively. Oral prednisolone was started on day 5 after the operation at the dose of 20 mg, being then tapered to 5-10 mg/d within 2-3 mo after transplant. Living donor recipients (n = 3) were prescribed FK-506 and MMF 7 d before transplant.

Eight patients underwent a desensitization protocol. Five patients received intravenous immunoglobulin (IvIg) 2 g/kg at transplant (0.5 g/kg immediately before transplant, and at day 1, 2 and 3) and 1-mo after transplant (1 g/kg in 2 consecutive days). One patient received a similar dose of IvIg and underwent plasmapheresis every other day (first session immediately before transplant, for a total of 6-9 sessions) and two other patients received additionally a dose of Rituximab (375 mg/m^2) on day 3 post-transplant.

Patients' data and outcomes

The data concerning patients' characteristics and transplantation variables was collected retrospectively. Estimated glomerular filtration rate (eGFR) was assessed using the 2006 Modification of Diet in Renal Disease (MDRD) equation and dialysis requirement in the first week post-transplant was defined as delayed graft function. Patients were followed until graft failure, death or end of follow-up (five years after transplant or December 31, 2015, which came first). Graft survival was evaluated considering graft failure censored for death with a functioning graft.

Follow-up

Graft biopsies were performed "for cause" only. Allograft rejection was classified according Banff classification (updated in 2013) and defined by biopsy where specimens were evaluated by light microscopy and immunofluorescence (with C4d staining). Mild acute cellular rejection (ACR Banff grade Ⅰ) was treated with 500 mg methylprednisolone for 3 d and increased maintenance immunosuppression. All other ACR were treated with ATG. AMR patients were treated with plasmapheresis every other day (the number of plasmapheresis sessions was 4 per protocol) and IvIg 100 mg/kg after each session. After the last plasmapheresis session, they received a high-dose IvIg (2 g/kg) divided in four daily doses and the same dose was repeated 1 mo later. If not used at transplant, patients received, additionally, one dose of rituximab (375 mg/m^2).

Statistical analysis

Categorical data were expressed as numbers (frequencies) and continuous data were described using median (interquartile range). Categorical data (demographic and medical characteristics) were compared using Pearson χ^2 test or Fisher's exact test, as appropriate. Continuous variables were compared with Mann-Whitney U test. Predictors of AMR were explored by univariate and multivariable (using a backward elimination method, with a P-value < 0.05 necessary for retention in the model) Cox regression. For graft survival curves was used the Kaplan-Meier method, and the comparison between groups was done by log-rank test.

RESULTS

Baseline characteristics

Baseline characteristics of DSA-Cw and DSA-A-B groups are given in Table 1. DSA-Cw patients tended to be younger compared to patients in DSA-A-B group (respectively, 39 years *vs* 48 years), (P = 0.061). There was no significant difference between groups concerning gender, history of previous transplant or previous pregnancies. However DSA-Cw patients had significantly higher prevalence of previous blood transfusions (75% *vs* 39%, P = 0.044).

Concerning donor characteristics and pretransplant immunological data, namely donor age, donor gender, type of donor transplant (living *vs* decease), peak PRA, and DSA number, none of these characteristics significantly differed between groups. Although DSA strength median was higher in DSA-A-B (MFI 7583) in comparison with DSA-Cw group (MFI 2939), this difference was not

Table 1 Baseline characteristics of donor-specific antibodies-Cw and donor-specific antibodies-A-B groups

	DSA-A-B *n* = 23	DSA-Cw *n* = 12	*P*
Recipient			
Age (yr), median (IQR)	48 (39-55)	39 (33-49)	0.061
Female gender, *n* (%)	13 (57)	6 (50)	0.713
Retransplant, *n* (%)	11 (48)	5 (42)	0.728
Previous blood transfusions, *n* (%)	9 (39)	9 (75)	0.044
Previous pregnancies, *n* (%)	8 (35)	8 (33)	1
Kidney-pancreas transplantation, *n* (%)	1 (4)	1 (8)	1
Donor			
Age (yr), median (IQR)	45 (36-56)	45 (32-54)	0.542
Female gender, *n* (%)	8 (35)	8 (33)	1
Living donor, *n* (%)	1 (4)	2 (17)	0.266
Pretransplant immunological data			
Peak PRA, median (IQR)	4 (0-80)	8 (0-52)	0.472
DSA number, median (range)	1 (1-3)	1 (1-2)	0.056
DSAsum MFI, median (IQR)	7583 (2320-12395)	2939 (2529-3650)	0.11
Transplant			
ABDR HLA mismatches, mean ± SD	3.22 ± 1.28	4.08 ± 1.16	0.056
FCXM-T + (*n* = 29), *n* (%)	1 (6)	3 (27)	0.139
FCXM-B + (*n* = 29), *n* (%)	2 (11)	0	0.512
ATG induction, *n* (%)	14 (61)	9 (75)	0.476
Tacrolimus (*vs* CsA), *n* (%)	20 (87)	12 (100)	0.536
Desensitized, *n* (%)	5 (22)	3 (25)	1
IvIg only, *n*	2	3	
IvIg + PP, *n*	1	0	
IvIg + Rtx + PP, *n*	2	0	

DSA: Donor-specific antibodies; MFI: Mean fluorescence intensity; IQR: Interquartile range; SD: Standard deviation; CKD: Chronic kidney disease; PRA: Panel reactive antibodies; HCV: Hepatitis C virus; CMV: Cytomegalovirus; HLA: Human leukocyte antigen; ATG: Anti-thymocyte globulin; CsA: Cyclosporin; IvIg: Intravenous immunoglobulin; PP: Plasmapheresis; Rtx: Rituximab.

Table 2 Clinical outcomes and follow-up

	DSA-A-B *n* = 23	DSA-Cw *n* = 12	*P*
Delayed graft function, *n* (%)	7 (30)	1 (8)	0.216
Acute rejection at 1-yr, *n* (%)	6 (26)	3 (25)	1
AMR at 1-yr, *n* (%)	6 (26)	2 (17)	0.685
ACR-only at 1-yr, *n* (%)	0	1 (8)	0.343
1 yr-eGFR (mL/min), median (IQR)	51 (46-60)	59 (47-64)	0.192
1 yr-ProtU, median (IQR)	0 (0-0.1)	0.1 (0-0.2)	0.163
Censored graft failure, *n* (%)	2 (9)	0	0.536
Follow-up time (mo), median (IQR) [range]	60 (45-60) [28-60]	18 (11-50) [3-60]	0.001

DSA: Donor-specific antibodies; AMR: Acute antibody-mediated rejection; ACR: Acute cellular rejection; eGFR: Estimated glomerular filtration rate; IQR: Interquartile range; ProtU: Proteinuria.

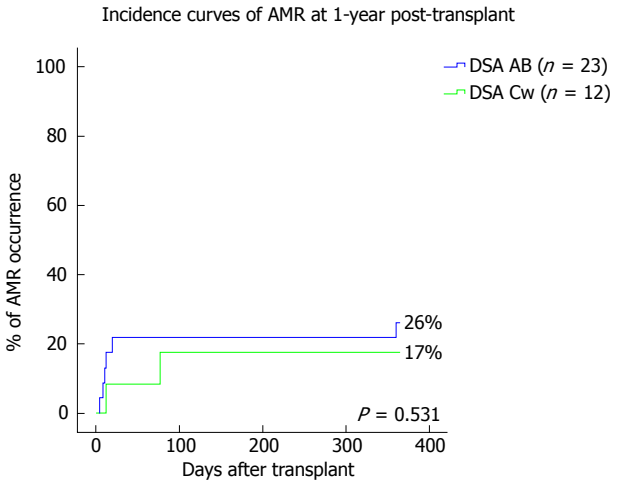

Figure 1 Incidence curves of antibody-mediated rejection at 1-year post-transplant. AMR: Antibody-mediated rejection; DSA: Donor-specific antibodies.

significant (P = 0.110).

Flow cytometry crossmatch (FCXM) was performed for 29 of 35 patients. Positive T- and/or B- cell FCXM was similarly uncommon between groups. Three (27%) patients had a positive T-cell FCXM in the DSA-Cw group and only one (6%) in the DSA-A-B group (P = 0.139). Only two patients had a positive B-cell FCXM and both belonged to the DSA-A-B group.

Immunosuppression and induction treatment were similar between groups. ATG induction was used in 14 (61%) and 9 (75%) patients from the DSA-A-B and DSA-Cw groups, respectively (P = 0.476). Additionally, 5 patients in the DSA-A-B group were desensitized: 2 of them using only IVIG, 1 with IVIG and plasmapheresis and another 2 combining IVIG, plasmapheresis and rituximab. In DSA-Cw group 3 patients were treated with IVIG.

Clinical outcomes

Transplant outcomes are detailed in Table 2. There was no difference in terms of acute rejection at one year between the two groups (6 and 3 cases, respectively in the DSA-A-B and the DSA-Cw groups; P = 1). All cases of acute rejection were diagnosed as AMR in the DSA-A-B group, while in the DSA-Cw group there were 2 cases of AMR and 1 of ACR. Figure 1 shows the incidence of AMR at one-year post-transplant, between DSA-A-B and DSA-Cw patients groups, (respectively, 26% and 17%, Log-rank P = 0.531) with no significant difference being detected. At one year, eGFR tended to be higher in DSA-Cw group (median 59 mL/min) compared to DSA-A-B group (median 51 mL/min), (P = 0.192) (Figure 2). Importantly, follow-up was significantly longer for the DSA-A-B group (median 60 mo) than in the DSA-Cw group (median 18 mo) (P < 0.001). Kidney graft survival at 5-years was also similar between groups (Figure 3, 91% for the DSA-A-B group *vs* 100% for the DSA-Cw group, P = 0.528).

Antibody-mediated rejection: Incidence, predictors and clinical impact

AMR occurred in 8 patients (23%) of the overall cohort. Possible associations between clinical and immunological data and AMR incidence through a Cox regression analysis is shown in Table 3. The sole independent predictor of AMR incidence was the DSA strength, both in uni- and multi-

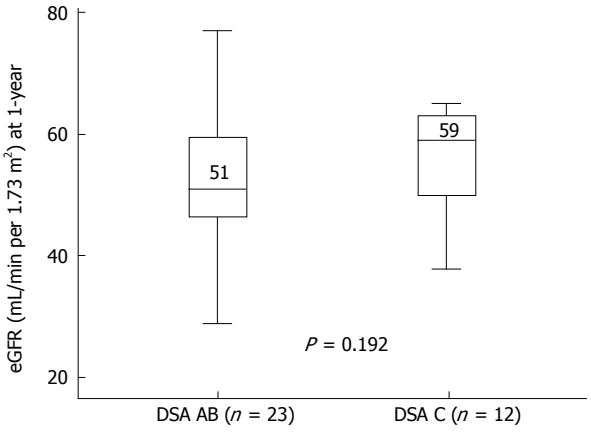

Figure 2 Graft function (estimated glomerular filtration rate at 1-year) post-transplantation according to donor-specific antibodies human leukocyte antigen loci. Boxes show the interquartile range of the values (median and percentile 25-75); whiskers show the lowest and the highest value within 1.5 times below or above the interquartile range, respectively. DSA: Donor-specific antibodies; eGFR: Estimated glomerular filtration rate.

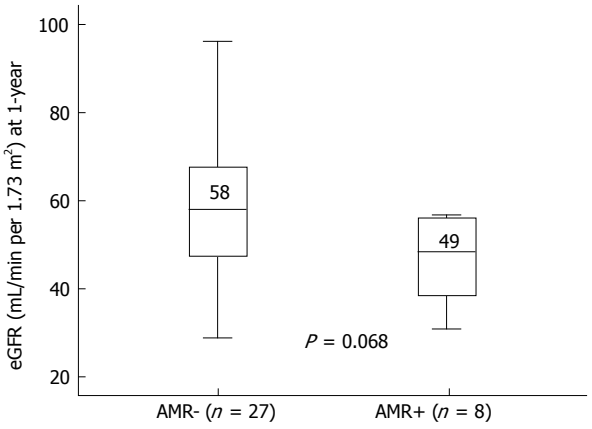

Figure 4 Graft function (estimated glomerular filtration rate at 1-year) post-transplantation according to antibody-mediated rejection occurrence. Boxes show the interquartile range of the values (median and percentile 25-75); whiskers show the lowest and the highest value within 1.5 times below or above the interquartile range, respectively. AMR: Antibody-mediated rejection; eGFR: Estimated glomerular filtration rate.

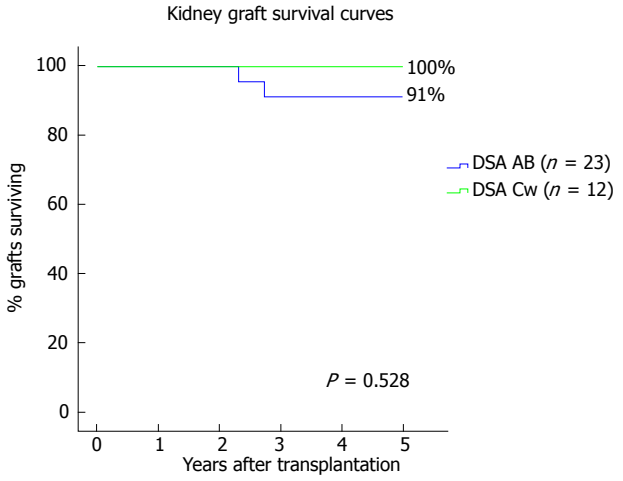

Figure 3 Kidney graft survival curves according with donor-specific antibodies human leukocyte antigen *loci*. DSA: Donor-specific antibodies.

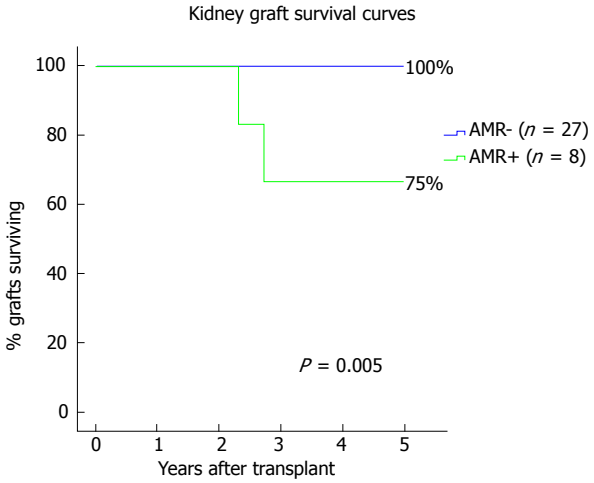

Figure 5 Kidney graft survival curves according with antibody-mediated rejection occurrence. AMR: Antibody-mediated rejection.

variable analysis (HR = 1.07 per 1000 increase in MFI, P = 0.034). At 1-year, eGFR was lower in AMR$^+$ (median 49 mL/min) in comparison with AMR$^-$ patients (median 58 mL/min) (P = 0.068), as shown in Figure 4. At the end of follow-up, kidney graft survival (Figure 5) was 75% in patients that experienced AMR and 100% in those who did not (Log-rank P = 0.005).

DISCUSSION

This retrospective study demonstrates that patients with preformed DSA solely antiHLA-Cw had a similar impact on post-transplant outcomes comparing to those patients with preformed antiHLA-A/-B DSA. Both groups had a relative high incidence of AMR at one year, 26% in the DSA-A-B group and 25% in DSA-Cw group. Also, the impact on graft outcomes measured by eGFR at one-year and graft survival at the end of follow-up was comparable between groups.

HLA-Cw molecules are scantily expressed at the cell surface compared with HLA-A and HLA-B *locus* products, but intracellular HLA-A, HLA-B and HLA-Cw alleles are expressed in similar quantities[3,11]. One reason pointed for this low amount at the cell surface is the fact that HLA-Cw alleles interact in a very stable way with the transporter associated with antigen processing (TAP) and they are kept in the endoplasmic reticulum, where they are degraded[11]. Another justification for finding low HLA-Cw at cell proposed by McCutcheon *et al*[3] is that HLA-Cw heavy chain mRNA is instable and rapidly degraded, resulting in a lower rate of protein. This fact, associated with the modest sensitivity of the lymphocytotocicity-based assays used in the past for identification of HLA-Cw antigens, probably explains why for many years they were considered less immunogenic and neglected in the matching systems of most kidney allocation procedures.

Table 3 Analysis of possible predictors of acute antibody-mediated rejection occurrence by univariable Cox regression

	HR for AMR	95%CI	*P*
Recipient			
Age (yr), per year	0.96	0.89-1.03	0.269
Female (*vs* male) gender	0.26	0.05-1.26	0.094
Retransplant	2.18	0.52-9.13	0.287
Previous blood transfusions	0.5	0.12-2.10	0.345
Previous pregnancies	0.24	0.03-1.99	0.187
Donor			
Age (yr), per year	1.01	0.96-1.06	0.684
Living donor	1.79	0.22-14.76	0.588
Pretransplant immunological data			
Peak PRA, per unit	1.01	1.00-1.03	0.149
DSA Cw (*vs* AB)	0.6	0.12-2.99	0.537
DSAsum MFI, per 1000[1]	1.07	1.01-1.15	0.034
Transplant			
ABDR HLA mismatches, per unit	0.84	0.50-1.41	0.512
ATG (*vs* basiliximab) induction	1.68	0.34-8.34	0.527
FCXM + (n = 29)	0.75	0.09-6.21	0.787
Desensitized	1.2	0.24-5.97	0.825
Delayed graft function	2.55	0.61-10.68	0.201

[1]Only independent predictor identified by multivariable Cox regression model (all variables included) using backward elimination (P-value < 0.050 needed for retention in the model). DSA: Donor-specific antibodies; AMR: Acute antibody-mediated rejection; MFI: Mean fluorescence intensity; ATG: Anti-thymocyte globulin; FCXM: Flow cytometry crossmatch.

Recent studies confirm their lower frequency. Bryan *et al*[6] in 2010 described in their sensitized transplant patients a 42% positivity to HLA-Cw, which was significantly lesser than sensitization to HLA-A (80%) and HLA-B (83%). In 2012, Ling *et al*[5], obtained similar results and showed that the frequency of antiHLA-Cw antibodies in sensitized patients was about 56%, lower than HLA-A (79%) and B (86%) antibodies. Our group evaluated 453 sensitized kidney transplantation candidates to determine the presence of antiHLA class Ⅰ and class Ⅱ antibodies, comparing how different sensitization events, such as pregnancy, transfusion or previous organ transplantation, affected the degree of HLA alloimmunization[12]. For antiHLA antibodies against class Ⅰ, if the sensitization event was previous transplant only, the antiHLA antibodies prevalence was 21.2% for -A, 28.8% for -B and 21.1% for -Cw; if the single sensitization event was previous transfusion, the antiHLA antibodies prevalence was 3.9% for -A, 5.5% for -B and 1.6% for -Cw. At last, if the sensitization event was pregnancy only, the antiHLA antibodies prevalence was 13.6% for -A, 11.1% for -B and 6.2% for -Cw.

In spite of their lower frequency, some reports have been published concerning their association with AMR and impact on graft function and survival[8,13,14]. Besides, the recent development of the solid-phase immunoassays, in particular the single-antigen flow bead (SAFB) assays, allowed us to detect and properly identify anti-HLA-Cw antibodies. Tambur *et al*[15] compared virtual flow-cytometry cross-match to actual cross-match and described that 40% of the cases with a positive actual flow-cytometry cross-match and negative virtual cross-match were explained by the presence of antiHLA-Cw antibodies. Gilbert *et al*[7] compared two groups of sensitized recipients, one group with only classical HLA-A, -B, -DR, -DQ antibodies (n = 176) and the other group with classical plus HLA-C and/or -DP antibodies (n = 27). They concluded that there was a significant increase in the number of AMR among the group with pre-transplant anti-Cw and -DP antibodies. However, they did not distinguish between pre-transplant anti-DP or anti-Cw antibodies, and they speculated that anti-DP antibodies seemed to be involved more often in poorer graft outcomes. Ling *et al*[5] investigated the clinical outcomes in kidney transplant patients with isolated Cw-DSA. They identified eight patients with pre-transplant DSA antiHLA-Cw, exclusively. During a median 6 mo of follow-up (range 3-24 mo), patient and graft survival was 100% without any acute rejection occurring. In this group, all the patients had induction therapy with thymoglobulin or basiliximab and additionally all patients received intravenous immunoglobulin, similar to patients with positive FCXM and/or cPRA > 50%. Even so, the median time of follow up was relatively short and may have underestimated the incidence of rejection. Aubert *et al*[9] evaluated retrospectively 22 renal transplant recipients with isolated antiHLA-Cw DSA at day 0 of renal transplant, comparing them with 88 allosensitized patients with no preformed DSA (control group), and followed for a period of 1 year. Acute AMR was diagnosed in six patients (27.3%) in patients with DSA-Cw *vs* 9% in those without DSA. In this study, the patients with DSA antiHLA-Cw received less-intensive immunosuppression than the control group of sensitized patients, including ATG induction (only 59.1%), and this may probably be a plausible explanation for this high rate of AMR. However they alert for the necessity of screening pre-transplant DSA HLA-Cw and subsequent modulation of immunosuppression in cases of positivity. More recently, Bachelet *el al*[10] investigated the clinical effect of DSA antiHLA-Cw and/or -DP, comparing 48 patients transplanted with isolated preformed DSA antiHLA-Cw and/or -DP with a group of HLA-sensitized recipients with no DSA (104 patients) and 47 kidney transplant recipients with preformed DSA antiHLA-A, -B, -DR, and/or -DQ. Two years after transplantation, the groups with DSA (both -Cw/-DP or -A/-B/-DR/-DQ) had similar incidence of AMR and graft survival (and worse than the group with no DSA), showing that preformed DSA anti-HLA-Cw and/or -DP were as deleterious as DSA anti-HLA -A/-B/-DR/-DQ.

Our data reached similar results of these previous studies, confirming that DSA-Cw is associated with a similar incidence of AMR and impact on graft survival in comparison with "classical" DSA against class Ⅰ [9,10].

We have also shown that patients that experienced AMR had a significant lower kidney graft survival in comparison to patients who did not (respectively, 75% *vs* 100%, Log-rank P = 0.005), with the sole independent predictor of AMR incidence being DSA strength. The negative impact of DSA for AMR occurrence and adverse results on kidney graft

survival has been previously established[2]. Lefaucheur *et al*[16] stated that it is the occurrence of AMR associated with DSA that has impact on graft survival, since graft survival of DSA-positive patients, in the absence of AMR, is the same as DSA-negative patients. Furthermore, DSA characteristics as number, class or strength may have a negative impact on graft outcomes[1,17-19]. Malheiro *et al*[20] showed that DSA strength (MFI) had a reasonable ability to predict AMR occurrence, with no cases of AMR occurring below a MFI < 3000. However when the MFI values increased from this value, also did the risk of AMR. Again, Aubert *et al*[9] in their retrospective study with 22 renal transplant recipients with preformed isolated antiHLA-Cw DSA, showed that the level of DSA at day 0 was predictive for AMR: Measurement of MFI was 4966 (978-17941) in the AMR group and 981 (530-8012) in the group of patients without AMR (P = 0.017).

This study has limitations. First, the small number of patients in the cohort limits our ability to generalize the results. Second, follow-up time difference may have limited the comparative analysis of graft survival according with DSA HLA loci. Contrarily, AMR incidence was not influenced by it, since it was analyzed at 1-year post-transplant. Third, there was no protocol biopsies performed in our patients and it is an important tool for HLA incompatible kidney transplantation[21,22]. Lastly, the limitations of SAB assay are well established and their reported MFI values should be considered for analyzing our results[23].

In summary, our data show that preformed DSA antiHLA-Cw exerts a deleterious effect in presensitized kidney transplant recipients that is similar when compared to antiHLA antibodies against other class I locus (antiHLA-A or -B). Also, the association between AMR occurrence and reduced graft survival is clear, with DSA strength being predictive of rejection. Therefore, HLA-C typing and respective antibody identification will benefit sensitized patients during organ allocation.

COMMENTS

Background

Classically, antibodies to major human leukocyte antigen (HLA) Class I (A and B) and Class Ⅱ (DR and DQ) antigens are considered to be responsible for most cases of AMR. Compared to other class Ⅰ antiHLA antibodies, antiHLA-Cw are considered less immunogenic.

Research frontiers

Preformed antiHLA-Cw donor-specific antibodies (DSA) seem to have the same impact on graft outcome as DSA against "classical" HLA *loci* (-A, -B, -DR and -DQ), suggesting that it should also be considered in transplant allocation systems and in immunologic risk stratification algorithms.

Innovations and breakthroughs

The clinical relevance of preformed antiHLA-Cw DSA in kidney transplant patients remains controversial, so the authors performed a retrospective study comparing 12 patients with DSA exclusively antiHLA-Cw with 23 patients with preformed DSA antiHLA-A and/or B. Antibody-mediated rejection occurrence and graft survival rates, respectively, at 1 and at 5-years of follow-up, were comparable between groups.

Applications

The data show that preformed DSA antiHLA-Cw exerts a deleterious effect in presensitized kidney transplant recipients that is similar when compared to antiHLA antibodies against other class Ⅰ locus (antiHLA-A or -B). Also, the association between AMR occurrence and reduced graft survival is clear, with DSA strength being predictive of rejection.

Terminology

HLA: Human leukocyte antigen; DSA: Donor-specific antibodies; AMR: Antibody-mediated rejection.

Peer-review

The topic is very interesting. The authors investigated the possible role of preformed donor-specific antibodies against HLA antigens, specially anti-Cw antibodies compared to standard anti A/B antibodies. The importance of Cw antibodies is still under investigation and this study is valuable about this topic. This article is worthwhile for publication.

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ORIGINAL ARTICLE

Retrospective Study

Tacrolimus confers lower acute rejection rates and better renal allograft survival compared to cyclosporine

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Abstract

AIM

To compare the impact of tacrolimus (FK) and cyclosporine (CYA) on acute rejection and graft survival and to assess the predominant causes of graft loss between patients receiving these two calcineurin inhibitors (CNIs).

METHODS

Retrospective review of 1835 patients who received a kidney transplant (KTX) between 1999-2012. Patients were grouped based on initial CNI utilized: 1195 in FK group, 640 in CYA group. Data on baseline characteristics, clinical outcomes, and causes of graft loss in both groups were analyzed.

RESULTS

Cumulative acute rejection rates were 14% in the FK *vs* 24% in the CYA group. Despite more marginal donor characteristics in the FK group, these patients had better graft survival rates compared to the CYA group. Three and five year graft survival rates were 88% and 84% respectively in the FK group compared to 79% and 70% respectively in the CYA group ($P < 0.001$). After multivariate analysis, which controlled for confounders, FK use was a strong predictor for lower acute rejection

rates [odds ratio (OR) 0.60, 95%CI: 0.45-0.79] and better renal allograft survival (OR 0.740, 95%CI: 0.58-0.94). Death with a functioning graft was the most common cause of graft loss in both groups. Common causes of death included cardiovascular disease, infections, and malignancies. Chronic allograft nephropathy was also found to be an important cause of graft loss, being more prevalent in the CYA group.

CONCLUSION

The use of FK-based maintenance immunosuppression therapy is associated with a significantly lower rate of acute rejection and better graft survival compared to CYA-based regimen. Individualizing immunosuppression through risk-stratified CNI choice may lead to improved outcomes across all spectra of KTX patients.

Key words: Tacrolimus; Cyclosporine; Renal allograft survival

Core tip: Tacrolimus (FK) has surpassed cyclosporine (CYA) as the calcineurin inhibitor (CNI) of choice for the vast majority of kidney transplant (KTX) programs. Yet, CYA continues to be an important alternative for patients intolerant to FK. FK is associated with significantly lower rate of acute rejection and better graft survival compared to CYA. Individualizing immunosuppression through risk-stratified CNI choice may lead to improved outcomes across all spectra of KTX patients.

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INTRODUCTION

Calcineurin inhibitors (CNIs) are the main immunosuppressive agents utilized in kidney transplantation[1]. Cyclosporine (CYA) and tacrolimus (FK) are currently the most widely used maintenance immunosuppressants for prevention of acute rejection in kidney transplant recipients. CYA-based regimen was more common in the era of 1990 until 2002, after which FK-based regimen became more commonly used in most transplant programs. In our transplant center, FK became the primary CNI of choice in 2005. FK and CYA show variable side effect profiles. Hypertension, hyperlipidemia, gum hypertrophy, and hirsutism occur more frequently with CYA use. On the other hand, a higher incidence of post-transplant diabetes mellitus is observed with FK therapy. Prolonged use of CNI may result in nephrotoxicity.

FK use is associated with less acute rejection compared to CYA, as documented in different studies[2,3]. Mayer *et al*[2] found that among 448 renal transplant recipients who were on triple therapy (FK or CYA + Azathioprine + Prednisone), patients who were in the FK group had a significant reduction in the frequency of acute rejection at 12 mo (FK 25.9% *vs* CYA 45.7%; $P < 0.001$). Ekberg *et al*[3] also found that at 12 mo post-transplant, the use of FK-based regimen is associated with less biopsy-proven acute rejection compared to CYA use (12.3% *vs* 25.8%, $P < 0.01$).

FK is frequently preferred in patients with high immunologic risk (highly sensitized, ABO-incompatible organ recipients), delayed graft function, and African American race. Data regarding graft survival based on the use of FK *vs* CYA is controversial with most studies showing similar graft survival rates with the use of either agent[4]. Vincenti *et al*[5] showed comparable patient (79.1% *vs* 81.4%; $P = 0.472$) and graft (64.3% *vs* 61.6%; $P = 0.558$) survival between treatment arms at 5 years of follow-up among FK and CYA-treated patients. However, after accounting for patients initially on CYA who crossed over to FK, the authors found significantly reduced graft failure in the FK group[5]. Gonwa *et al*[6] showed that among 223 kidney transplant recipients who experienced delayed graft function, patients who used FK-based therapy had a better 3-year graft survival compared to CYA use (84.1% *vs* 49.9%, $P = 0.02$). Given these conflicting findings, this study aims to compare rates of acute rejection and graft loss among patients who receive FK and CYA.

MATERIALS AND METHODS

Patients

This was a retrospective cohort study of 1835 patients who received a KTX between 1999-2012 at a single center. Patients were grouped based on the type of CNI they were prescribed: 1195 patients utilized FK-based immunosuppression whereas 640 patients were on a CYA-based regimen. All patients received an antimetabolite and prednisone in combination with CNI. The initial CYA dose was 4-5 mg/kg PO BID. Target CYA levels were 350-400 ng/mL for weeks 1-4, 250-350 ng/mL for weeks 5-12, 200-300 ng/mL within the first year post-transplant, and 100-200 ng/mL thereafter. Initial FK doses were given at 0.025-0.05 mg/kg PO BID. Target FK levels were kept between 8-12 ng/mL within the first four weeks post-transplant, then 6-10 ng/mL within the first year post-transplant, and 4-6 ng/mL subsequently. Characteristics of recipients (age, race, sex, BMI, etiology of kidney disease, history of heart disease, diabetes, hypertension, years on dialysis, panel reactive antibody, preemptive transplant, living donor transplant), and donors [age, race, kidney donor risk index (KDRI)] were compared between groups. Characteristics of the kidney transplant (cold ischemia time, induction agent) as well as clinical outcomes (cumulative acute rejection rate, delayed graft function, three, and five year graft survival) were also analyzed. The Banff '97 criteria were used to define the

Table 1 Characteristics of patients in tacrolimus and cyclosporine group *n* (%)

Parameter	Cyclosporine *n* = 640	Tacrolimus *n* = 1195	*P*-value
Mean recipient age (yr)	49 ± 12	50 ± 13	0.059
Race			0.96
Non-African American	281 (44)	526 (44)	
African-American	359 (56)	669 (56)	
Sex			0.78
Male	371 (58)	693 (58)	
Female	269 (42)	502 (42)	
BMI	26 ± 7	28 ± 5	0.462
Etiology of kidney disease			
DM	172 (26.9)	375 (31.4)	0.044
HTN	317 (49.5)	559 (46.8)	0.26
FSGS	36 (5.6)	78 (7.3)	0.177
IgA nephropathy	24 (3.8)	34 (2.8)	0.291
Polycystic kidney	63 (9.8)	89 (7.4)	0.076
History of DM	186 (29)	394 (33)	0.092
History of HTN	595 (93)	1135 (95)	0.122
History of heart disease	134 (21)	227 (19)	0.38
Years on dialysis	3 ± 2.4	3 ± 2.9	0.01
PRA	5%	17%	< 0.010
Preemptive transplant	122 (19)	239 (20)	0.49
Living donor transplant	122 (19)	179 (15)	0.27
CIT (h)	13 ± 9	16 ± 9	0.621
Mean donor age (yr)	31 ± 18	36 ± 16	< 0.010
KDRI	0.9 ± 0.6	1.3 ± 0.4	< 0.010
African-American donor	122 (19)	203 (17)	0.27
Induction therapy			< 0.010
Cytolytic agents	70 (11)	550 (46)	
IL-2 receptor antagonist	570 (89)	645 (54)	

DM: Diabetes mellitus; HTN: Hypertension; FSGS: Focal segmental glomerulosclerosis; PRA: Panel reactive antibody; CIT: Cold ischemia time; KDRI: Kidney donor risk index.

different grades of rejection. Based on center protocol, Banff 1A and 1B rejection episodes were treated with Methylprednisolone Ⅳ. Rejection episodes with Banff 2A grade or higher were treated with anti-thymocyte globulin. Subset analysis was conducted on subjects who had graft loss to retrospectively investigate the factors leading to graft loss. For patients who died, causes of death were presented as overall prevalence of infections (encompassing sepsis, bacterial, fungal, CMV, and other viral infections), malignancies (encompassing solid organ tumors, hematologic malignancies, and post-transplant lymphoproliferative disorder), and cardiovascular diseases (encompassing acute myocardial infarction and cerebrovascular accident). Cause of death classified under "other" includes accidents, unknown, or undocumented. Non-adherence was defined as documentation in the medical record by a provider that a patient was not taking their immunosuppressive regimen as prescribed. Under immunosuppression was defined as evidence of kidney transplant injury related to rejection that led or contributed to graft loss.

Statistical analysis

The statistical review was performed by a clinician with advanced biostatistical training and experience.

Two-sided independent student's *t*-test was used to compare continuous data while the χ^2 test was used to compare categorical data. A two-sided *P*-value of less than 0.05 was considered statistically significant.

Multivariate survival analysis, using both logistic and Cox regression, was used to assess the association between CNI choice and acute rejection (logistic), graft survival (Cox), and patient mortality (Cox), while controlling for additional transplant variables known to influence outcomes or those that differed across CNI choice. In a subset analysis of patients who had graft loss, causes of graft loss, and causes of death were compared between the two groups using standard univariate comparative statistics. All analyses were conducted using SPSS version 21.0 (IBM Corp, Armonk, NY).

RESULTS

Patient characteristics

Table 1 displays demographic characteristics of the two groups. Mean recipient age, race, BMI, etiology of kidney disease, comorbidities, and dialysis vintage, were similar between the two groups. Patients on FK had higher PRA compared to patients on CYA group (17% *vs* 5%, $P < 0.01$). Rates of living donor transplants were similar between the two groups. Among patients who received a deceased donor transplant, KDRI was higher in those who received FK. More patients in the FK group received induction agent with depleting antibodies (46% *vs* 11%, $P < 0.01$).

Table 2 Clinical outcomes

Parameter	Tacrolimus *n* = 1195	Cyclosporine *n* = 640	*P*-value
Mean glomerular filtration rate	56 ± 19	46 ± 17	0.09
Delayed graft function *n* (%)	179 (15)	115 (18)	0.049
Acute rejection (biopsy proven) *n* (%)	167 (14)	154 (24)	< 0.010
Three years graft survival	88%	79%	< 0.010
Five years graft survival	84%	70%	< 0.010

Table 3 Multivariate analysis of factors associated with acute rejection

Variable	Hazard ratio	95%CI	*P*-value
CNI tacrolimus	0.6	0.45-0.79	< 0.001
Retransplant	1.43	0.91-2.24	0.123
PRA	1	0.99-1.00	0.529
Cytolytic induction	0.5	0.36-0.69	< 0.001

CNI: Calcineurin inhibitor; PRA: Panel reactive antibody.

Table 4 Multivariate analysis of factors associated with graft loss

Variable	Hazard ratio	95%CI	*P*-value
CNI tacrolimus	0.74	0.58-0.94	0.012
History of DM	1.41	1.13-1.76	0.002
History of HTN	0.56	0.34-0.94	0.029
Delayed graft function	2.1	1.66-2.66	< 0.001
Acute rejection	1.59	1.26-2.01	< 0.001

CNI: Calcineurin inhibitor; DM: Diabetes mellitus; HTN: Hypertension.

Clinical outcomes

Patients in the FK group had better clinical outcomes in terms of delayed graft function (DGF) rate (15% *vs* 18%, $P = 0.049$), cumulative biopsy proven acute rejection rates for Banff 1A and higher, as well as antibody-mediated rejection (14% *vs* 24%, $P < 0.01$), three year graft survival (88% *vs* 79%, $P < 0.010$), and five year graft survival (84% *vs* 70%, $P < 0.01$) (Table 2). FK was a strong predictor of lower acute rejection rates. After multivariate analysis, which accounted for recipient immunologic risks (age, gender, re-transplant, PRA, HLA mismatches, cold ischemic time, induction), donor characteristics (deceased status, ECD, age, race) and delayed graft function, FK continued to be strongly associated with lower acute rejection rates, as compared to CYA (OR = 0.60, 95%CI: 0.45-0.79; $P < 0.001$) (Table 3). Further analysis showed that even after controlling for all other variables, including delayed graft function and acute rejection, FK remained a strong and statistically significant predictor of graft survival (OR = 0.740, 95%CI: 0.58-0.94; $P = 0.012$) (Table 4) (Figure 1).

Graft loss

During the study period, there were 106 patients in the FK group and 123 in the CYA group who had graft loss. Death with a functioning graft was the cause of graft loss in the majority of these patients. The leading causes of death among the patients include cardiovascular disease, infections, and malignancies (Table 5). The contribution of non-adherence and underimmunosuppression in patients who had graft loss was not significantly different between the FK and CYA groups.

DISCUSSION

The utilization of potent immunosuppressive medications such as CYA and FK has led to progressive improvement in renal allograft survival. Two large studies on kidney transplant recipients showed that the incidence of acute rejection is much lower with FK-based immunosuppression compared to CYA-based regimen[2,3]. Our study demonstrated similar findings of lower acute rejection rates in patients using FK compared to those on CYA. Acute rejection rate was significantly lower in the FK group despite the relatively higher degree of sensitization, as evidenced by higher PRA, in this group. Multivariate analysis showed that FK was a strong predictor for lower acute rejection rates while controlling for recipient, donor, and transplant characteristics.

The shortage of deceased donor kidneys and the growing number of patients on the waiting list has driven the increased utilization of organs with relatively marginal donor characteristics. Donor factors affect initial graft function and survival[7]. Donor factors that may influence graft survival include age, gender, hypertension, and cardiovascular disease[8]. The KDRI is a comprehensive metric that was recently developed to assess the relative risk of graft failure associated with various combinations of donor characteristics. Kidneys with the highest KDRI quintile are associated with lower graft survival[9]. Although many trials have shown similar graft survival outcomes with FK when compared with CYA-based regimen[4], some studies showed better survival and outcomes with FK-based immunosuppression[6]. Our

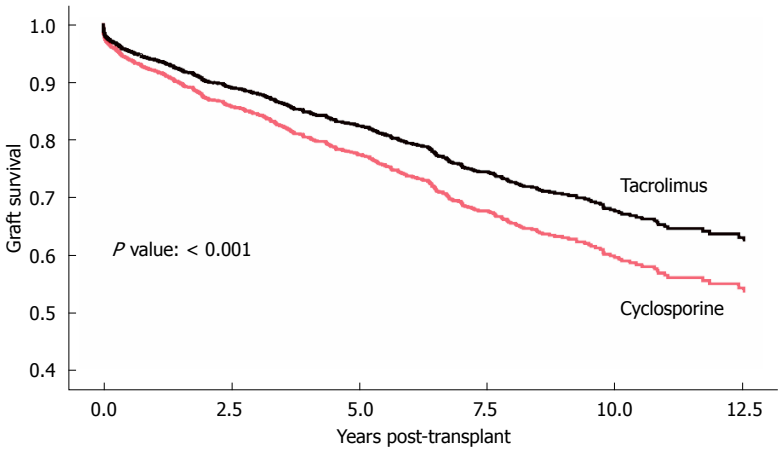

Figure 1 Kaplan Meier curve showing effect of tacrolimus *vs* cyclosporine on graft survival.

Table 5 Graft loss *n* (%)

Parameter	Tacrolimus *n* = 106	Cyclosporine *n* = 123	*P*-value
Death with functioning graft	61 (58)	66 (54)	0.55
Cause of death			0.85
Cardiovascular disease	19 (18)	19 (15)	
Infections	10 (9)	9 (7)	
Malignancy	10 (9)	9 (7)	
Others	33 (31)	41 (33)	
Causes of graft loss			0.44
Chronic allograft nephropathy	18 (17)	29 (24)	
Acute rejection	14 (13)	11 (9)	
Acute on chronic rejection	8 (8)	13 (11)	
Recurrent disease	1 (1)	1 (1)	
Death	63 (59)	68 (55)	
Component of non- adherence	15 (14)	20 (16)	0.65
Component of underimmunosuppression	21 (20)	25 (20)	0.92

study showed that although patients in the FK group received kidneys from more marginal donors (higher KDRI), the three year and five year graft survival was still more superior in this group compared to the CYA group (Figure 1).

The risk of infections after kidney transplant depends on the net state of immunosuppression. As FK was shown to be associated with less acute rejection compared to CYA[10], it may concurrently cause more intense immunosuppressive effects compared to CYA. Thus, risk of infections after kidney transplant may be higher with FK compared to CYA. This may be exemplified by the higher incidence of polyomavirus (BK) viremia in patients on FK-based regimen compared to CYA[11]. Progression of BK viremia may lead to BK nephropathy, which can then eventually cause premature renal allograft failure[11]. However, in our subjects who had graft loss, we did not observe a significant difference in the prevalence of infections (including BK) in the FK and CYA groups.

The use of maintenance immunosuppressive medications among transplant recipients increases the long-term risk of malignancy, compared with that of the general population. The overall level of immunosuppression appears to be the principal factor that increases the risk of post-transplant malignancy. Both FK and CYA are associated with an increased risk of malignancy following kidney transplant[12,13]. No direct comparison between these two agents has been reported regarding the incidence of malignancy following kidney transplant. However, FK was found to have higher incidence of *de novo* malignancy after liver transplant compared to CYA[14]. In our study, we did not find a significant difference in the prevalence of malignancies between the two groups.

Cardiovascular disease is a leading cause of mortality among kidney transplant recipients. Death from cardiovascular disease is the most common cause of renal allograft loss[15]. CNIs potentially contribute to increased risk of cardiovascular events indirectly by the development of new-onset diabetes mellitus, hypertension, and hyperlipidemia. Clinical trials have shown a higher incidence of post-transplant diabetes mellitus with FK. However, the risk of hypertension and hyperlipidemia is slightly higher with CYA than FK. No direct comparison has been done between FK and CYA regarding the incidence of cardiovascular disease. In our study, we found that FK was associated with a slightly higher prevalence of cardiovascular disease compared to CYA, although the difference was not statistically significant.

In conclusion, FK is associated with lower prevalence of acute rejection compared to CYA. It confers better three and five year graft survival even with the use of

organs with marginal deceased donor characteristics. An individualized approach to the choice of CNI needs to be employed in order to achieve the best possible outcome while minimizing adverse effects. The use of either FK or CYA should be individualized according to the patient' s comorbid conditions and immunological risk.

COMMENTS

Background

Calcineurin inhibitors (CNIs) [cyclosporine (CYA) and tacrolimus (FK)] are currently the most widely used maintenance immunosuppressants for prevention of acute rejection following kidney transplantation. However, data on the impact of these CNIs on acute rejection rate and graft survival have remained equivocal.

Research frontiers

The choice of immunosuppressive regimen that will achieve the best renal allograft outcomes remains an important focus in the care of kidney transplant recipients.

Innovations and breakthroughs

The data showed lower acute rejection rates and better graft survival in patients on FK compared to those on CYA.

Applications

The use of either FK or CYA should be individualized based on patient's comorbidities and immunological risk.

Terminology

FK: Tacrolimus; CYA: Cyclosporine; CNI: Calcineurin inhibitor; DGF: Delayed graft function; DM: Diabetes mellitus; HTN: Hypertension; FSGS: Focal segmental glomerulosclerosis; PRA: Panel reactive antibody; CIT: Cold ischemia time; KDRI: Kidney donor risk index; GFR: Glomerular filtration rate.

Peer-review

The authors are presenting their experience in the use of CNIs in the immunosuppression post renal transplantation.

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ORIGINAL ARTICLE

Retrospective Study

Quality of life 10 years after liver transplantation: The impact of graft histology

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Abstract

AIM

To evaluate the relationship between the state of transplanted liver graft and the recipient quality of life (QOL) of histologically proven lesions in a 10-year post liver transplantation (LT) cohort of patients.

METHODS

Seventy-two recipients with a functional first graft at 10 years post-LT underwent liver biopsy and completed a QOL questionnaire. Logistic regression analysis was used to explore associations between histological, clinical and

QOL criteria.

RESULTS

Ten years after LT, fibrosis was detected in 53% of patients, and affected the general health perception, while ductopenia, present in 36%, affected the well-being (P = 0.05). Hepatic steatosis (HS) was present in 33% of patients and was associated with the worst QOL score on multiple domains. When compared to patients without HS, patients with HS had significantly higher incidence of fibrosis (P = 0.03), hepatitis C virus (HCV) infection (P = 0.007), and more patients had retired from their job (P = 0.03). Recurrent or *de novo* HCV-associated fibrosis and patient retirement as objective variables, and abdominal pain or discomfort and joint aches or pains as subjective variables, emerged as independent determinants of HS.

CONCLUSION

Long-term liver graft lesions, mainly HS presumably as a surrogate marker of HCV infection, may have a substantial impact on QOL 10 years after LT.

Key words: Liver transplantation; Quality of life; Liver biopsy; Hepatic steatosis; Liver fibrosis

Core tip: Objective and subjective parameters are helpful in the accurate assessment of long-term outcome in liver transplantation recipients. The main finding of this study was that histological lesions in the transplanted liver 10 years after liver transplantation can affect the recipient quality of life. Hepatic steatosis had the most significant impact on quality of life and this was independent of alcohol consumption, fibrosis, diabetes and body mass index. The strongest determinants of a worse quality of life in patients with hepatic steatosis were hepatitis C virus infection and retirement from job irrespective of patient-age.

Karam V, Sebagh M, Rifai K, Yilmaz F, Bhangui P, Danet C, Saliba F, Samuel D, Castaing D, Adam R, Feray C. Quality of life 10 years after liver transplantation: The impact of graft histology. *World J Transplant* 2016; 6(4): 703-711 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i4/703.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i4.703

INTRODUCTION

The goal of liver transplantation (LT) is to ameliorate not only survival, but also quality of life (QOL) while minimizing the effects of disease and costs of care. Analysis of data from the European Liver Transplant Registry (ELTR) shows that 38% of the patients transplanted in 1991 were still alive with their first graft at least 10 years post LT[1]. The increasing proportion of recipients alive at long-term follow up has incited transplant professionals to focus on long term morbidity-free survival and an acceptable QOL.

The QOL is increasingly recognized as an important measure of outcome after solid organ transplantation[2-4]. We showed in a previous study that the challenge of maintaining long-term well-being is achieved to a greater extent in liver transplant recipients than in other solid-organ transplant recipients[4]. However, short term and long term QOL in liver transplant recipients is still inferior to that of the general population[3,4].

Studies of long-term survivors have been mainly based on clinical data and histological follow up at long term, or with respect to indication for LT, immunosuppressive regimen or recipient and donor criteria[5-10]. In previous studies, we justified the use of biopsies in the follow-up protocol to adjust treatments, not only in HCV-infected patients (in whom fibrosis progression was rapid and non-linear), but in all recipients[11,12].

No study has been published assessing the relationship between the state of transplanted liver graft and the recipient QOL of histologically proven lesions in a 10-year post LT cohort of patients.

MATERIALS AND METHODS

Patients

Between September 1989 and December 1992, 485 LT were performed in 432 patients at Paul Brousse Hospital (Villejuif, France). During the 10th year post LT, among the 145 patients who were alive with a first functional graft, 126 accepted to complete the QOL questionnaire, and among these 72 accepted to have a liver biopsy done. For the purpose of this study, only the 72 subjects who underwent both a 10-year post LT liver biopsy and completed the QOL auto-questionnaire were included.

Questionnaire

QOL data was obtained using the NIDDK questionnaire[2]. The questionnaire includes 21 disease-specific items assessing symptoms related to chronic liver disease. We used a validated French version of the questionnaire developed previously using the back-translation method

Five domains of QOL; physical distress (PHD), psychological distress (PD), personal function (PF), social/role function (SRF), and general health perception (GHP) are well represented in the questionnaire. Each symptom is numerically graded according to severity and then a composite overall score is calculated from all domains[3,4].

Histological evaluation

Prospectively obtained reperfusion biopsies and ten-year post LT liver biopsies were reviewed by the same experienced pathologist (MS) who was blinded to clinical information.

Portal tracts, hepatic veins and parenchyma were systematically analysed according to a preformed format. Fibrosis was staged on a five-point scale: 0, none; 1, portal fibrosis without septa; 2, few septa; 3, numerous septa without cirrhosis; 4, cirrhosis. Ductopenia evaluated on

liver biopsy was analysed according to the Banff criteria[13], and ductopenia was considered as significant when the percentage of bile duct lost exceeded 20%. Steatosis was scored according to the percentage of biopsy tissue involved. Patients were considered in Hepatic Steatosis (HS) group when the percentage of steatosis exceeded 10%. Minimal changes were defined as the absence of all the above cited lesions or the existence of only one of the following criteria: steatosis < 10%, sinusoidal fibrosis, or minimal bile duct or lobular inflammation. The final diagnosis was established by joint review of records; biochemical, virological, and immuno-histochemical data.

Statistical analysis

Continuous variables are given as mean ± SD. Comparisons of continuous variables were performed with the Mann-Whitney test and those of nominal variables with χ^2 contingency test or Fisher's exact test when appropriate. Logistic regression was conducted to examine determinants of HS. We conducted two separate regressions: (1) with the objective (clinical) variables; and (2) with the subjective (QOL) variables. A *P*-value of < 0.05 was considered statistically significant.

RESULTS

Characteristics of patients who were not included in the study

Only 72 patients accepted to complete the QOL questionnaire and undergo liver biopsy amongst the 145 patients who survived for a minimum of 10 years with a first functional graft. Since this could create a selection bias in our study, we compared the selected and unselected patients with respect to characteristics at the time of LT and at 10-years post-transplant. Clinico-demographic characteristics like age at transplantation, sex, donor age, ABO group, CMV and reperfusion biopsy status, indication for LT, liver enzyme tests at the time of 10 year control of included patients were not statistically different from the non-included patients. Moreover, comparison of all domains of QOL has not shown any statistically significant difference between the selected and unselected patients (data not shown).

Patient characteristics at the time of LT

The mean age at time of transplantation was 35 ± 19 years and proportion of female patients was 52%. Mean donor age was 27 ± 11 years. The main indications for LT were PBC (25%), acute liver failure (24%) and viral cirrhosis (20%) [mostly hepatitis C virus (HCV) related (12%)]. The reperfusion biopsies showed steatosis (≥ 10%) in 18% of patients and reperfusion injury related lesions in 86% of reperfusion biopsies. Twenty-six percent of these lesions were classified as mild while 60% were of moderate to severe-degree (Table 1).

Patient status and histological findings 10 years after transplantation

As regards co-morbidities present in the recipients at follow-up, thirty eight (53%) patients had arterial hypertension and 7 (10%) suffered from diabetes mellitus (mostly type Ⅱ). According to the body mass index (BMI), 9 (13%) patients were underweight, 50 (69%) patients were within normal limits, 10 (14%) were overweight and 3 (4%) were obese. Fifteen (21%) patients consumed alcohol with 1.1 ± 0.3 drinks/day (one drink = 1 bottle of beer or 1 glass of wine or 1 mixed drink, the equivalent of 1.25 grams of alcohol) and 12 (17%) were tobacco smokers with 1.9 ± 0.8 cigarettes/day (Table 2).

Forty one patients (57%) had HCV infection, amongst them 35 (49%) had *de novo* infection whereas 6 (8%) had recurrent HCV infection. Most patients had been transplanted before the screening of blood and organ of donors for HCV serology began (pre HCV era). The predominant HCV genotype in our study cohort was genotype 1 (60%), mostly 1b subtype (51%). The proportion of other genotypes was; genotype 2 (12%), genotype 3 (9%) and genotype 4 (6%). In 13% of cases HCV-infection was established by RNA revelation. At the time of biopsy and QOL evaluation, none of the patients was being treated with interferon.

The immunosuppression was mainly using Cyclosporine-based (96%) in the study population.

The main histological findings were as follows: (1) fibrosis F1-F4 (*n* = 38, 53%), with F1 (*n* = 16, 22%), F2 (n = 13, 18%), F3 (*n* = 4, 6%). Cirrhosis (F4) was found in 7% (*n* = 5) of cases; (2) ductopenia (*n* = 26, 36%) with a mean percentage of bile duct loss of 40% ± 20%; and (3) steatosis (*n* = 24, 33%) with a mean percentage of 19 ± 17 %, which was mostly macrovacuolar (*n* = 23, 32%). Combined fibrosis and steatosis was found in 24% (*n* = 17) of patients. Only 23% (*n* = 16) of biopsies contained minimal-change lesions (as defined above).

Relation between QOL and histological lesions

Overall-QOL was not affected by fibrosis or ductopenia (Figure 1A and B). Nevertheless, GHP score was lower in patients with fibrosis (*P* = 0.02) and well-being score was lower in patients with ductopenia (*P* = 0.05). The overall-QOL score was the lowest in HS patients (*P* = 0.007) (Figure 1C). HS impaired particularly the PHD (*P* = 0.002), PD (*P* = 0.01) and GHP (*P* = 0.05). According to these results, we focused our study on the group of patients with HS.

Profile of patients with hepatic steatosis

As the worst QOL score on multiple domains was associated strikingly with HS we made a detailed analysis to compare the group with steatosis on 10 year liver biopsy, with those without. There were no statistically significant differences between the groups with respect to data at the time of LT except for recipient age (32 ± 21 years *vs* 42 ± 12 years; *P* = 0.04) (Table 1). At 10 year post LT follow-up, the BMI (22.6 ± 3.4 *vs* 22.3 ± 3.9), rate of diabetes (13% *vs* 9%), rate of arterial hypertension (54% *vs* 54%) and immunosuppressive dosage were not statistically higher in HS group. No difference was found

Table 1 Relationship between various parameters at the time of liver transplantation and the incidence of hepatic steatosis on 10-year post-liver transplantation biopsy

	All subjects n = 72	No HS n = 48	HS n = 24	P^1
Age (yr)	35 ± 19	32 ± 21	42 ± 12	0.04
Gender (female)	52%	60%	71%	NS
Disease				
Acute hepatic failure	24%	27%	17%	NS
Primary biliary cirrhosis	25%	25%	25%	NS
HBV-related cirrhosis	8%	6%	12%	NS
Autoimmune cirrhosis	7%	6%	8%	NS
Biliary atresia	5%	8%	0%	NS
HCV-related cirrhosis	12%	6%	25%	NS
Metabolic disease (Wilson disease)	1%	2%	0%	NS
Alcohol related cirrhosis	1%	2%	0%	NS
Primary sclerosing cholangitis	4%	4%	4%	NS
Cryptogenic cirrhosis	2%	4%	0%	NS
Hepatocellular carcinoma	8%	8%	8%	NS
ABO compatible	97%	96%	100%	NS
Donor age (years)	27 ± 11	27 ± 12	27 ± 10	NS
Donor gender (female)	41%	42%	39%	NS
Urgency	25%	27%	21%	NS
Cold ischemic time (min)	410 ± 212	406 ± 215	429 ± 214	NS
Reperfusion biopsy[2]				
Steatosis (≥ 10%)	18%	15%	22%	NS
% of steatosis	24 ± 15	31 ± 16	16 ± 8	NS
Reperfusion lesions				
Mild	26%	31%	17%	NS
Moderate to severe	60%	50%	79%	NS

Continuous data are represented as mean ± SD, and categorical data as percentage. [1]Comparison between HS and No HS; [2]Reperfusion biopsy not done in 10 cases (9 in Non HS group and 1 in HS group). HS: Hepatic steatosis; NS: Not significant; HCV: Hepatitis C virus.

in liver function tests (Table 2).

For the 24 patients with HS, three of the studied objective variables were statistically significant when compared to patients without HS at 10 years post LT: Fibrosis (71% *vs* 44%, $P = 0.03$), HCV infection (79% *vs* 46%, $P < 0.007$) (Table 2), and patient retirement (50% *vs* 21%, $P = 0.03$) (Table 3). Fibrosis was present in 17 (71%) patients and was mainly related to HCV infection. The HCV genotype 1 was predominant and represented 63%, mostly 1b subtype (42%). Despite the equally distributed mean age and the percentage of more than 60 years old patients in the two groups (29% *vs* 27%, P = ns), retired recipients were more prevalent in the HS group (46% *vs* 21%, $P = 0.03$).

Regarding the subjective QOL variables, a detailed analysis showed that the HS has an impact on 17 symptoms belonging to each one of the 5 domains of QOL (Table 4). The most affected physical symptoms were: Abdominal pain or discomfort ($P < 0.0001$), joint aches or pains ($P < 0.001$) and change in facial appearance ($P < 0.001$). Nervousness/anxiety was the most affected psychological symptom followed by a feeling of being depressed, sad or blue ($P < 0.01$). As regards PF, the health of HS patients currently limits their ability to perform vigorous activities such as running, heavy lifting or sport ($P < 0.001$). The SRF was affected by the patients' decreased interest in sex ($P = 0.003$). Finally, bodily pain during the last month represented the worst symptom of GHP ($P < 0.01$).

In multivariate regression analysis, two objective variables emerged as independent determinants of HS: HCV infection ($P < 0.01$) and patient retirement ($P = 0.04$). So also, two subjective variables were significantly associated with HS: Abdominal pain or discomfort ($P < 0.01$) and joint aches or pains ($P = 0.04$) (Table 5).

DISCUSSION

The developments in surgical techniques, immunosuppressive treatment modalities and better patient care have led to an increasing number of long-term survivors after LT, yet the QOL of transplant recipients does not always return to normal. The constant need for drug ingestion and monitoring the high incidence of recurrent or intervening diseases after LT, all seem to impair QOL[14,15]. Nevertheless, reported data shows that most of the QOL parameters are better after transplantation than before[2,3,16]. This study is an attempt to identify those factors which prevent long term liver transplant survivors from returning to a near normal lifestyle, with a specific focus on the relationship of QOL with graft histological status. One can recognize that a key challenge specific to this study could be its face validity, *i.e.*, comparison of histologic changes to QOL, which in the absence of advanced histologic changes is not intuitively related. In order to attenuate the relative fluctuations liver biopsies were reviewed by the same experienced pathologist (MS) who was blinded to clinical information. Moreover, we used the NIDDK questionnaire considered as one of the most appropriate and validated instruments for QOL

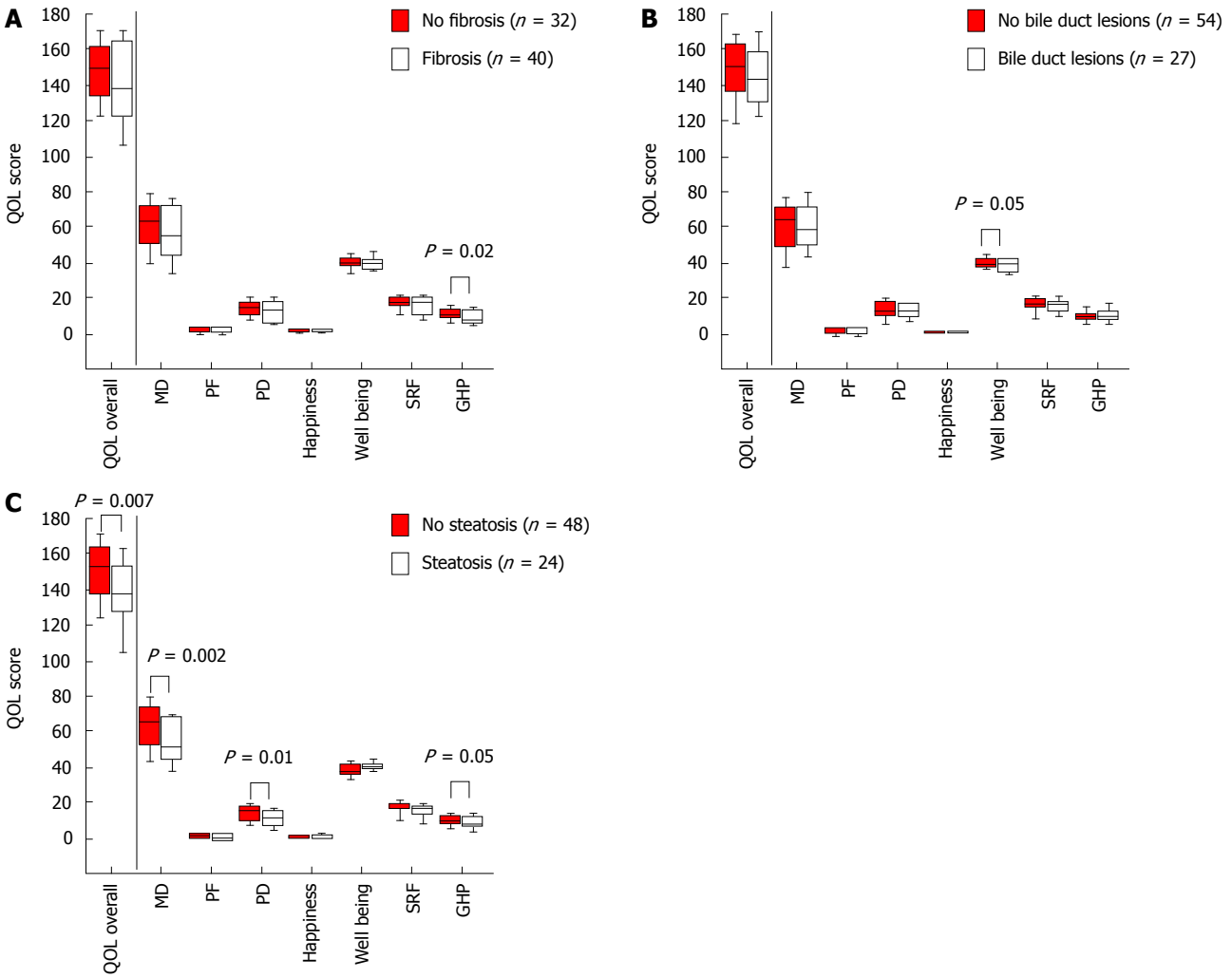

Figure 1 Relationship between quality of life domains and histological findings on 10 year post-liver transplantation liver biopsy. A: Fibrosis; B: Bile duct lesions; C: Steatosis. QOL: Quality of life; PF: Personal function; SRF: Social/role function; GHP: General health perception.

evaluation in transplant recipients[17].

The main finding of this study was that histological lesions (especially HS) in the transplanted liver 10 years after LT can affect the recipients' QOL. Overall-QOL was not affected by fibrosis or ductopenia, but there was a significant decrease in GHP score in patients with fibrosis and in well-being score in patients with ductopenia. HS had the most significant impact on overall-QOL score and this was independent of alcohol consumption, fibrosis, diabetes and BMI.

Post-LT development of HS in recipients has only been analyzed in few studies so far[18]. Post-transplant metabolic syndrome and graft NAFLD are being increasingly recognized as long term problems in LT recipients[19,20]. Patients with post-LT NAFLD develop at the least an increased risk of cardiovascular events, rejection and infection[21]. Recently, a retrospective study reported that the reasons for long term steatosis in liver allografts may be related to seven factors either present alone or in combination, such as graft steatosis at the time of transplantation, HCV infection, recurrence of NAFLD or alcoholic liver disease, metabolic syndrome, diabetes mellitus and *de novo* NAFLD[22]. Most of these factors are known risk factors for NAFLD in the non-transplant setting also.

For the determination of such potential underlying factors, we compared the groups with and without HS in our series. We could not find any significant difference between the groups, neither with respect to known metabolic risk factors not related to LT (such as incidence of diabetes, hypertension or recipient BMI) nor with respect to transplant-related factors (such as donor liver steatosis, reperfusion injury, alcohol abuse and immunosuppressive dosage). Only three of the objective variables were significantly different; HCV infection, fibrosis and patient retirement irrespective of age.

The post-transplant setting is a good background for the development of one or several components of the metabolic syndrome[18], *de novo* NAFLD seems to be one of the most probable reasons for HS. For instance, high incidence of hypertension and hyperlipidemia in patients on Cyclosporine-based immunosuppressive regimen and diabetes mellitus in patients on Tacrolimus-based

Table 2 Relationship between various parameters at 10-year post-liver transplantation and the incidence of hepatic steatosis on 10-year post-liver transplantation biopsy

	All subjects *n* = 72	No HS *n* = 48	HS *n* = 24	*P*[1]
Age at the time of survey	49 ± 15	47 ± 15	53 ± 12	NS
≥ 60 yr aged patients	28%	27%	29%	NS
Histological lesions				
Steatosis	33%	-	100%	
Macrovacuolar	28%	-	82%	
Microvacuolar	1%	-	4%	
Combined Mac-Mic	4%	-	14%	
Initial and 10-yr maintained steatosis	8% (5 pat.)	0%	22%	0.002
Fibrosis (F1-F4)	53%	44%	71%	0.03
F1-F2	40%	35%	50%	NS
F3-F4	13%	8%	21%	NS
Combined fibrosis-steatosis	24%	0%	71%	< 0.0001
HCV(+) Fibrosis	44%	31%	71%	< 0.001
Bile duct lesions	36%	42%	25%	NS
Minimal change	23%	27%	17%	NS
Other potential steatosis factors				
BMI (kg/m^2)	22.4 ± 3.8	22.3 ± 3.9	22.6 ± 3.4	NS
Underweight (BMI ⩽ 18.5)	13%	17%	4%	
Normal weight (BMI = 18.5-24.9)	69%	65%	79%	NS
Overweight (BMI = 25-29.9)	14%	14%	13%	
Obesity (BMI ⩾ 30)	4%	4%	4%	
HCV infection (*de novo* or recurrence)	57%	46%	79%	0.007
Arterial hypertension	53%	52%	54%	NS
Glycemia (mmol/L)	5.4 ± 2.0	5.1 ± 0.9	6.2 ± 3.2	NS
Diabeties mellitus	10%	8%	13%	NS
Maintenance immunosuppresssion				
Cyclosporine A	96%	96%	96%	NS
Dosage (mg)	129.8 ± 58.1	135.0 ± 61.5	119.5 ± 50.4	NS
Prednisolone	93%	96%	88%	NS
Dosage (mg)	6.8 ± 3.1	6.9 ± 3.2	6.7 ± 2.9	NS
Azathioprine	43%	40%	50%	NS
Dosage (mg)	48.4 ± 15.7	51.3 ± 15.5	43.8 ± 15.5	NS

Continuous data are represented as mean ± SD, and categorical data as percentage. [1]Comparison between HS and No HS. HS: Hepatic steatosis; NS: Not significant; BMI: Body mass index.

Table 3 Social life factors and hepatic steatosis at 10-year biopsy

	All subjects *n* = 72	No HS *n* = 48	HS *n* = 24	*P*[1]
Work				
Employed	33%	39%	23%	NS
Homemaker	13%	17%	4%	NS
Student full/part-time	3%	4%	0%	NS
Unemployed	20%	19%	23%	NS
Retired	30%	21%	50%	0.03
No. of years worked	17.9 ± 12.7	16.4 ± 12.6	20.9 ± 12.5	NS
Alcohol and smoking				
Alcohol consumption	21%	17%	30%	NS
No. of drinks[2]/d in drinkers	1.1 ± 0.3	1.0 ± 0.0	1.2 ± 0.4	NS
Tobacco smokers	17%	15%	21%	NS
Cigarettes/d in smokers	1.9 ± 0.7	2.1 ± 0.7	1.6 ± 0.9	NS

Continuous data are represented as mean ± SD, and categorical data as percentage. [1]Comparison between HS and No HS; [2]One drink = 1 bottle of beer or 1 glass of wine or 1 mixed drink. HS: Hepatic steatosis; NS: Not significant.

regimen are well-known side effects[23]. We acknowledge that at the time of this study almost all patients (96%) were on Cyclosporine and our results may not be applicable to patients who are on Tacrolimus.

Interestingly, in our series HS in the 10 year allograft biopsies were related to HCV infection, rather than NAFLD or other causes. HCV infection is well known to highly influence the rate of not only liver fibrosis but also HS. In the non-transplant setting steatosis is a very common lesion in chronic HCV infection[24], and

Table 4 Univariate analysis of subjective variables associated with hepatic steatosis at 10-year biopsy

QOL criteria	Univariate *P*
Physical distress	
Muscle weakness	0.04
Abdominal pains or discomfort	< 0.0001
Abdominal swelling or bloating	0.04
Joint aches or pains	< 0.001
Headaches	0.03
Poor or blurred vision	0.03
Change in facial appearance	< 0.001
Fluid retention or swelling of ankles	0.02
Psychological distress	
Sleeplessness or insomnia	0.03
Nervousness, anxiety	0.009
Feeling depressed, sad or blue	< 0.01
Low satisfaction with life as a whole	0.02
Personal function	
Health currently limits the kind of vigorous activities such as running, heavy lifting or sport	< 0.001
Social and role function	
Decreased interest in sex	0.003
Problem with sex life	0.04
General health perception	
Bodily pain during the last month	< 0.01

Table 5 Multivariate analysis of independent factors of hepatic steatosis at 10-year biopsy

Factors	Multivariate *P*
Objective factors	
Retirement	0.04
Hepatitis C virus infection (*de novo* or recurrence)	< 0.01
Subjective factors	
Abdominal pains or discomfort	< 0.01
Joint aches or pains	0.04

the pathogenesis of steatosis may differ according to the genotype of HCV. Strong clinical and experimental evidence suggests that steatosis in patients infected with genotype 3 is partly related to a direct cytopathic effect, whereas in genotype 1, steatosis is mainly related to an associated metabolic syndrome and insulin resistance[25]. Because the predominant HCV genotype in our patients with HS was the genotype 1 (63%), mostly 1b subtype, and genotype 3 represented 16% (all in HS group), we can consider that both mechanisms were involved.

HCV seems to dominate other risk factors in our study. One explanation for this findings may be that a type Ⅱ error occurred because of the relatively small sample size (n = 72 and only 24 in HS group). Otherwise, HS is presumably a surrogate for chronic hepatitis C, which is more directly affecting the QOL from chronic viral infection than the presence of histologic steatosis.

Unfortunately, HCV recurs in nearly all liver transplant recipients, and the reduction in long-term survival observed in these patients is the result of progressive fibrosis and evolution into cirrhosis[26-30]. Among the recipients with HCV infection, those who achieved 10 year post-transplant survival in our series can probably be categorized as "slow fibrosers". Fibrosis was present in 71% of our patients with HS and was mainly related to HCV infection.

Other symptoms like changes in facial appearance, fluid retention or swelling of ankles, and headaches affected the QOL of long term survivors. These symptoms are probably associated to the long term medication that patients require after LT. Moreover, in addition to muscle weakness, these physical affections presumably had a repercussion on PD; predominantly nervousness, anxiety, sadness or depression associated with sleeplessness or insomnia. As a consequence, PD, SRF, and GHP also worsened in patients with HS.

The impact of HS on QOL has been already reported in non-transplanted patients. Recent studies demonstrated the negative impact of NAFLD on the physical and psychological function[31-33]. Newton *et al*[31] refuted the misconception that symptoms associated with NAFLD are entirely related to excessive weight, a concept that supported by our data. It is well recognized that the major risk factor for HS is excessive consumption of food, alcohol, or both. However, many people who over-consume do not have fatty livers, and steatosis can develop in those who do not engage in these behaviors. Thus, genetic or environmental factors or both could influence one's susceptibility to hepatic triglyceride accumulation[34-36].

Future perspectives in the transplant setting must inevitably imply the host and the graft. At the present time, the gold standard for diagnosis remains liver biopsy but its costs and risks limit its practice in the non-transplant setting. Some demographic factors, blood tests, and imaging studies can be used to predict a higher risk of steato-hepatitis or advanced fibrosis, but are of limited sensitivity and specificity. More accurate predictors and scoring systems would allow identification of those who would benefit most from liver biopsy and monitor disease progression and response to therapy[19].

In conclusion, we could demonstrate that in patients with long-term follow-up after LT, HS is the most important histological finding that has an impact on the patients' quality of life. Interventions are needed to restore and optimize QOL in patients with *de novo* or recurrent HS during long-term follow-up. Future research should focus on identifying factors that lead to the development of HS after LT.

COMMENTS

Background

The goal of liver transplantation is to ameliorate not only survival, but also quality of life (QOL) while minimizing the effects of disease and costs of care. The increasing proportion of recipients alive at long-term follow up has incited transplant professionals to focus on long term morbidity-free survival and an acceptable QOL. In this study the authors evaluated the relationship between the state of transplanted liver graft and the recipient QOL of histologically proven lesions in a 10-year post liver transplantation (LT) cohort of patients.

Research frontiers

Studies of long-term survivors have been mainly based on clinical data and follow up at long term with respect to indication of LT, immunosuppressive

regimen or recipient and donor criteria. Few studies assessed the graft histology by long-term graft biopsy protocol and, to our knowledge; no report assessing the relationship between the histological state of long-term transplanted liver graft and the recipient QOL has been published.

Innovations and breakthroughs

The results of this study showed a potential impact of graft's steatosis on the QOL of transplant patients 10 years after surgery.

Applications

These results are encouraging and may represent the beginning of further studies in the area and, consequently the establishment of a specific care of these patients.

Peer-review

An interesting experience on the histological explore the outcome of 10-year liver transplantation. Manuscript is well written.

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ORIGINAL ARTICLE

Retrospective Study

Stabilization of estimated glomerular filtration rate in kidney transplantation from deceased donors with acute kidney injuries

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Abstract

AIM

To evaluate and compare the outcomes of kidney transplant (KT) from deceased donors among standard criteria, acute kidney injury (AKI) and expanded criteria donors (ECDs).

METHODS

This retrospective study included 111 deceased donor kidney transplant recipients (DDKT). Deceased donors were classified as standard criteria donor (SCD), AKI donor and ECD. AKI was diagnosed and classified based on change of serum Cr by acute kidney injury network (AKIN) criteria. Primary outcome was one-year estimated glomerular filtration rate (eGFR) calculated from Cr by CKD-EPI. Multivariate regression analysis was done by adjusting factors such as type of DDKT, %Panel-reactive antibodies, cold ischemic time, the presence of delayed graft function and the use of induction therapy. Significant

factors that can affect the primary outcomes were then identified.

RESULTS

ECD group had a significantly lower eGFR at one year (33.9 ± 17.3 mL/min) when compared with AKI group (56.6 ± 23.9) and SCD group (63.6 ± 19.9) (P < 0.001). For AKI group, one-year eGFR was also indifferent among AKIN stage 1, 2 or 3. Patients with AKIN stage 3 had progressive increase of eGFR from 49.6 ± 27.2 at discharge to 61.9 ± 29.0 mL/min at one year. From Kaplan-Meier analysis, AKI donor showed better two-year graft survival than ECD (100% *vs* 88.5%, P = 0.006). Interestingly, AKI group had a stable eGFR at one and two year. The two-year eGFR of AKI group was not significantly different from SCD group (56.6 ± 24.5 mL/min *vs* 58.6 ± 23.2 mL/min, P = 0.65).

CONCLUSION

Kidney transplantations from deceased donors with variable stage of acute kidney injuries were associated with favorable two-year allograft function. The outcomes were comparable with KT from SCD. This information supports the option that deceased donors with AKI are an important source of organ for kidney transplantation even in the presence of stage 3 AKI.

Key words: Acute kidney injury donor; Rising of terminal serum creatinine; Acute kidney injury network stage; Deceased donor; Estimated glomerular filtration rate stabilization; Stabilize allograft function

Core tip: Many concerns about problems from using kidneys donated from donors who had acute kidney injury (AKI) before organ procurement lead to underutilization of such kidneys. Several kidneys have unnecessary been discarded in recent year. Here, we describe the comparable allograft and patient outcomes between using kidney from standard criteria donor and donor with AKI. Kidney transplantations from deceased donors with variable stages of acute kidney injuries were associated with favorable allograft function. This information supports the option that deceased donors with AKI are an important source of organ for kidney transplantation and can remedy the problem of organ shortage.

Wiwattanathum P, Ingsathit A, Kantachuvesiri S, Arpornsujaritkun N, Tirapanich W, Sumethkul V. Stabilization of estimated glomerular filtration rate in kidney transplantation from deceased donors with acute kidney injuries. *World J Transplant* 2016; 6(4): 712-718 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i4/712.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i4.712

INTRODUCTION

An increasing tendency to perform kidney transplant (KT) from deceased donors other than standard criteria donor (SCD) is the result of disparity between the number of patient being in the waiting list for transplantation and utilized donor pool[1]. Types of non-ideal deceased donor include donors with acute kidney injury (AKI) and expanded criteria donor (ECD) are being used for expanding donor pool[2]. However, there are concerns about worse allograft outcomes when using kidneys form AKI donors. Therefore, a significant number of kidneys from AKI donors with high terminal serum creatinine level have been discarded. Hence, the plan to solve problem of organ shortage cannot be accomplished.

Increased incidence of delayed graft function (DGF)[3,4] is a significant disadvantage of using kidneys from AKI donors. This can lead to increased hospital stay and cost of treatment or even worse allograft function[5] when compare with KT from SCD. In addition, it is uncertain whether KT from AKI donor is associated with increased risk of acute rejection or allograft loss when compare with KT from using kidney from standard deceased donor[3,4]. Since AKI can occur from different causes and have different severities, the outcomes of KT from donors with AKI may be varied. Theoretically, KT from donors with mild degree of AKI may have favorable outcomes than KT from severe AKI. However, it is not universally agreed to use kidneys from donors with AKI. There are studies reporting association of discarding kidney in the presence of AKI of deceased donor[6]. We conducted a study aimed to determine outcomes of kidney transplantation from deceased donors with variable degrees of acute kidney injuries.

MATERIALS AND METHODS

Patients

A retrospective cohort of 243 KT recipients from our single center hospital during 1st January 2012 to 31st December 2013 was reviewed. Inclusion criteria were (1) deceased donor kidney transplant (DDKT) recipient; (2) Age ⩾ 15 years old; (3) Negative lymphocytotoxic cross match result at the time of transplantation; and (4) Panel-reactive antibodies (PRA) luminex < 20%. Exclusion criteria were: (1) recipients who had combined solid organ transplantation; and (2) donor whose terminal serum creatinine increased ⩾ 0.3 mg/dL but not ⩾ 1.5-fold from baseline. From these inclusion and exclusion criteria (excluded 115 cases due to living related kidney transplantation, 8 cases due to age < 15 years, 1 case due to combined solid organ transplant and 8 cases due to terminal serum creatinine increased ⩾ 0.3 mg/dL but not ⩾ 1.5-fold from baseline), total 111 KT recipients who received DDKT were enrolled in the study. This study was approved by the study center Institutional Review Board/Ethics Committee.

Study procedure

Baseline transplantation data and the clinical outcomes at two-year were collected from all patients then compared

outcomes by statistical analysis. Study populations were stratified into 3 groups according to the donor status: (1) Standard criteria deceased donor (SCD); (2) Deceased donor with AKI donor; and (3) Expanded criteria deceased donor (ECD). AKI donor was recognized by a rising of serum creatinine more than 0.3 mg/dL and defined by AKI Network criteria (AKIN criteria[7]) based on baseline to terminal serum creatinine (Cr) as follows: Stage 1, increase in Cr $\geqslant$ 1.5 to < 2-fold increase; stage 2, 2 to < 3 fold increase and stage 3, $\geqslant$ 3-fold increase. However, we did not included AKI donors who have terminal serum creatinine less than 1.5 fold from baseline to ensure that degree of AKI was significant enough to have impacts on transplantation outcomes. ECD was defined by any donor over the age of 60, or a donor over the age of 50 with two of the following: A history of high blood pressure, a creatinine greater than or equal to 1.5 mg/dL, or death resulting from a stroke. All other donors were classified as SCD.

Outcomes

Primary outcome was estimated glomerular filtration rate (eGFR) at one year as calculated from Cr by CKD-EPI equation. Secondary outcomes were eGFR at discharge and two year, rate of DGF (defined as requirement of dialysis within 7 d after transplantation), two-year allograft and patient survival.

Statistical analysis

Continuous variables were described as mean values (SD) and median values (range) for data with normal distribution and non-normal distribution respectively. Categorical variables were described as frequency and percentage. Student *t* test (or Mann-Whitney *U* test) was used to compare the difference between groups for continuous data. A χ^2 test (or Fisher's exact test) was used to compare the difference between groups for categorical data. Multivariate regression analysis was used to determine independently significant factors (type of DDKT, %PRA, cold ischemic time, the presence of DGF and the use of induction therapy) that may affect one-year eGFR. Allograft survival and patient survival were presented by Kaplan Meier analysis. All analyses were performed using Stata statistical software, version 13.0 (Stata Corp., Collage Station, TX). $P < 0.5$ was considered significant. The statistical review of the study was performed by a biomedical statistician.

RESULTS

A total of 119 DDKT recipients were enrolled. Eight recipients receiving kidney from AKI donors whose terminal serum creatinine increased $\geqslant$ 0.3 mg/dL but not $\geqslant$ 1.5-fold from baseline and were excluded. One hundred and eleven patients were included in the analysis. There were 32 recipients in SCD group, 51 in AKI group and 28 in ECD group. Recipient and donor characteristics are shown in Table 1. All recipient baseline characteristics were similar among 3 groups. Donor age was older in ECD group than the other groups. Most donors were male and the proportion was highest in AKI group. Basiliximab (Simulect®) was commonly used for induction in both SCD (34.4%) and AKI group (47.1%). Antithymocyte globulin (ATG) was frequently used in ECD group (39.9%). However, the different in prescribing induction therapy was not statistically significant ($P = 0.19$). Maintenance immunosuppressive regiments were shown in Table 1. The combination of cyclosporine and everolimus was more commonly used in AKI and ECD donor when compared with standard criteria deceased donor ($P = 0.05$).

eGFR at discharge was 64.1 ± 22.1, 52.5 ± 22.9 and 35.5 ± 17.9 mL/min for SCD, AKI and ECD group. eGFR at one year was 63.6 ± 19.9, 56.6 ± 23.9 and 33.9 ± 17.3 mL/min for SCD, AKI and ECD group. eGFR at two year was 58.6 ± 23.2, 56.6 ± 24.5 and 29.9 ± 19.2 mL/min in SCD, AKI and ECD group respectively (Table 2). Two-year eGFR was significant lower in ECD group ($P < 0.001$) when compared with the other groups but was not different between SCD group and AKI group ($P = 0.65$). For AKI group, two-year eGFR was also indifferent among degree of AKI as classified by AKIN stage 1, 2 or 3 (Table 3). Two-year eGFR for AKI group with AKIN stage 1, 2 and 3 was 53.4 ± 24.3, 54.0 ± 21.4 and 64.0 ± 29.4 mL/min ($P = 0.79$). While two-year eGFR in both SCD and ECD groups decreased over time after transplantation, two-year eGFR in AKI group had tendency to improve over time after transplantation especially in AKIN stage 3 (Table 3). In AKIN stage 3 group, two-year eGFR progressively improved form 49.6 ± 27.2 mL/min after transplant to 64.0 ± 29.4 mL/min. However, this change was not statistically different ($P = 0.12$). Univariate regression analysis showed that the use of ECD and presence of DGF were significantly associated with decreased of eGFR at one year by univariate model. However, multivariate regression analysis showed that use of ECD is the only factor that was associated with declining one-year eGFR (Table 4).

Rate of DGF was lowest in SCD group and highest in ECD group. DGF occurred 31.2%, 56.9% and 77.8% for each group ($P = 0.001$). Rate of acute rejection was not differed among the three groups (Table 2). Two-year allograft survival was 100%, 100% and 88.5% for each group (Figure 1, $P = 0.01$). Two-year patient survival rate was similar among three groups (Figure 2). Cardiovascular death was responsible for cause of death in 1, 3 and 1 recipient in SCD, AKI and ECD group respectively. Infection related death was responsible for cause of death in 1 recipient both from SCD and ECD group. Rate of CMV and BK virus infection were not difference among 3 groups (Table 2).

DISCUSSION

Our findings suggest that transplantation from deceased donors with AKI have comparable outcome when compared with SCD. The outcomes include both eGFR and

Table 1 Baseline characteristics

DDKT (*n* = 111)	SCD (32)	AKI (51)	ECD (28)	[a]*P*-value	[b]*P*-value
Recipients					
Age year (mean ± SD)	42.7 ± 13.8	43.9 ± 12.0	43.1 ± 12.3	0.68	0.67
Male *n* (%)	19 (59.4)	35 (68.6)	16 (57.1)	0.48	0.55
Pre KT dialysis				1	1
Hemodialysis *n* (%)	26 (81.3)	42 (82.4)	23 (82.1)		
Peritoneal dialysis *n* (%)	6 (18.8)	9 (17.7)	5 (17.9)		
Comorbid *n* (%)					
DM	2 (6.25)	8 (15.7)	3 (10.7)	0.3	0.48
HT	30 (93.8)	49 (96.1)	25 (89.3)	0.67	0.41
CAD	1 (3.1)	1 (1.9)	1 (3.6)	1	1
Cause of ESRD *n* (%)				0.91	0.73
Unknown (no biopsy)	23 (23.2)	33 (31.8)	18 (18.9)		
Diabetic nephropathy	1 (0.9)	1 (1.3)	1 (0.8)		
IgA nephropathy	1 (1.9)	2 (2.6)	3 (1.5)		
Chronic glomerulonephritis	2 (0.9)	1 (1.3)	0 (0.8)		
Blood group *n* (%)				0.14	0.38
A	4 (12.5)	13 (25.5)	7 (25.0)		
B	13 (40.6)	13 (25.5)	7 (25.0)		
AB	4 (12.5)	2 (3.9)	3 (10.7)		
O	11 (34.4)	23 (45.1)	11 (39.3)		
PRA - % median (range)	0 (0.85)	0 (0.0)	0 (0.0)	0.03	0.04
Second KT *n* (%)	2 (6.25)	3 (5.88)	1 (3.57)	1	1
Total HLA mismatch - (mean ± SD)	2.5 (1.2)	2.3 (1.1)	2.1 (1.1)	0.52	0.76
Donors					
Age, year (mean ± SD)	33.9 ± 14.8	41.0 ± 12.0	61.2 ± 7.0	0.02	< 0.001
Male *n* (%)	24 (75.0)	44 (86.3)	17 (60.7)	0.25	0.04
Terminal serum creatinine (mg/dL) - median (range)	0.91 (0.73, 1.13)	2.22 (1.65, 3.20)	1.28 (0.99, 2.70)		< 0.001
Cold ischemic time, minute (mean ± SD)	1099 ± 291	1129 ± 294	1261 ± 242	0.65	0.5
Immunosuppressive drugs					
Induction *n* (%)				0.11	0.19
No	16 (50.0)	16 (31.4)	9 (32.1)		
ATG	5 (15.6)	11 (21.6)	11 (39.3)		
Simulect	11 (34.4)	24 (47.1)	8 (28.6)		
Maintenance *n* (%)				0.05	0.005
Tacrolimus/mycophenolate/prednisolone	16 (50.0)	27 (52.9)	13 (46.4)		
Cyclosporin A/mycophenolate/prednisolone	15 (46.8)	16 (31.4)	5 (17.9)		
Cyclosporin A/everolimus/prednisolone	0	7 (13.7)	8 (28.6)		
Everolimus/mycophenolate/prednisolone	1 (3.1)	0	0		

[a]*P*-value compared between SCD and AKI; [b]*P*-value compared among SCD, AKI and ECD. DDKT: Deceased donor kidney transplant; SCD: Standard criteria donor; AKI: Acute kidney injury; ECD: Expanded criteria donor; KT: Kidney transplant; DM: Diabetes mellitus; HT: Hypertension; CAD: Cardiovascular disease; ESRD: End stage renal disease; HLA: Human leukocyte antigen; ATG: Antithymocyte globulin.

two year patient survival. In addition, eGFR of AKI group did not decline after two year follow up. In contrast, eGFR in ECD group significantly declined after two year. This finding supports the view that kidneys with AKI may have recovery after a period of time.

In native kidney, after injury subsides, kidney can repair itself and restore normal or sub-normal function over time depends on severity and duration of injury[8]. Our finding suggests that these processes also occur in transplanted kidney. As shown in AKI group, one-year eGFR had progressive increase from baseline and stable at two-year follow up in all three groups. However, there are difficulties to predict the ability of each kidney allograft regarding the ability to recovery from acute kidney injuries. A calculation of "Kidney Donor Profile Index"[9,10] has been proposed to predict the risk of graft loss after deceased donor kidney transplantation. The involved donors' parameters include age, height, weight, ethnicity, history of hypertension, history of diabetes, causes of death, serum creatinine, HCV status and donation after circulatory death status. However, the calculations of KDPI use a single value of serum creatinine and may or may not be indicative the presence of AKI in the donors. Evidences from some studies showed worse allograft function from AKI donor. These suggested that not all kidneys from AKI donor were suitable for transplantation. Researches providing such information are necessary and useful for making decision on which kidney should be used or discarded.

In the recent years, kidneys form AKI donor were underutilization. As shown in some studies that there are high discard rate of deceased donor with high serum creatinine. About 20%-30% of kidneys from AKI donors were discarded and sometime more than 40 percent were discarded when terminal serum creatinine > 2.0 mg/dL[3,6,11]. In contrast, our study has shown that KT from deceased donors with AKI is associated with comparable clinical outcomes with standard criteria deceased donors.

Table 2 Transplantation outcomes

Outcomes	SCD (32)	AKI (51)	ECD (28)	[a]*P*-value	[b]*P*-value
Cr at discharge - mg/dL (mean ± SD)	1.35 ± 0.51	1.70 ± 0.84	2.41 ± 1.00	0.04	< 0.001
Cr at 1 yr - mg/dL (mean ± SD)	1.35 ± 0.50	1.59 ± 0.75	2.64 ± 1.38	0.14	< 0.001
Cr at 2 yr - mg/dL (mean ± SD)	1.52 ± 0.63	1.68 ± 1.06	3.29 ± 2.12	0.47	< 0.001
eGFR at discharge - mL/min (mean ± SD)	64.1 ± 22.1	52.5 ± 22.9	35.5 ± 17.9	0.03	< 0.001
eGFR at 1 yr - mL/min (mean ± SD)	63.6 ± 19.9	56.6 ± 23.9	33.9 ± 17.3	0.19	< 0.001
eGFR at 2 yr - mL/min (mean ± SD)	58.6 ± 23.2	56.6 ± 24.5	29.9 ± 19.2	0.65	< 0.001
DGF *n* (%)	10 (31.2)	29 (56.9)	21 (77.8)	0.03	0.001
Length of stay - d (mean ± SD)	24.4 ± 8.3	31.1 ± 14.7	37.9 ± 15.3	0.02	0.002
Nephrectomy *n* (%)	0	2 (3.9)	2 (7.4)	0.52	0.27
Acute rejection	5 (15.7)	10 (19.6)	6 (27.4)	0.70	0.8
ACR	2 (6.3)	6 (11.8)	2 (7.1)		
ABMR	1 (3.1)	3 (5.9)	3 (10.7)		
ACR + ABMR	2 (6.3)	1 (1.9)	1 (3.6)		
Graft loss *n* (%)	0	0	3 (11.5)	NS	0.01
Death *n* (%)	2 (6.3)	3 (5.1)	3 (10.7)	0.63	0.57
CMV *n* (%)	7 (5.2)	6 (8.3)	5 (4.5)	0.23	0.46
BK virus nephropathy *n* (%)	1 (3.1)	3 (5.69)	0	1	0.69

eGFR: Estimated glomerular filtration rate; DGF: Delayed graft function; ACR: Acute cellular rejection; ABMR: Antibody mediated rejection; CMV: Cytomegalovirus.

Table 3 Estimated glomerular filtration rate classified by acute kidney injury network stage

eGFR - mean ± SD	SCD (*n*) (32)	AKIN stage (*n*)			*P*-value
		1 (18)	2 (21)	3 (12)	
eGFR at discharge - mL/min	64.1 ± 22.1	49.8 ± 20.7	57.1 ± 23.7	49.6 ± 27.2	0.87, 0.07
eGFR at 1 yr - mL/min	63.6 ± 19.9	52.9 ± 21.2	57.1 ± 21.5	61.9 ± 29.0	0.47, 0.92
eGFR at 2 yr - mL/min	58.6 ± 23.2	53.4 ± 24.3	54.0 ± 21.4	64.0 ± 29.4	0.79, 0.54

AKIN: Acute kidney injury network; eGFR: Estimated glomerular filtration rate; SCD: Standard criteria donor.

Table 4 Univariate and multivariate regression analysis of factors associated with the change of one-year estimated glomerular filtration rate

Factors	Univariate			Multivariate		
	B-coefficient	*P*-value	95%CI	B-coefficient	*P*-value	95%CI
Type of donor						
SCD	Reference	NA		Reference	NA	
AKI	-6.73	0.17	-16.41, 2.94	-3.7	0.49	-14.52, 7.13
ECD	-29.76	< 0.001	-41.67, -17.85	-25.43	< 0.001	-38.80, -12.05
PRA > 20%	3.49	0.64	-7.37, 14.34	3.62	0.53	-7.82, 15.05
DGF	12.3	0.008	3.22, 21.39	6.17	0.18	-2.91, 15.25
HLA mismatch ≥ 3	-2.99	0.53	-12.45, 6.46	-7.12	0.11	-15.77, 1.53
CIT > 24 h	-14.72	0.03	-27.99, -1.45	-9.54	0.14	-22.19, 3.12
Received Induction	4.49	0.36	-5.28, 14.25	1.8	0.72	-8.09, 11.70

The B-coefficient values were calculated from univariate and multivariate regression analysis. SCD: Standard criteria donor; AKI: Acute kidney injury; ECD: Expanded criteria donor; PRA: Panel reactive antibody; DGF: Delayed graft function; HLA: Human leukocyte antigen.

Thus, our results show that kidneys from AKI donor are important source for organ transplantation and should not be discarded.

The limitation of our study is that there may be selection bias regarding the quality of kidneys when compare with other studies[3,6]. Pre-implantation biopsy and organ perfusion machine are not routinely used in this study for the organ procurement process. These can lead to more kidneys being used when organ retrieval process was satisfied as judged by the clinician.

In summary, kidney transplantations from deceased donors with variable stages of acute kidney injuries were associated with favorable two-year allograft function and survival. The outcomes were comparable with KT from those of standard criteria deceased donors. This information supports the option that deceased donors

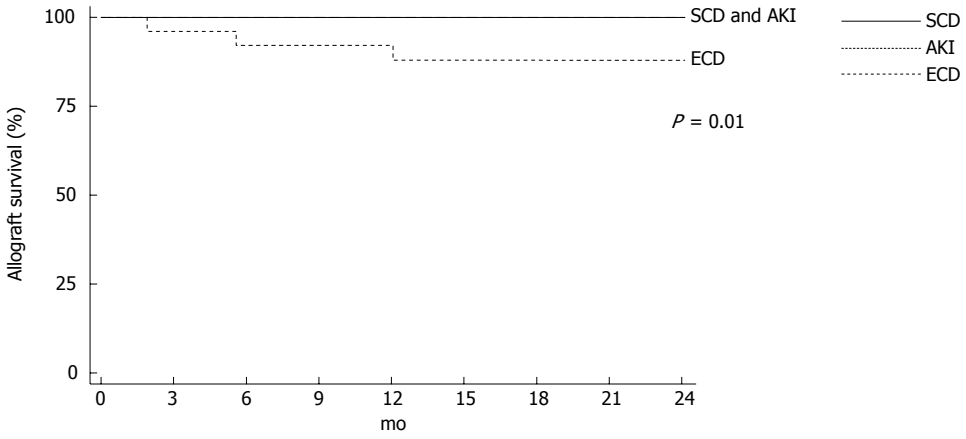

Figure 1 Comparison of two year actuarial allograft survival of standard criteria deceased donor, acute kidney injury donors and expanded criteria donors. SCD: Standard criteria donor; AKI: Acute kidney injury; ECD: Expanded criteria donor.

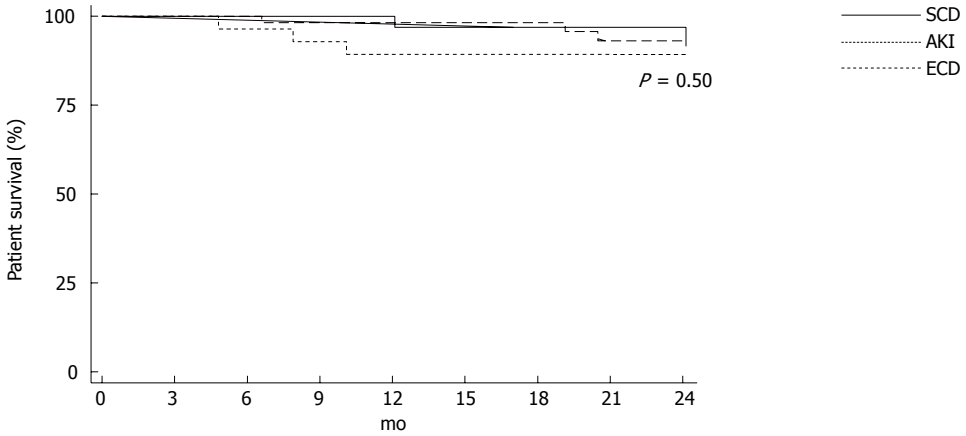

Figure 2 Comparison of two year actuarial patient survival of standard criteria deceased donor, acute kidney injury donors and expanded criteria donors. SCD: Standard criteria donor; AKI: Acute kidney injury; ECD: Expanded criteria donor.

with AKI are an important source of organ for kidney transplantation even in the presence of stage 3 AKI. However, not all kidneys from AKI donor may be used for transplantation. Further studies are required to determine and clarify the optimal use of kidneys with AKI and the precise parameters that can identify suitable kidneys form AKI donor suitable for proceeding to transplantation.

COMMENTS

Background

Organ shortage is a common problem worldwide. Kidney transplantations from non-ideal deceased donors are a potential option to minimize this problem. Acute kidney injury (AKI) donor and expanded criteria donor (ECD) are important sources of deceased donors. However, there are several challenging issues about the outcomes of using kidney form AKI donors or ECD. This can lead to the discard of using deceased donors with high terminal serum creatinine (Cr). The "old to old" concept has been proposed to be the model of allocating kidneys from ECD. However, there is no consensus guideline regarding the use of kidneys from AKI donors. The authors therefore evaluate the outcomes of kidney transplant from deceased donors with several stages of AKI and compare with that of standard criteria donors (SCDs) and ECDs.

Research frontiers

Results from some studies showed worse allograft function when transplantation from AKI donor. These suggest that not all kidneys from AKI donor were suitable for transplantation. Researches providing such information are necessary and useful for decision whether which kidney should be used or discarded.

Innovations and breakthroughs

Many kidneys from AKI donors were discarded because of concerning about poor allograft outcomes. This study showed that kidney transplantation from deceased donors with variable stage of acute kidney injuries was associated with equivalent allograft function and survival when compare with SCD.

Applications

Kidneys from AKI donors are important sources of organ for transplantation that can mitigate the problem of organ shortage.

Terminology

KT: Kidney transplant; SCD: Standard criteria donor; AKI: Acute kidney injury; ECD: Expanded criteria donor; DM: Diabetes mellitus; HT: Hypertension; CAD: Cardiovascular disease; ESRD: End stage renal disease; PRA: Panel reactive antibody; HLA: Human leukocyte antigen; Cr: Creatinine; eGFR: Estimated glomerular filtration rate; ATG: Antithymocyte globulin; DGF: Delayed graft function; ACR: Acute cellular rejection; ABMR: Antibody mediated rejection; CMV: Cytomegalo virus.

Peer-review

The article is well written and relevant.

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ORIGINAL ARTICLE

Retrospective Study

Acute antibody-mediated rejection after intestinal transplantation

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Author contributions: Wu GS cared for the patients and collected and summarized the data and wrote the article; Cruz Jr RJ cared for the patients; Cai JC helped for the interpretation of antibody data revising the manuscript.

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Abstract

AIM

To investigate the incidence, risk factors and clinical outcomes of acute antibody-mediated rejection (ABMR) after intestinal transplantation (ITx).

METHODS

A retrospective single-center analysis was performed to identify cases of acute ABMR after ITx, based on the presence of donor-specific antibody (DSA), acute tissue damage, C4d deposition, and allograft dysfunction.

RESULTS

Acute ABMR was identified in 18 (10.3%) out of 175 intestinal allografts with an average occurrence of 10 d (range, 4-162) after ITx. All acute ABMR cases were presensitized to donor human leukocyte antigens class Ⅰ and/or Ⅱ antigens with a detectable DSA. A positive cross-match was seen in 14 (77.8%) cases and twelve of 18 patients (66.7%) produced newly-formed DSA following ITx. Histological characteristics of acute ABMR include endothelial C4d deposits, interstitial hemorrhage, and severe congestion with focal fibrin thrombin in the lamina propria capillaries. Multivariate analysis identified a liver-free graft and high level of panel reactive antibody

as a significant independent risk factor. Despite initial improvement after therapy, eleven recipients (61.1%) lost transplant secondary to rejection. Of those, 9 (50%) underwent graft removal and 4 (22.2%) received second transplantation following acute ABMR. At an average follow-up of 32.3 mo (range, 13.3-76.4), 8 (44.4%) recipients died.

CONCLUSION

Our results indicate that acute ABMR is an important cause of intestine graft dysfunction, particularly in a liver-exclusive graft and survivors are at an increased risk of developing refractory acute rejection and chronic rejection. More effective strategies to prevent and manage acute ABMR are needed to improve outcomes.

Key words: Intestinal transplantation; C4d deposition; Donor-specific antibody; Acute antibody-mediated rejection

Core tip: Antibody-mediated rejection (ABMR) has appeared to be an important cause of allograft failure after intestinal transplantation (ITx). This study aimed to evaluate the incidence, risk factors and clinical outcomes of acute ABMR after ITx. The incidence of acute ABMR after ITx was as high as 10.3% in our series, which was closely associated with poor graft and patient survival. Our results indicate that acute ABMR is an important cause of intestinal graft failure, especially in a liver-free allograft and survivors are at an increased risk of developing chronic rejection. Effective strategies to prevent and treat acute ABMR are needed to improve outcomes.

Wu GS, Cruz Jr RJ, Cai JC. Acute antibody-mediated rejection after intestinal transplantation. *World J Transplant* 2016; 6(4): 719-728 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i4/719.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i4.719

INTRODUCTION

Intestinal transplantation (ITx) has increasingly become a viable option for most patients with irreversible intestinal failure. Short-term patient and graft survival have improved to a great extent due to advances in surgical technology and immunosuppressive management[1,2]. However, long-term outcomes after ITx have been inferior to other solid-organ transplants, especially with less intestinal allograft survival more than 10 years[3,4]. Allograft failure secondary to acute and chronic rejection remain a significant impediment to the success of ITx[5].

Traditionally, intestine transplant rejection has been considered as a T-cell-mediated course that can be effectively controlled with T-cell targeted immunosuppressive agents. Harmful effects of antibodies to human leukocyte antigens (HLA) in intestinal allograft rejection have not been studied thoroughly although these HLA antibodies were often detectable after ITx[6-8]. To date, HLA antibodies are believed to be a major risk factor for hyperacute rejection, acute antibody-mediated rejection (ABMR) and chronic ABMR after kidney or heart transplantation[9]. Several case reports imply that HLA antibodies are also associated with lung, liver, or pancreas allograft dysfunction[10-12]. Increasing data suggest that an early diagnosis and prompt management of acute ABMR are essential for improving patient and graft outcomes[13,14].

The impact of HLA antibodies has got less attention in the assessment of acute intestinal allograft rejection. Similar to other solid-organ transplantation, many patients who require ITx become sensitized and form alloantibodies that originate either from previous exposure to blood products, pregnancies, transplants, and/or infections or *de novo* formation of donor-specific antibody (DSA) following transplantation[15,16]. In recent years, we and others have shown that the presence of DSA was closely associated with the incidence and severity of intestinal allograft rejection and decreased the overall graft and patient survival[17,18]. Although hyperacute rejection, caused by preformed DSA, rarely occurs in highly sensitized recipients after ITx[19], clinicopathological findings consistent with acute ABMR have increasingly been recognized as an important form of rejection[20,21]. Currently diagnostic standards for acute ABMR after ITx have not been set up yet and its incidence and clinical significance have remained unknown.

The diagnostic standards for acute ABMR in a kidney or heart transplant have been well-established. According to the guidelines, acute ABMR is defined by circulating DSA, C4d deposition, tissue pathology and clinical allograft dysfunction. In this series, we reviewed our institutional experience to identify recipients with acute ABMR that fulfill the criteria for kidney transplantation, and to evaluate the rate, risk factors and consequences after acute ABMR.

MATERIALS AND METHODS

Patient selection

Since August 2003, patients who received small bowel transplants at the University of Pittsburgh Medical Center have started to have a routine serum DSA specificities determinations, by either the purified HLA antigen-based ELISA or the Luminex single-antigen bead analysis. We performed a retrospective electronic medical records review of patients who underwent a small bowel transplant between August 2003 and May 2010. The clinical charts were reviewed as needed for additional data and the Institutional Review Board approved this study.

Donor and recipient demographics are summarized in Table 1. The transplant type consisted of a liver-exclusive transplant (isolated intestine graft and modified multivisceral graft without liver) and a liver-inclusive full multivisceral transplant. T cell complement-dependent lymphocytotoxic cross-match (CDC-XM) was performed by anti-human globulin (AHG)-enhanced method and

Table 1 Donor and recipient demographic and clinical characteristics

Characteristic	Transplants (n = 175)
Donor characteristics	
Age (yr)	25.4 ± 9.9
Gender (% male)	77.7
Nonwhite race (%)	16.6
Cold ischemic time (h)	7.6 ± 1.5
Recipient characteristics	
Age at transplantation (yr)	43.0 ± 12.5
Gender (% male)	38.9
Nonwhite race (%)	5.9
Primary diagnoses, n (%)	
Vascular occlusion	59 (33.7)
Crohn's disease	34 (19.4)
Neoplastic disorders	28 (16.0)
Motility disorders	21 (12.0)
Others	33 (18.9)
Donor/recipient sex mismatches (%)	56.6
Donor CMV positive/recipient negative (%)	21.9
Type of graft liver-free/liver-inclusive (%)	61.1/38.9
Two mismatches in HLA loci A/B/DR (%)	39.1/82.1/66.9
PRA at transplantation (≥ 10%) Class Ⅰ (%)	40
Class Ⅱ (%)	26.3
Positive T/B cell cross-match (%)	25.7
Preformed DSA (%)	30.3
Retransplantation (%)	6.7
Induction, n (%)	
None	41 (23.4)
Zenapax	3 (1.7)
Thymoglobulin	7 (4.0)
Campath-1H	124 (70.9)
Follow-up (mo; range)	37.5 ± 22.7 (0.7 to 81.5)

CMV: Cytomegalovirus; PRA: Panel reactive antibody; HLA: Human leukocyte antigens; DSA: Donor-specific antibody.

B cell CDC-XM was performed by extended-incubation/modified Amos technique. In our practice, a positive CDC-XM was not considered as a contraindication to ITx. HLA panel reactive antibody (PRA) was determined by LAT ELISA assay. The HLA antibodies were checked by the purified HLA antigen-based ELISA prior to April 2007 and have since then been replaced by the Luminex single-antigen bead assay. A value of the mean fluorescence intensity (MFI) ≥ 1000 was considered positive. We did not routinely follow up DSA levels post-transplant and indications for DSA monitoring were usually higher PRA levels, refractory rejection, or suspicious of acute ABMR.

The majority of patients underwent induction therapy with alemtuzumab (Campath-1H; Genzyme, Cambridge, MA) (n = 124), administered at day 0 (30 mg each dose) and some patients received antithymocyte globulin (ATG; Genzyme, Cambridge, MA) (n = 7), the IL-2 receptor antagonist basiliximab (Simulect; Novartis, East Hanover, NJ) (n = 3) or no induction therapy (n = 41) during the early period of this study. The basic immunosuppressive regimen was tacrolimus (Prograf; Astellas, Deefield, IL) and steroids. The 12-h trough levels of tacrolimus during the initial six months were targeted at 10-15 ng/mL with Campath-1H or ATG induction therapy, and 15-25 ng/mL with Simulect induction or without any treatment. Maintenance immunosuppression was similar between a positive and negative CDC-XM. All patients with a positive preformed DSA were given a single-dose of intravenous immunoglobulin (IVIG) at 2 g/kg body weight on day of transplantation. A 5-d steroid tapering was also given followed by a 10-20 mg daily dose for at least 6 mo. Recipients with acute ABMR underwent steroid boluses and/or OKT3. No patients were given plasmapheresis or anti-B cell treatment for acute ABMR.

Diagnosis of rejection

Surveillance ileal biopsies were routinely performed twice per week for the first 2 to 3 wk after transplantation and then once a week thereafter, with increased frequency in case of clinical indications. A diagnosis of acute ABMR was based upon the criteria, including: (1) clinical evidence of graft dysfunction; (2) histological evidence of tissue damage (vascular congestion, submucosal hemorrhage, neutrophilic margination, and platelet-fibrin thrombi in the lamina propria microvasculature)[7]; (3) focal (5%-50%) or diffuse (> 50%) linear C4d deposition; and (4) circulating anti-HLA antibodies[9,22,23]. A C4d staining was done on formalin-fixed paraffin-embedded tissue when acute ABMR was clinically or histologically suspected. The histological criteria for diagnosis of acute cellular rejection (ACR) were as described previously[24]. A new rejection episode was defined by newly occurred clinical symptoms and histological evidence of acute rejection with at least 1 normal mucosal biopsy between rejection episodes. A determination of chronic rejection was based upon clinical symptoms and was further confirmed by a full-thickness specimen of partially or totally resected allografts to reveal evidence of vasculopathy and mesenteric lymphoid depletion with mesenteric sclerosis[25].

Statistical analysis

Results are shown as means and ranges, unless otherwise stated. Categorical variables were assessed with the use of the χ^2 test or, when appropriate, Fisher's exact test. Continuous variables were analyzed with the use of the Student's t-test. Survival time was analyzed with the Kaplan-Meier method and differences were assessed by log-rank test. All data were analyzed using MedCalc for Windows, version 12.5 (MedCalc Software, Ostend, Belgium).

RESULTS

Diagnosis of acute ABMR after ITx

During the study period, 164 adults underwent 175 consecutive small bowel transplants; 11 (6.7%) patients underwent retransplantation. Donor characteristics, recipient profiles, and perioperative features are summarized in Table 1. We identified 18 cases (10.3%) that fulfilled all the criteria for acute ABMR proposed by the National Conference. Of these, 16 of 164 cases (9.8%) developed acute ABMR after primary transplantation and 2 of 11 cases (18.2%) developed acute ABMR after retransplantation (Figure 1). Recipient age at the time

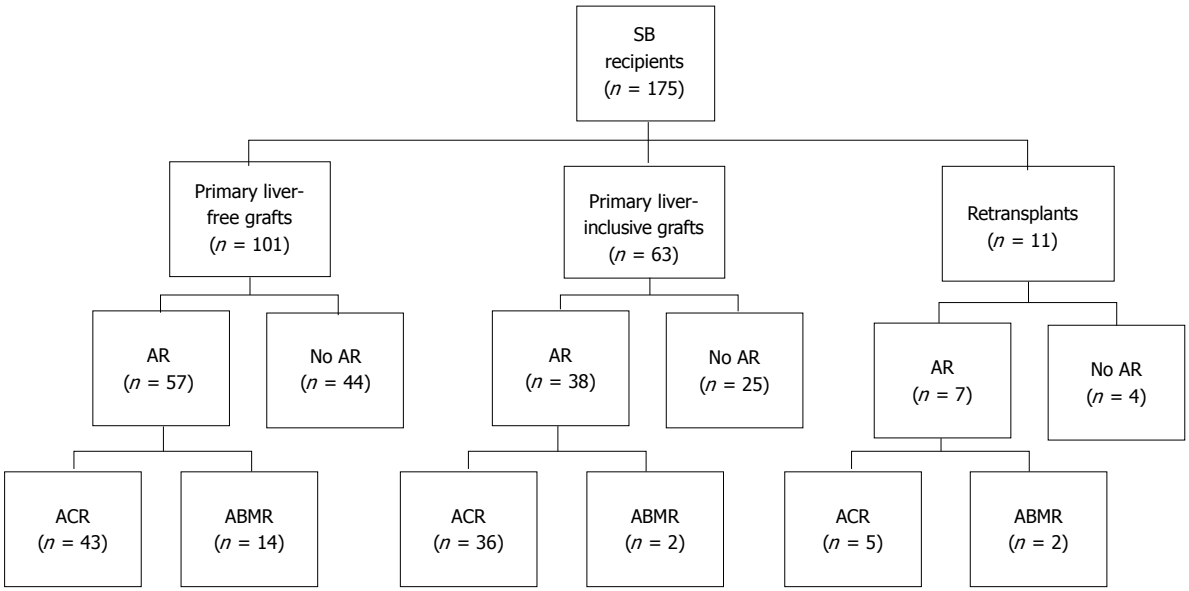

Figure 1 Patient distribution according to graft type and acute rejection type. SB: Small bowel; AR: Acute rejection; ACR: Acute cellular rejection; ABMR: Acute antibody-mediated rejection.

of transplantation was 25.4 ± 9.9 years old and thirteen cases (72.2%) were female. All patients were sensitized to HLA class Ⅰ (median PRA 78.5%, range 11%-100%) and/or HLA class Ⅱ antigens (median PRA 67.0%, range 1%-100%). A CDC-XM was positive in 14 (77.8%) recipients, in which anti-donor antibody titer was ≥ 1:8 in 7 cases (50.0%). Three recipients (16.7%) underwent splenectomy at the time of transplantation (2 at primary transplantation and 1 at retransplantation). Recipients developed acute ABMR at a median time of 10.0 d (range, 4-162 d). Fourteen patients presented within 30 d after transplantation (early acute ABMR) and the remaining 4 presented beyond 30 d (late acute ABMR). Fifteen patients developed a single episode of acute ABMR and three developed repeat episodes of ABMR (Table 2).

In all cases, we established the diagnosis of acute ABMR based on the combination of clinical evidence of graft dysfunction, histological findings, and the presence of DSA. Six (37.5%) patients with acute ABMR occurring within a week displayed evidence of graft dysfunction by severe mucosal vascular congestion and diffuse mucosal hemorrhage during endoscopic examination. The other clinical presentation includes fever, abdominal pain or distention, increased stomal output or other non-specific symptoms. The prominent pathological findings in the cases with early ABMR were vascular congestion, focal hemorrhage with focal platelet-fibrin thrombi, and capillary neutrophilic infiltration in the lamina propria and the submucosa. The mucosal biopsies obtained from acute ABMR occurring within a week showed severe vascular congestion along with diffuse mucosal hemorrhage without any evidence of crypt and epithelial injury or apoptosis. These changes gradually returned to normal by 2 to 3 wk in most cases after treatment. Four cases with late ABMR exhibited less prominent vascular congestion and hemorrhage but showed significant fibrin thrombi or neutrophilic margination (Table 2). There was no evidence of any significant vasculitis in the biopsies we evaluated. Seventeen cases showed a diffuse C4d deposition in the lamina propria and the submucosal capillaries (Figure 2). One case with a liver-inclusive transplant showed focal C4d deposition of the intestinal allograft but with significant vascular disturbance. Four of the 18 patients had pure acute ABMR without concomitant ACR within a year and the remaining 14 patients had concomitant ACR either before ABMR (*n* = 3), at the time of ABMR (*n* = 4), or after a diagnosis of ABMR (*n* = 9) (Table 2).

All 18 patients had DSA at the time of transplant: 10 to Class Ⅰ HLA, 1 to Class Ⅱ HLA only, and 7 to both Class Ⅰ and Class Ⅱ HLA. DSA was persistent in 3 cases at the time of the second episode of acute ABMR (Table 2). These antibodies were detected in fourteen cases by the purified HLA antigen-based ELISA and in the remaining four cases by the Luminex single-antigen bead assay.

Treatment, graft loss and patient death

Our treatment approach evolved over time and the regimen was individualized based on severity of illness, clinical course and response to therapy (Table 3). All patients were initially given intravenous steroids. Thirteen patients required additional OKT3 (*n* = 10), ATG (*n* = 1), Campath-1H (*n* = 1), or Campath-1H followed by OKT3 (*n* = 1) to reverse acute ABMR.

During the study period, post-transplant HLA antibodies were checked in 158 (90.3%) cases. *De novo* DSA was detected in twelve of the 18 patients (66.7%): 7 to Class Ⅱ HLA, 5 to both Class Ⅰ and Class Ⅱ HLA. The presence of *de novo* DSA was markedly higher in the cases with acute ABMR compared to 7.6% (5 of 66) in the cases without rejection ($P < 0.0001$) or

Table 2 Characteristics of 18 patients with diagnosis of acute antibody-medicated rejection

Case	Tx type	POD (d)	XM		DSA at time of Tx and/or rejection	*De novo* DSA	Vascular alterations	C4d	#ACR≤ 360 d
			T-cell	B-cell					
1	MV + K	4	1:32	1:16	A1, A25, B8, B18	DR51	++	Focal	0
2	SB	5	1:256	1:512	B7, B44, BW4, DQ1, DR10	DR15, DR51	+++	Diffuse	2
3	SB	5	1:2	1:2	B60	DR16	++	Diffuse	3
4[1]	SB	6	1:8	1:8	B35, B60	A31, DQ7, DR11	+++	Diffuse	0
		109			A24, B60[4]	DR14, DR52	++	Diffuse	
5	SB	7	Neg	1:1	A3, B18, DR17	None	++	Diffuse	0
6	MV	7	Neg	1:8	A26, B70, DR52	None	++	Diffuse	1
7	MMV	9	1:32	1:8	A2	None	+++	Diffuse	4
8	SB	10	Neg	Neg	DR52	None	++	Diffuse	1
9	MMV	10	1:2	1:2	B13, BW4, DR7, DR53	DQ1	++	Diffuse	2
10[1]	SB + K	11	Neg	1:4	A32, B8	A1, DR17	++	Diffuse	4
		52			A32, DQ4[4]		++	Diffuse	
11	SB + P	14	Neg	1:4	A3, B64	DQ7	++	Diffuse	0
12[1]	SB	15	Neg	Neg	A2, B50	DQ8, DR53, DR4	+	Diffuse	5
		112			B7, B50[4]		+	Focal	
13	SB	41	1:256	1:8	A3, BW4, B53	DR18	+++	Diffuse	2
14	MMV	84	1:4	1:1	A25, B14, B18	None	++	Diffuse	1
15	SB	140	Neg	Neg	A2[4]	A28, B78, A30, DQ7, 9	+	Diffuse	1
16	SB	162	1:2	1:2	A24, B44[4]	DQ1, CW5	++	Diffuse	2
17[2]	SB	4	Neg	Neg	A28, B78, A30, DQ7, 9	B44, B58, DR4	+++	Diffuse	2
18[3]	MMV	18	1:1	1:8	A11, B7, DR12, DR17, DQ2	None	++	Diffuse	1

Type of transplant: SB: An isolated small bowel; MMV: A modified multivisceral graft; MV: A full multivisceral graft; P: Pancreas; K: Kidney. [1]Patients with repeat ABMR; [2]Patient with a history of ABMR after prior transplant; [3]Patient with prior transplant; [4]DSA detected at time of rejection. POD: ABMR days post-transplant; XM: Cross-match; DSA: Donor-specific antibody; ACR: Acute cellular rejection.

Table 3 Treatment and outcome of 18 patients with acute antibody-mediated rejection

Case	Treatment	Graft status/survival (mo)	Re-Tx/graft type	Patient status/survival (mo)
1	ST/IVIG/OKT3	CHR/30.5	None	Dead (liver failure)/30.5
2	ST/IVIG/OKT3/Campath	CHR/13.5	None	Dead (ruptured pseudo-aneurysm)/18.6
3	ST/OKT3	Functioning/75.9	None	Alive/75.9
4[1]	ST/OKT3	CHR/5.4	Yes/MV	Dead (pneumonia)/43.0
5	ST/OKT3	Functioning/17.7	None	Alive/17.7
6	ST/OKT3	Functioning/56.4	None	Alive/56.4
7	ST/OKT3/Campath	ACR/31.7	None	Dead (pneumonia)/31.7
8	ST/Campath	Functioning/30.3	None	Dead (unknown)/30.3
9	ST	CHR/35.4	Yes/MV	Alive/55.4
10[1]	ST/OKT3	ACR/13.2	None	Dead (sepsis)/15.5
11	ST	Functioning/52.6	None	Alive/52.6
12[1]	ST/ATG	CHR/22.6	None	Alive/22.6
13	ST	AHR/2.7	Yes/MV	Alive/76.4
14	ST	Functioning/22.5	None	Alive/22.5
15	ST/OKT3	CHR/4.8	None	Dead (sepsis)/32.8
16	ST	CHR/12.3	None	Alive/46.2
17[2]	ST/OKT3	CHR/12.6	Yes/SB	Dead (GI bleeding)/13.3
18[3]	ST/OKT3	Functioning/37.6	Yes/MV	Alive/37.6

Type of transplant: SB: An isolated small bowel; MV: A full multivisceral graft. [1]Patients with repeat ABMR; [2]Patient with a history of ABMR after prior transplant; [3]Patient with prior transplant. ST: Steroids; IVIG: Intravenous immunoglobulin; ACR: Acute cellular rejection; CHR: Chronic rejection.

21.6% (16 of 74) in the cases with ACR ($P < 0.001$). Graft failure occurred in 12 (66.7%) of the 18 patients with acute ABMR. The causes of graft loss were chronic rejection in 8 cases, severe ACR in 2, persistent ABMR in 1, and unknown etiology in 1 (Table 3). Nine cases underwent enterectomy due to rejection. Of those, *de novo* DSA was detectable in 7 cases prior to enterectomy and was persistent after graft removal. Two cases had undetectable levels of *de novo* DSA by the ELISA assay before enterectomy but became detectable after enterectomy. The presence of *a* newly-formed DSA was closely associated with graft loss ($P < 0.0001$). Compared with no rejectors, intestinal graft survival was significantly lower in patients with acute ABMR ($P = 0.0001$) or ACR ($P = 0.0009$). Graft survival was lower in acute ABMR than in ACR but the differences between them did not reach statistical significance ($P = 0.088$) (Figure 3A). Patient survival was worse in acute ABMR

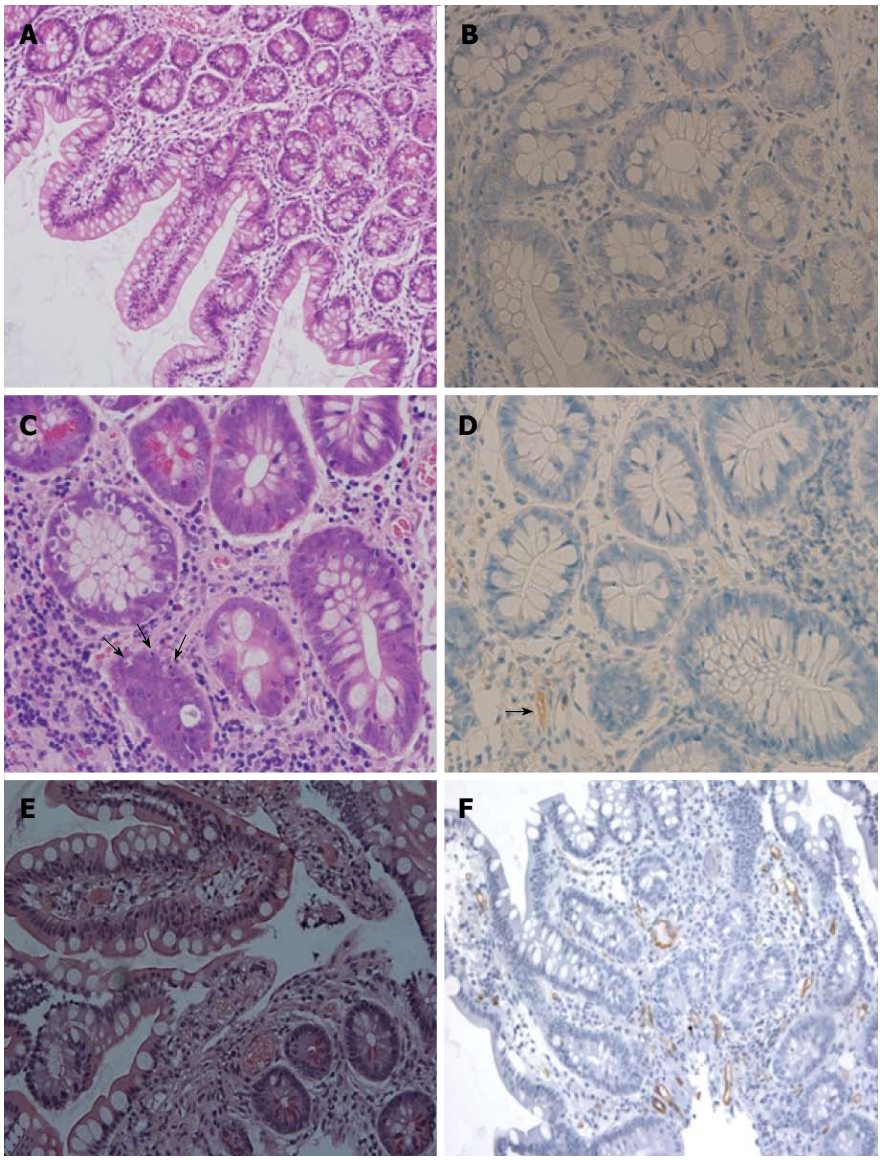

Figure 2 Histopatholgy of intestinal allograft. A and B: No rejection: normal mucosal architecture of small bowel biopsy after transplantation. No staining for C4d is seen in the capillaries of the lamina propria; C and D: Acute cellular rejection (ACR): There is mononuclear infiltration, crypt epithelial injury, and apoptotic bodies (arrows) in the lamina propria. Weak and focal staining for C4d (arrows) is sometimes present in a patient with ACR; E and F: Acute antibody-mediated rejection (ABMR): There is prominent hemorrhage and congestion with scattered fibrin thrombin in the lamina propria. Widespread and bright staining for C4d is present in the capillaries of the lamina propria. Magnifications: × 200 in A, E and F; × 400 in B, C and D. A, C, E: H and E; B, D, F: C4d.

or ACR than in no rejectors (P = 0.0264). There were no statistical differences in patient survival between ABMR and ACR (Figure 3B).

A total of five patients underwent repeat transplants, including a liver-inclusive graft in 4 and a liver-free intestinal graft in 1 patient. Of those, three patients had acute ABMR after primary transplantation and two patients had acute ABMR after retransplant. Four patients underwent repeat transplants after a diagnosis of acute ABMR and one with prior history of a liver-free graft developed acute ABMR after a liver-inclusive retransplant.

At an average follow-up of 32.3 mo (range, 13.3-76.4 mo), eight of the 18 (44.4%) patients died. The causes of patient death were sepsis in 4, massive gastrointestinal bleeding in 2, chronic liver failure in 1 and unknown etiology in 1 (Table 3). Patient 1 with a positive CDC XM developed acute ABMR in the intestinal allograft 4 d after a liver-inclusive intestine combined with kidney transplant. He responded well with a combination of steroids, IVIG and OKT3 therapies. One year after transplantation, he had progressively elevated liver enzymes with circulating *de novo* DSA and he subsequently died due to chronic liver failure. In three patients undergoing liver-inclusive retransplants after primary graft loss, one patient died secondary to Aspergillus pneumonia, while the other three patients were alive with a well-functioning graft at the time of last follow-up. Two patients (#17 and #18) with a prior history of primary graft loss due to rejection developed acute ABMR after retransplantation. Patient 17 had acute ABMR 4 d after a liver-free retransplant and soon developed chronic rejection within a year with persistent *de novo* DSA and subsequently died due to massive lower GI bleeding. Patient 18 had acute ABMR 18 d after liver-inclusive retransplant, which was successfully treated with steroids and OKT3. The higher levels of preformed DSA gradually declined in this case after transplantation and she was well with functioning graft with no evidence of *de novo* DSA by the Luminex assay at the time of the last follow-up.

Risk factors for acute ABMR

In the univariate analysis, younger recipients at the time

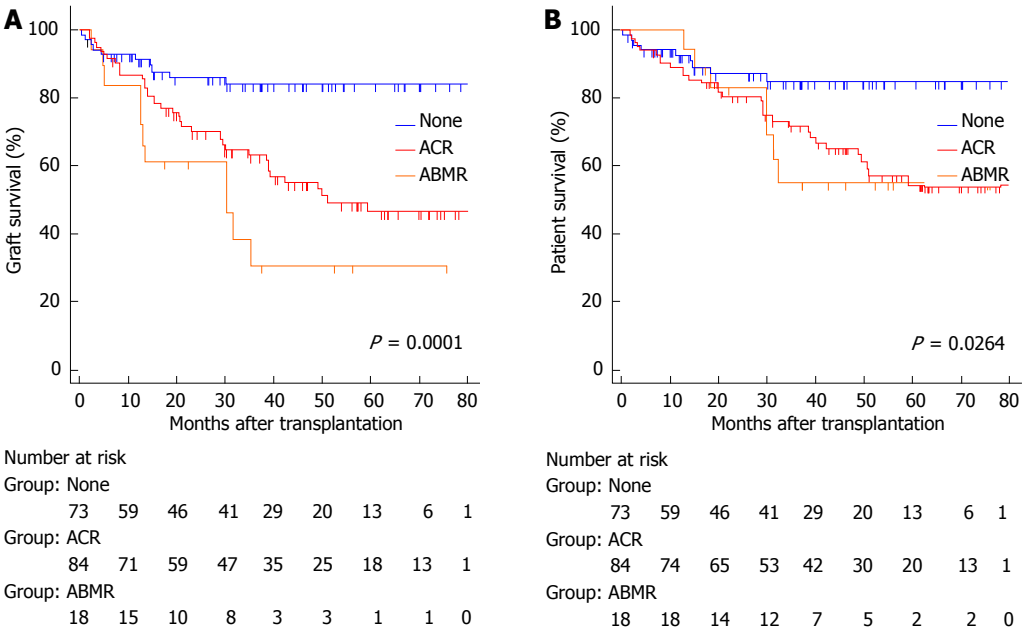

Figure 3 The Kaplan-Meier graft (A) and patient (B) survival for no acute rejection (none) (solid line), acute cellular rejection (dotted heavy line), and acute antibody-mediated rejection (dotted light line). The overall comparison was significantly different in graft (log-rank $P = 0.0001$) and patient survival (log-rank $P = 0.0264$). The patients with antibody-mediated rejection (ABMR) or acute cellular rejection (ACR) had significantly lower graft and patient survival than those without rejection. The graft survival was worse in ABMR than in ACR but the differences between them did not reach statistical significance ($P = 0.088$).

of transplant, liver-free graft, and the presence of DSA were closely related to acute ABMR. The presence of the spleen in recipients tended to be associated with acute ABMR but no statistical significance was observed ($P = 0.071$) (Table 4). In the multivariate analysis, only the presence of DSA and a liver-free graft were significantly associated with the development of acute ABMR (Table 5). Donor age, gender, cold ischemic time, cytomegalovirus donor and recipient serology, HLA mismatches, previous transplants, and induction therapy were not significantly associated with acute ABMR, and their inclusion in the multivariate model did not exclude liver-free grafts or the presence of DSA as independent risk factors.

DISCUSSION

We believe that the established diagnostic standards for acute ABMR in kidney or heart transplants can help identify acute ABMR after ITx. We found the incidence of acute ABMR of the intestinal allograft is 10.3%, on the basis of the presence of circulating DSA, evidence of C4d deposit, acute tissue injury, and clinical graft dysfunction. To our knowledge, this is the first largest series of investigation to date to retrospectively assess the incidence of acute ABMR after ITx. The most important finding in this study was that acute ABMR is closely associated with increased graft loss and poor outcomes. Our rate of intestinal graft acute ABMR is comparable to the incidence of acute ABMR reported in the kidney transplant that ranges from 7.7% to 41%, depending on the level of pre-transplant recipient sensitization status[9,26,27]. Given the frequency and poor prognosis of acute ABMR after ITx, every effort should be made to set up or eliminate this diagnosis in the setting of graft dysfunction to more specifically direct immunosuppressive management.

C4d deposition along graft capillaries has become a critical component to the diagnosis of acute AB MR in a kidney or heart transplant. However, the clinical relevance of a positive C4d staining in an intestinal allograft remains uncertain. Post-transplant microvascular lesions in a small intestinal allograft at early time periods might be related to higher pre-transplant PRA levels or a positive CDC-XM[7]. Other studies concluded that C4d deposition had no clinical significance when assessing acute ABMR in a small bowel allograft[28,29]. Unfortunately these studies either did not correlate with the HLA antibody levels or they did not detect DSA in small-sized heterogeneous populations. Therefore, the above studies did not have sufficient evidence to include or exclude C4d as a useful marker to detect acute ABMR in intestinal allografts. Our previous publications showed that a diffuse C4d deposition was very common in CDC-XM positive recipients with the presence of DSA, while focal and trace C4d deposition was often seen in CDC-XM negative recipients in the setting of no histological evidence of ACR or evidence of ACR but in absence of DSA[18]. Our current study further demonstrates that a diffuse C4d deposition is strongly associated with vascular disturbances after ITx, indicating that it is a useful marker for a diagnosis of ABMR after ITx. Our results suggest that a diffuse C4d staining, in conjunction with the presence of DSA, clinicopathological findings and significant clinical improvement after initial treatment, strongly supports a diagnosis of acute ABMR. The clinical relevance of focal and weak C4d staining in an intestinal

Table 4 Pretransplant risk factors for acute antibody-mediated rejection (univariate analysis)

Variables	Non-ABMR (n = 157)	ABMR (n = 18)	OR	95%CI	P
Donor age (yr)	25.6 ± 10.2	24.0 ± 6.5	0.98	0.93-1.04	0.549
Female donor, n (%)	33 (20.8)	6 (37.5)	0.44	0.15-1.29	0.133
Cold ischemic time (h)	7.72 ± 1.52	7.58 ± 1.11	0.94	0.66-1.33	0.711
Recipient age	43.7 ± 12.4	36.9 ± 12.0	0.96	0.92-0.99	0.028
Female recipient, n (%)	94 (59.8)	13 (72.2)	0.57	0.19-1.69	0.299
Donor CMV positive/recipient negative, n (%)	34 (21.4)	4 (25.0)	1.12	0.69-1.81	0.642
Donor/recipient sex mismatches, n (%)	88 (55.3)	11 (68.7)	1.78	0.59-5.35	0.308
HLA mismatches ⩾ 4, n (%)	107 (67.3)	12 (75.0)	1.46	0.45-4.74	0.531
Prior transplant, n (%)	9 (5.7)	2 (12.5)	2.38	0.47-2.12	0.296
Campath-1H induction, n (%)	113 (71.1)	12 (75.0)	0.72	0.30-1.73	0.468
Liver-free graft, n (%)	92 (58.5)	15 (83.3)	3.53	1.08-12.7	0.031
Presence of spleen, n (%)	99 (63.1)	15 (83.3)	2.93	0.81-10.55	0.071
Anti-HLA antibodies					
Positive CDC-XM, n (%)	30 (19.1)	14 (77.8)	21.17	5.76-77.81	< 0.0001
PRA Ⅰ ⩾ 10%, n (%)	53 (33.8)	18 (100)	33.36	4.32-257.52	< 0.0001
PRA Ⅱ ⩾ 10%, n (%)	32 (20.4)	14 (77.8)	13.67	4.21-44.36	< 0.0001
Presence of DSA, n (%)	37 (23.6)	18 (100)	55.14	7.09-428.38	< 0.0001

ABMR: Antibody-mediated rejection; CMV: Cytomegalovirus; CDC-XM: Complement-dependent lymphocytotoxic cross-match; PRA: Panel reactive antibody; DSA: Donor-specific antibody.

Table 5 Pretransplant risk factors for acute antibody-mediated rejection (multivariate analysis)

Variables	OR	95%CI	P
Liver-free graft	8.791	2.011-38.480	0.004
PRA class Ⅰ	16.302	3.092-85.801	0.001
PRA class Ⅱ	6.023	1.490-24.253	0.012

PRA: Panel reactive antibody.

allograft should be further evaluated in future studies. We suggest that a C4d staining be routinely included in intestinal biopsies in sensitized recipients or in the setting o f appearance of a newly-formed DSA after ITx.

The rate of pretransplant sensitization in our current study was 30.3%, higher than 10%-15% in kidney or heart transplant recipients, indicating that intestinal recipients are an high immunological risk group. The causes of sensitization may be from previous operations, multiple blood transfusions, infections, pregnancies or retransplantation. Sensitization increases the risk of a positive CDC-XM and is associated with rejection and poor outcomes. Our results further showed that a positive CDC-XM significantly increases the risk of acute ABMR and is closely associated with graft loss, particularly in a liver-free transplant recipient. In the setting of anti-donor antibody titer ⩾ 1:8, all four recipients lost grafts early on after a liver-free transplant. Similar to other solid organ transplants, our findings confirmed a close association between preformed DSA and acute ABMR, indicating that preformed DSA is a prerequisite for the occurrence of acute ABMR after ITx. In our series the majority of patients had preformed class Ⅰ DSA prior to transplantation, often directed at the A, B locus, whereas the majority of *de novo* DSA post-transplant were against class Ⅱ, which were often associated with late graft failure. The mechanisms underlying the difference between class Ⅰ and class Ⅱ in this clinical setting is unknown. Based on our results, we suggest special attention should be paid to recipients with high immunological risk in terms of implementing pre-transplant desensitization strategies, avoiding positive cross-match transplantation, increasing maintenance immunosuppression, and frequently monitoring DSA post-transplant.

In our series, younger recipient age was associated with acute ABMR, but this significance no longer exist when we adjusted for other factors. Our analysis identified a liver-free transplant as an independent risk factor for the occurrence of acute ABMR after ITx. Our previous paper demonstrated that the liver is relatively insensitive to antibody-mediated damage and the inclusion of a liver graft with the intestine appears to be protective in recipients of high immunological risk[30]. In contract to a liver-free graft, no or only a single mild episode of rejection occurred in three highly sensitized recipients after a liver-inclusive transplant. Our findings further confirmed that the liver as a component of multivisceral transplants might ameliorate or prevent early and late intestinal allograft loss. A liver-inclusive transplant may offer a better long-term patient and graft survival in immunological high-risk recipients. As optimized approaches for depleting HLA antibodies have not yet been set up in ITx, the use of a liver-inclusive graft may be a valuable option in highly sensitized recipients, especially in the setting of retransplantation after primary graft loss due to rejection.

Despite the initial clinical improvement in many patients, long-term outcomes were dismal because of a high incidence of chronic rejection. In this series, although a combination of steroids and T-cell targeted OKT3 achieved the initial resolution after a diagnosis of acute ABMR, the majority of grafts failed due to subsequent severe ACR or chronic rejection. Clearly, additional studies are required to identify effective strategies to control acute ABMR. The antibody-directed regimens, such as

IVIG and anti-CD20, should be routinely implemented in highly sensitized recipients prior to transplantation. A new therapy, such as the proteasome inhibitor "bortezomab" and complement-targeted treatment with C1 or C5 inhibitor, has yielded encouraging preliminary results, but the long-term efficacy and safety remain to be seen.

Our study has several important limitations, including its retrospective nature, inconsistent antibody detection methods, and experience at a single institution. Although we identified 18 cases among 175 transplants over a 7-year period, the true incidence of acute ABMR may be higher after ITx. It is likely that less severe cases were unrecognized due to our evolving antibody detection methods, lack of standardized definition, and our unawareness of the importance of acute ABMR during the early study period. An additional important limitation of this study is that C4d staining of biopsies was not routinely performed in recipients with ACR to evaluate its sensitivity and specificity. Furthermore, the lack of consistent DSA monitoring post-transplantation limits our ability to assess sub-clinical acute ABMR. However, we sought to characterize a convincing series of cases to develop a preliminary definition that may serve as a foundation for future studies.

Our results indicate that acute ABMR is an important cause of intestinal graft dysfunction, particularly in a liver-exclusive transplant. After acute ABMR, patients are at an increased risk of developing refractory acute rejection and chronic rejection. Preventive and effective therapeutic approaches are needed to manage acute ABMR in intestinal transplant recipients.

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COMMENTS

Background

Antibody-mediated rejection (ABMR) is the major cause of kidney transplant failure. The incidence and clinical significance of acute ABMR after intestinal transplantation (IATx) remain unknown.

Research frontiers

ABMR has increasingly become an important area for research and clinical investigation. The aim of this study aimed to assess the incidence, risk factors and clinical outcomes of acute ABMR after ITx.

Innovations and breakthroughs

This is the first largest series of study to retrospectively examine the incidence of acute ABMR after ITx. The incidence of acute ABMR after ITx is 10.3% in the series. Both a liver-free graft and a high level of panel reactive antibody were identified as a significant independent risk factor for acute ABMR. Without appropriate management, acute ABMR was closely associated with increased graft loss and poor clinical outcomes.

Applications

The results suggest that acute ABMR must be included in the differential diagnosis of acute rejection after ITx. The prevention of acute ABMR should include desensitization, avoiding a positive cross-match donor, and considering the liver as part of an intestinal graft in highly sensitized recipients. Future studies are required to develop the optimal approaches to managing acute ABMR in ITx recipients.

Terminology

Acute ABMR is identified on the basis of circulating donor-specific antibody, C4d deposition, tissue injury and clinical allograft dysfunction after ITx.

Peer-review

This is a well written, well designed retrospective review study on acute ABMR after intestinal transplantion, put through an electronic analysis of medical records of 18 patients diagnosed with ABMR out of 175 liver-free small bowel and modified multivisceral graft transplantations, during a 7-year period.

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ORIGINAL ARTICLE

Observational Study

Persistent Epstein-Barr viral load in Epstein-Barr viral naïve pediatric heart transplant recipients: Risk of late-onset post-transplant lymphoproliferative disease

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Abstract

AIM

To examine the risk of late-onset post-transplant lymphoproliferative disorder (PTLD) in the presence of persisting high Epstein-Barr virus (EBV) in EBV naïve pediatric heart transplant (HT) recipients.

METHODS

A retrospective review of the medical records of the 145 pediatric HT recipients who had serial EBV viral load monitoring at our center was performed. We defined EBV naive patients whose EBV serology either IgM or IgG in the blood were negative at the time of HT and excluded passive transmission from mother to child in subjects less than 6 mo of age.

RESULTS

PTLD was diagnosed in 8 out of 145 patients (5.5%); 6/91 (6.5%) in those who were EBV seropositive and 2/54 (3.7%) in the EBV naïve group at the time of HT (P = 0.71). We found 32/145 (22%) patients with persistently high EBV load during continuing follow-up; 20/91 (22%) in EBV seropositive group *vs* 12/54 (22%) in EBV naïve group (P = 0.97). There was no significant association between pre-HT serostatus and EBV load after transplant (P > 0.05). In the EBV seropositive group, PTLD was diagnosed in 15% (3/20) of patients with high EBV *vs* 4.2% (3/71) of patients with low or undetectable EBV load (P = 0.14) whereas in EBV naïve patients 8.3% (1/12) of those with

high EBV load and 2.3% (1/42) with low or undetectable EBV load (P = 0.41). There was a highly significant association between occurrence of PTLD in those with high EBV load and duration of follow up (4.3 ± 3.9 years) after HT by Cochran-Armitage test for the entire cohort (P = 0.005). At least one episode of acute rejection occurred in 72% (23/32) of patients with high EBV *vs* 36% (41/113) patients with low or undetectable EBV after HT (P < 0.05).

CONCLUSION

There is an association between persistently high EBV load during post-HT follow up and the occurrence of late-onset PTLD in pediatric HT recipients irrespective of serostatus at the time of transplant. The occurrence of allograft rejection increased in patients with high EBV load presumably due to reduction in immunosuppression.

Key words: Pediatric heart transplantation; Epstein-Barr virus; Post-transplant lymphoproliferative disorder; Immunosuppression; Allograft rejection

Core tip: Post-transplant lymphoproliferative disorder (PTLD) after heart transplantation is a severe complication where there is still limited information is available. There are many publications on estimations of PTLD frequency in different settings and types of patient, as well as the factors associated with its appearance and prognosis. But, most studies do not take into account the length of follow-up which may be misleading given that patients are exposed to the risk of immunosuppression over a long period of follow-up. This study is unique that, it is a single center study span over a period of 18 years in which maintenance immunosuppression therapy and management of rejection episodes remained same throughout. Although, a single center study result cannot be generalized, however it adds to the existing literature for risk stratification of these patients based on whole blood Epstein-Barr virus (EBV) polymerase chain reaction (PCR) after accounting for the time since transplant and patients' pre-transplant EBV serostatus. This paper also highlights the risk of acute rejection after reduction or alteration in immunosuppression in patients with high EBV load by PCR without any effect on the occurrence of PTLD.

Das B, Morrow R, Huang R, Fixler D. Persistent Epstein-Barr viral load in Epstein-Barr viral naïve pediatric heart transplant recipients: Risk of late-onset post-transplant lymphoproliferative disease. *World J Transplant* 2016; 6(4): 729-735 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i4/729.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i4.729

INTRODUCTION

Post-transplant lymphoproliferative disorder (PTLD) is the most common malignancy occurring in 3.5%-9% of pediatric HT recipients[1-4]. It is characterized by uncontrolled proliferation of lymphoid lineage cells, the vast majority of which are B-cell lymphomas, in a context of posttransplant immunosuppression. In some situations, reducing the immunosuppression can reverse the proliferation, thus differentiating it somewhat from truly irreversible malignancies. Most but not all PTLD cases have a strong relationship with Epstein-Barr virus (EBV).

The development of PTLD is influenced by a variety of factors including the type, intensity, and cumulative amount of immunosuppression, and the EBV status of the donor and recipient. Children are at greatest risk for the development of PTLD since they are often seronegative at transplant and acquire a primary EBV infection post-heart transplant (HT) in the setting of immunosuppression. Nevertheless, the factors that account for whether or not a particular child develops EBV-associated PTLD are undetermined. Diagnosis and effective treatment of PTLD is hampered by our inability to determine which children are at risk of developing EBV-associated PTLD.

The onset of PTLD is usually preceded by an elevated EBV load in the peripheral blood which is highly sensitive but not a specific marker for development of PTLD in renal transplant recipients[5,6]. Routine long-term post-HT EBV monitoring identifies a group of children who carry persistent viral loads for months to years after solid organ transplants[5-8]. Patients with a persistently elevated level of circulating EBV may have an increased risk of PTLD[2,9]. Previously, a single center study reported that a high EBV load did not predict PTLD in early post-heart and heart-lung transplant period[10]. However, another single center study suggested that exposure to EBV and higher intensity immunosuppression was associated with increased risk of PTLD in pediatric HT recipients[11]. A more recent study has shown that early onset PTLD in solid organ transplant recipients appears mainly as an EBV-driven disease especially favored by insufficient immunosurveillance[12,13]. These contradictory findings leave the long-term clinical significance of chronic high EBV load unknown. We hypothesized that patients with persistently high EBV viral load at any time after their transplant in the setting of immunosuppression may be at increased risk for development of PTLD.

The objective of the study was to examine the risk of late-onset PTLD (> 1 year post-transplant) in the presence of persisting high EBV and to determine whether patients' serostatus at the time of HT changed the risk.

MATERIALS AND METHODS

All pediatric HT patients transplanted between 1995 and 2013 who had known EBV serology at the time of transplant were included in this retrospective descriptive study. For this study, we defined EBV naive patients

whose EBV serology either IgM or IgG in the blood were negative at the time of HT and excluded passive transmission from mother to child in subjects less than 6 mo of age. Data collection included demographics, clinical data, pre-HT EBV serological status, serial post-HT EBV load, diagnosis of PTLD and acute rejection episodes during post-HT follow-up. The presence of EBV virus in whole blood was measured by quantitative polymerase chain reaction (PCR) using a cut-off of 1000 copies/mL according to our institution protocol.

Viral (EBV) load testing was done in whole blood using PCR every 2 wk for 3 mo, every month for 3 mo, every 3 to 6 mo for a duration of 1 year after HT, and thereafter annually. Additional EBV PCR levels were drawn if EBV PCR was rising, with any increased immunosuppression for treatment of allograft rejection, or if clinically indicated by symptoms such as protracted fever, gastrointestinal symptoms, unexplained elevated liver enzymes, lymphadenopathy, tonsillar hypertrophy, obstructive sleep apnea, unexplained anemia, pancytopenia, atypical lymphocytes or eosinophilia, persistent headache or focal neurological symptoms.

Our protocol for follow-up of patients based upon EBV PCR positivity included: (1) EBV PCR < 1000 copies/mL: No change in immunosuppression, routine follow-up as per above protocol; (2) EBV PCR 1000-9999 copies/mL: No change in immunosuppression, repeat EBV PCR every 2 wk; and (3) EBV PCR ≥ 10000 copies/mL (> 2 consecutive tests and remains positive for > 12 mo): We reduced immunosuppression with a goal for tacrolimus trough level 3-5 ng/mL, cyclosporine trough level 50-75 ng/mL, and decreased mycophenolate mofetil/azathioprine dose to half of the initial dose and closely monitored for any signs of acute rejections. For this study analysis, we divided all patients into 3 groups based on EBV viral load as a continuous value anytime during post-TX follow-up: group Ⅰ: Negative EBV or EBV PCR < 1000 copies/mL; group Ⅱ: EBV PCR 1000-9999 copies/mL; and group Ⅲ: EBV PCR ≥ 10000 copies/mL (persistently positive for > one year). During follow-up, patients who had transiently increased EBV PCR in excess of 10000 but did not persist for a year were included in group Ⅱ.

PTLD was defined according to the 2008 World health Organization (WHO) classification system, but early lesions such as lymphoid hyperplasia with scattered positive in situ hybridization using EBV encoded RNA detected by the Epstein-Barr early region (EBER) immunostaining assay[14] was excluded for this study as PTLD. All biopsy proved polymorphic, monomorphic and classical Hodgkin lymphoma-type PTLD patients were evaluated by our oncology service and treatment was guided as per oncology protocol.

Acute Allograft rejection was defined as ISHLT grade 2R or higher or an episode of clinically significant decline in cardiac function treated with steroid bolus or anti-T cell therapies. Endomyocardial biopsy was performed per our institutional protocol for all patients and frequency of biopsy was not modified based on high EBV load or reduction of immunosuppression. However, patients whose immunosuppression was decreased as a result of high EBV load were monitored closely clinically and by echocardiogram for any graft dysfunction.

All patients received basiliximab (simulect) and methyl prednisone for induction at the time of transplant as per our institution protocol since 2001. Between 1995-2000, our induction therapy was only methyl prednisone. Maintenance immunosuppression includes triple therapy of tacrolium/cyclosporine, mycophenolate mofetil (MMF), and steroids. Steroids were withdrawn after one year routinely unless there are more than one rejection episode within first year after transplant. This study was approved by our institutional IRB.

Statistical analysis

Descriptive analyses of the continuous and categorical data were performed using mean, standard deviation, median, quartiles, frequency and proportion as appropriate. Fisher's exact test and χ^2 tests were used to test binary variables between two groups. Cochran-Armitage test and logistic regression were used to test the association between post-HT EBV load, duration of follow-up and incidence of PTLD. The statistical analyses were performed with SAS 9.3.

RESULTS

A total of 145 patients were followed from 1995 to 2013 for mean 4.3 ± 3.9 years (interquartile range 1.5 to 6.0 years) post-HT. Mean age at HT was 6.6 ± 6.3 years, median age 4.8 years with interquartile range 0.69 to 12.0 years. EBV was first detected at a median of 1 year (range 0.1 to 16 years) post-HT. Patients were then sub-grouped based on age at transplant into 0-6 mo, 6 mo to 1 year, 1-7 years and 7-20 years *vs* EBV load as shown in Table 1. The proportions of high EBV load are 38.8%, 27.4%, 18.4% and 15.4% in age group 0-6 mo, 6 mo to 1 year, 1-7 years and 7-20 years, respectively. Cochran-Armitage test with square root transformation to age and the logistic regression showed that patients' age at HT was negatively associated with high EBV load ($P = 0.03$), which means patients with younger age had high risk for high EBV during follow up. One year old was chosen as the threshold for younger patients. χ^2 test showed that patients 1 year old or younger were more likely to have high EBV during follow up than patients older than 1 year old ($P = 0.01$). The relative risk for developing high EBV load in patients having transplant at 1 year old or younger is 2.16 (95%CI: 1.19-3.92) over patients having transplant at older age irrespective of their pre-HT EBV serological status.

The clinical characteristics of individual PTLD patient are described in Table 2. All patients were treated by reducing immunosuppression; five patients received rituximab, two patients received chemotherapy and one patient received chemotherapy plus radiation therapy. Three patients underwent tumor resection and all patients survived the treatment of PTLD. One patient died two years after treatment of PTLD due to non-cardiac cause.

Table 1 Outcomes (post-transplant lymphoproliferative disorder and number of rejections) by Epstein-Barr virus serological status at heart transplant

	Group Ⅰ (EBV PCR negative or < 1000) (*n* = 66)	Group Ⅱ (EBV PCR 1000-9999) (*n* = 47)	Group Ⅲ (EBV PCR ⩾ 10000) (*n* = 32)
EBV Naïve at HT	29/66 (44%)	13/47 (28%)	12/32 (37%)
Age at transplant			
0 up to < 6 mo	9	10	12
6 mo < 1 yr	3	5	3
1 yr up to < 7 yr	28	8	8
⩾ 7 yr up to 20 yr	26	24	9
Post-HT Follow-up (yr)	4.5 ± 3.2	4.8 ± 4.2	4.6 ± 5.3
No of PTLD	1	3	4
No of total Rejections	40	42	48
Number of patients with ⩾ 1 episodes of rejections	19	22	23

EBV: Epstein-Barr virus; PCR: Polymerase chain reaction; HT: Heart transplant; PTLD: Post-transplant lymphoproliferative disorder.

Table 2 Characteristics of individual patient diagnosed with post-transplant lymphoproliferative disorder

Patient (gender)	Year of HT	HT to PTLD (yr)	EBV serology at HT	EBV load at PTLD	Organ involved in PTLD	CD20 positivity	EBER status of PTLD	Histological diagnosis	Treatment
1 (M)	1995	16	Positive	< 10000	Retroperitoneal lymph node	Neg	Neg	Hodgkin Lymphoma	Chemotherapy
2 (F)	1996	14	Positive	< 10000	Cervical Lymph node	Neg	Neg	Diffuse large B-cell lymphoma	Chemotherapy
3 (M)	1999	12	Positive	> 10000	Retroperitoneal lymph node	Pos	Neg	Polymorphic PTLD	Rituximab
4 (M)	2001	3	Positive	> 10000	Pharynx	Neg	Pos	Intermediate between Hodgkin and large cell lymphoma	Chemotherapy plus Radiation
5 (F)	2009	3	Negative	> 10000	Cervical Lymph node	Pos	Pos	Polymorphic PTLD	Rituximab
6 (F)	2000	14	Positive	< 1000	Brain-Temporal Lobe	Pos	Pos	Polymorphic PTLD	Rituximab
7 (M)	2000	3	Negative	< 10000	Small intestine	Pos	Pos	Polymorphic PTLD	Rituximab
8 (F)	2005	6	Positive	> 10000	Retroperitoneal lymph node	Pos	Pos	Large B-cell Lymphoma	Rituximab

HT: Heart transplant; Neg: Negative; Pos: Positive; EBER: Epstein-Barr virus encoded small RNA; PTLD: Post-transplant lymphoproliferative disorder.

Figure 1 describes the distribution of patients' EBV serological status at the time of HT, EBV viral load by PCR post-HT, and number of patients who developed PTLD for the entire cohort. Out of 145 patients, 54 (37%) were EBV seronegative and 91 (63%) were EBV seropositive at the time of transplant and 22% from each group developed persistently high EBV viral load during follow-up after HT (P = 0.97). There were 6 cases (6.4%) of PTLD in EBV seropositive group *vs* 2 cases (3.9%) in EBV naive group (P = 0.71). In the EBV seropositive group, PTLD was diagnosed in 15 % (3/20) of patients with persistently high EBV *vs* 4.2% (3/71) of patients with low or undetectable EBV load (P = 0.14) whereas in EBV naïve patients PTLD was diagnosed in 8.3% (1/12) who had persistently high EBV load and 2.3% (1/42) with low or undetectable EBV load (P = 0.41). There was no significant association between pre-HT serostatus and post-HT EBV viral load after transplant (P > 0.05).

For the entire cohort of 145 patients, we found 65/145 (44.8%) had negative or EBV < 1000 copies/mL (Group I), 48/145 (33%) had EBV load between 1000-9999 copies/mL (Group Ⅱ), and 32/145 (22%) patients had EBV load ⩾ 10000 copies/mL (Group Ⅲ) during follow-up irrespective of initial serological status at the time of transplant. PTLD was diagnosed in 8 out of 145 patients (5.5%) at a median of 4.4 years (mean 7.5 ± 6.5 years, interquartile range 2.7 to 13.7 years) after heart HT. PTLD was diagnosed in 12.5% (4/32) of patients with persistently high EBV *vs* 3.5% (4/113) of patients with low or undetectable EBV load (P = 0.07 by Fisher's exact test). High viral load could predict PTLD with sensitivity 50% (95%CI: 15.7%-84.30%), specificity 79.66% (95%CI: 71.8%-85.97%), positive likelihood ratio 2.45 (95%CI: 1.14-5.27), negative likelihood ratio 0.63 (95%CI: 0.31-1.26) and positive predictive value 12.5% (95%CI: 3.51%-2.88%). There is a significant association between persistently high EBV load during a sum of follow up over 11 ± 7 years after HT and the occurrence of PTLD by Cochran-Armitage test (P = 0.005).

There was at least one episode of acute rejection

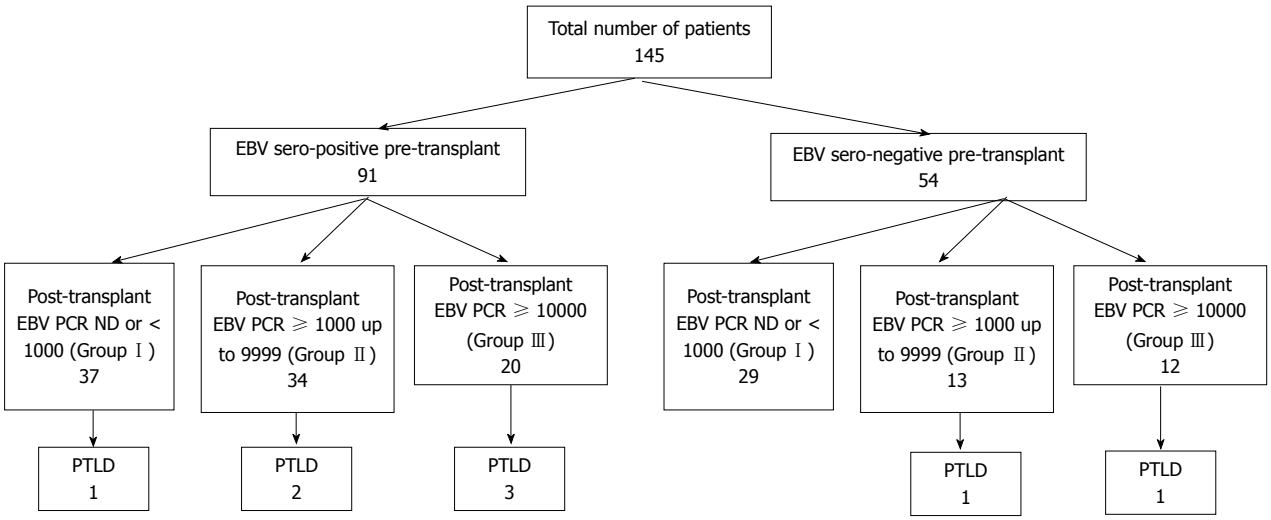

Figure 1 Patients' Epstein-Barr virus serostatus pre-heart transplant, post-transplant Epstein-Barr virus viral load as determined by whole blood polymerase chain reaction and incidence of post-transplant lymphoproliferative disorder for the entire cohort. EBV: Epstein-Barr virus; PTLD: Post-transplant lymphoproliferative disorder; PCR: Polymerase chain reaction.

(Grade 2R) in 23 patients with high EBV load after reduction of their immunosuppression (Table 1). On the other hand, 41 patients with low or negative EBV load who had no change in their immunosuppression had at least one episode of rejection. Thus, a larger proportion of patients 72% (23/32) with persistently high EBV load had acute rejections *vs* 36% (41/113) patients with low or negative EBV load ($P < 0.05$). Furthermore, there was an increase in frequency of total rejection episodes in patients with persistently high EBV load by 150% (48/32) *vs* 72.5% (82/113) in patients with low or negative EBV load ($P < 0.05$).

DISCUSSION

The incidence of PTLD in our study at 5.5% is similar with other series reported[1]. The occurrence of PTLD is dependent on the transplanted organ type and patient-specific risk factors. The strongest risk factor for PTLD is the development of primary EBV infection after transplantation[2,12,15,16]. Schubert *et al*[2] have reported 8.2% incidence of PTLD in pediatric HT recipients and the EBV association was 83% as a risk factor for development of PTLD. EBV monitoring in peripheral blood using PCR has been reported to have variable sensitivity and lack of specificity as an indicator of risk for developing PTLD[5,10,17-19]. Among pediatric HT recipients studied by Bingler *et al*[20] those with high EBV load were more likely to develop late-onset PTLD, occurring as long as 8.4 years after HT. In this study, we showed that patients who underwent HT at younger age (Table 1) are at higher risk for development of high EBV load over time ($P = 0.05$) irrespective their serological status at the time of HT. This observation is of clinical importance because many potential risk factors for development of PTLD such as persistent EBV viremia and overall immunosuppression are a function of duration of follow-up and may not be observed in early post-HT period. The occurrence of PTLD is highest in younger patients; age may not be an independent risk factor but may depend upon the likelihood of the recipient being exposed to long-term immunosuppression.

One of the limitations of the current study is the fact that we had incomplete data on the donor EBV status. Therefore, we could not determine whether high EBV load was the result of primary infection derived from community exposure or related to donor transmission. Asymptomatic high EBV load also predicts other adverse outcomes, such as graft dysfunction or acute rejection[20,21]. Jabs *et al*[21] showed that EBV viremia occurring immediately after renal transplant was associated with subsequent rejection episodes, and they speculate that T cell responses to viral infection might cross-react with the graft. In another study, Smith *et al*[22] have showed that subclinical cytomegalovirus and EBV viremia occurring in the early post-transplant period was associated with higher incidence of allograft injury. The authors did not find evidence of significant viral replication in the renal allograft at 2 years after transplant, suggesting that graft dysfunction is not related to chronic infection[22]. We found a higher rate of rejection episodes (mostly grade 2R) in pediatric HT recipients with persistently high EBV load compared to those patients with low or negative EBV PCR. The mechanisms of rejection are not clear from this study but may include viral cytopathic effects, increased expression of alloantigen, adhesion molecule expression by endothelial cells, or indirect inflammatory effects due to cytokine release, or a combination of multiple mechanisms leading to allograft injury.

In our practice, we do reduce maintenance immunosuppression in patients who have persistently raised EBV PCR ⩾ 10000 copies/mL of whole blood. A link between EBV load and level of immunosuppression in adult HT patients was noted[23]. We hypothesize that reduction of immunosuppression has probably contributed for higher allograft rejection episodes in patients with high

EBV load. Therefore, we recommend close monitoring for allograft rejection must be done after reduction of immunosuppressive therapy.

In this study, we have used methyl prednisone as induction therapy from 1995 through 2000 and basiliximab and methyl-prednisone as induction therapy from 2001 through 2013. Our standard maintenance immunosuppression (tacrolimus/cyclosporine, MMF or azathioprine and steroids) and consistent decrease in immunosuppression strategy in response to a high EBV support to the notion that it is overall immunosuppression exposure during the life time of the patient which compromises anti-tumor and anti-viral immunosurveillance capacity and thus facilitate development of PTLD. We have not used sirolimus or evorlimus routinely and we cannot comment regarding the effect of proliferation signal inhibitors on EBV PCR or PTLD from this study.

This study must be viewed in light of some limitations. It was a single-center retrospective study and thus findings may not be generalizable. Some patients were transferred to another center and also transitioned to an adult HT program, thus complete follow-up data for a small portion of patients were not available. However, this is a well-studied patient population in which maintenance immunosuppression therapy and management of rejection episodes remained same throughout and we followed our standardized institutional protocol strictly.

In conclusion, there is an association between persistently high EBV load and the occurrence of late-onset PTLD in pediatric HT recipients especially considering cumulative incidences at different lengths of follow-up. Patients $\leqslant$ 1 year of age at the time of HT are more likely to have persistently high EBV PCR during follow up than patients > 1 year of age at the time of transplant irrespective of their EBV serological status. Reduction of immunosuppression in the face of persistently high EBV load did not change the proportions of patient who had late-onset PTLD but did increase the risk of allograft rejection significantly. Based on our findings, there is a need for research to better determine other factors that might be predictive of PTLD. Currently, there is a multicenter study sponsored by National Institute of Allergy and Infectious Diseases examining the role of viral (EBV) and immunological biomarker associated with development of PTLD after transplantation[24]. This study will provide further insight to identify surrogate markers that can predict development of PTLD.

COMMENTS

Background

Post-transplant lymphoproliferative disorder (PTLD) is a significant complication after heart transplantation. Most but not all PTLD cases have a strong relationship with Epstein-Barr virus (EBV). This condition straddles the disciplines of transplantation, immunology, oncology, and virology. PTLD presents significant problems for the clinician because it is difficult to predict and has high morbidity and mortality rates. In addition, it has the potential for graft loss due to disease itself or the need to reduce immunosuppression, which increases the risk of graft rejection.

Research frontiers

The goal of this study is to review a single center experience of late-onset PTLD in the presence of persisting high EBV in pediatric heart transplant recipients.

Innovations and breakthroughs

This study showed that there is an association between persistently high EBV load during post-transplant follow up and the occurrence of late-onset PTLD in pediatric heart transplant recipients. The occurrence of PTLD is highest in younger patients; age may not be an independent risk factor but may depend upon the likelihood of the recipient being exposed to long-term immunosuppression. The incidence of allograft rejection increased in patients with high EBV load presumably due to reduction in immunosuppression.

Applications

Late-onset PTLD is less likely to be associated with patients' EBV serostatus at the time of transplant. Also, late-onset PTLD may be more likely extra-nodal and heterogeneous.

Terminology

Detection of persistently high EBV viral DNA by polymerase chain reaction (PCR) from the peripheral blood is associated with late-onset PTLD in pediatric heart transplant recipients.

Peer-review

The research is methodological well performed, clearly written, and the data is honestly presented.

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ORIGINAL ARTICLE

Observational Study

Psychological perspective of medication adherence in transplantation

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Abstract

AIM

To identify the risk factors and the post-transplant psychological symptoms that affect adherence to therapy in a population of kidney transplant recipients.

METHODS

The study examined the psychological variables likely responsible for the non-adherent behavior using a psychological-psychiatric assessment, evaluation of the perception of patients' health status, and an interview regarding the anti-rejection drug therapy assumption. The study included 74 kidney transplant recipients.

RESULTS

Individuals with a higher level of education and more years since transplantation showed better mental balance. Regarding gender, women appeared to be less adherent to therapy. Further, the years since transplantation adversely affected the proper pharmacological assumption. Adherence to therapy did not significantly change with the mental health index.

CONCLUSION

The biopsychosocial illness model provides a conceptual

frame of reference in which biological, psychological, and social aspects take on the same importance in the adherence to treatment protocols. For effective management, it is necessary to understand the patients' personal experiences, their assumptions about the disease, health status perception, and mood, and to identify any "barriers" that could cause them to become noncompliant.

Key words: Transplantation; Adherence; Mental health; Psychological assessment; Psychiatric assessment

Core tip: Therapeutic Adherence after transplantation is of fundamental importance for the patient's short- and long-term well-being and assumes a set of adaptations to a new lifestyle. The authors in this study analyzed the psychological characteristics of a sample of transplant recipients and different temperament styles, yet not studied in other research on transplantation. The results suggested that different temperaments influence in different ways the treatment compliance and showed that the transplant experience change behaviors and quality of life based on the personality and temperament characteristics. In conclusion, post-transplant psychological support positively affects adherence to treatment, and coping strategies of the subject.

De Pasquale C, Veroux M, Fornaro M, Sinagra N, Basile G, Gozzo C, Santini R, Costa A, Pistorio ML. Psychological perspective of medication adherence in transplantation. *World J Transplant* 2016; 6(4): 736-742 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i4/736.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i4.736

INTRODUCTION

The theme of therapeutic adherence (TA) plays a central role in research on education and health promotion[1]. Adherence to immunosuppressive therapy after transplantation is of fundamental importance for the patients' well-being both short- and long-term and assumes a set of adaptations to a new lifestyle. The treatment effectiveness and transplant success not only depend on the correct choice of immunosuppressive drugs, but also on the patients' active participation in the therapeutic program that often includes psychological support and appropriate motivation[2,3].

Non-adherence to therapy in transplant patients is one of the emerging causes of early and late graft loss. Patients with an organ transplant must take immunosuppressive drugs daily for the prevention of acute and chronic rejection. There is an obvious relationship between the discontinuity in the use of immunosuppressive drugs and the incidence of transplant failures in the medium and long term[4]. Non-adherence to the transplant medication regimen can lead to graft rejection, post-transplant mortality, increase in healthcare costs, and decrease in quality of life[5,6]. One meta-analysis found non-adherence to medication across all organ transplants to be 22.6%[7]. An estimated 50% of late acute rejections and 15% of graft losses are associated with non-adherence[8]. An essential aspect to ensure full adherence to the treatment is the assessment of transplant recipient needs and his/her expectations while establishing a good therapeutic alliance[9]. Many studies evaluating the relationship between the healthcare team and the patient highlighted the need for a relationship based on trust and clarity for the sharing of information regarding the treatment course[10,11]. Even psychological and psychosocial aspects can alter the response to treatment[12,13]: Mood disorders, high levels of anxiety, hostility, and the presence of "unstable" personality traits are associated with an increased risk of non-adherence to medical prescriptions in kidney transplant recipients[14-16].

Adherence to therapy thus is a complex variable and influenced by many factors: Socio-demographics, psychological characteristics, transplant recipient self-efficacy, factors related to immunosuppressive therapy, and the doctor-patient relationship. The aim of this study is to identify the risk factors and post-transplant psychological symptoms that affect adherence to therapy in a population of kidney transplant recipients.

MATERIALS AND METHODS

The study examined the psychological variables that are likely responsible for the non-adherent behavior using a psychological-psychiatric assessment, evaluation of the perception of patients' health status, and an interview regarding the anti-rejection drug therapy assumption. The psychological-psychiatric assessment involved the use of the following tests:

The Symptom Checklist-90-Revised (SCL-90-R) evaluated psychological symptoms. It is a relatively brief self-report psychometric instrument (questionnaire) published by the Clinical Assessment division of the Pearson Assessment and Information group. It is one of the most widely used measures of psychological distress in clinical practice and research and is designed to evaluate a broad range of psychological problems and symptoms of psychopathology. According to the overview given by the publisher, the SCL-90-R is normed on individuals 13 years and older. It consists of 90 items and takes 12-15 min to administer. The following primary symptom dimensions are assessed: Somatization (SOM), obsessive-compulsive (OBS), interpersonal sensitivity (INT), depression (DEP), anxiety (ANX), hostility (HOS), phobic anxiety (PHOB), paranoid ideation (PAR), psychoticism (PSY), and a category of "additional items" that helps clinicians assess other aspects of the patient's symptoms[17,18]. A large number of studies have been conducted demonstrating the reliability, validity, and utility of the instrument[19-22].

Personality study has provided an analysis of the

Table 1 Demographic data (n = 74 kidney transplant responders)

Years since transplantation procedure, mean ± SD (range)	5.39 ± 3.74 (1.00-14.00)
Education	
Basic	36%
High school	56%
University	8%
Occupation	
Employed	31.17%
Unemployed	56.82%
Retired	12.01%

temperament variables by the TEMPS-A (Temperament Evaluation of Memphis, Pisa and San Diego Autoquestionnaire). The features of temperament as well as its intensity may exert a constructive or destructive impact on the quality of life[23]. The TEMPS-A contains 110 items (109 in the version for males) measuring affective temperament traits occurring throughout life of the subject, as represented by five dimensions: Depressive (DT), cyclothymic (CT), hyperthymic (HT), irritable (IT), and anxious (AT). Questions about the various types are grouped together. The TEMPS-A measures the severity of the temperament traits ranging from 0 to 1. The calculation of points for each temperament is done by dividing the sum of points obtained in a given subscale by the number of questions contained therein[24,25].

Quality of life was examined with the Short Form Health Survey (SF-36) that assesses the degree of self-perceived psychological well-being. The SF-36 consists of eight subscales: Vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health. Subscales are presented as scores between 0 and 100; a lower score indicates more disability and a higher score less disability. The two considered variables in this study were the physical index score (PIS) and mental index score (MIS). The validity and reliability of the SF-36 has been confirmed in patients with renal disease[26,27].

Therapeutic adherence was studied through the Basel Assessment of Adherence to Immunosuppressive Medication instrument (BAASIS), which was developed to assess adherence to immunosuppressive medication in adult transplant patients. The instrument measures patients' taking, skipping, timing (± 2 h from the prescribed time, TM), and dose reduction of drugs. The recall period is limited to four weeks. The BAASIS comprises four questions with a 6-point scale for responses ranging from never (0) to every day (5). In addition, the BAASIS has a visual analogue scale (VAS) ranging from 0% (medication never taken as prescribed) to 100% (medication always taken as prescribed)[28,29].

The current study included 74 kidney transplant recipients (32 females, 43.25%), with a mean age of 48.3 ± 13.6 years (range 22-75). Demographic data regarding years since transplant procedure (first transplantation), occupation, level of education are presented in Table 1. All patients underwent a standardized immunosuppressive protocol with tacrolimus, mycophenolate mofetil, and steroids. The basic psychological-psychiatric assessment excluded the presence of lifetime psychiatric disorders (axis I) according to the Diagnostic and Statistical Manual for Mental Disorders (5th ed., DSM-5) or concomitant use of drugs that could influence cognitive and emotional aspects[30]. All patients provided written informed consent after the procedures were fully explained by a trained physician (MD, psychiatrist) or a psychologist.

The data were examined for normality and transformed if necessary. Pearson's R correlation test was performed using the "Statistical Package for Social Sciences" (SPSS, Version 17). The P value of less than 0.05 ($P < 0.05$) was considered statistically significant. In addition, we applied multivariate linear regression analysis to predict the outcome variable (BAASIS total score, BT) from predictor variables (patterns of personality and demographic characteristics).

RESULTS

The current study included 74 kidney transplant recipients (32 females, 43.25%), with a mean age of 48.3 ± 13.6 years (range 22-75). Demographic data regarding years since transplant procedure (first transplantation), occupation, level of education are presented in Table 1. All patients underwent a standardized immunosuppressive protocol with tacrolimus, mycophenolate mofetil, and steroids. Correlations by the Pearson coefficient between results of the SCL-90-R, SF-36 (physical and mental index score), and demographic characteristics of the sample are shown in Figure 1. Individuals with a higher level of education (E) and with more years of transplantation (YT) showed higher mental balance (E/MIS r = 0.61; YT/MIS r = 0.48). Specifically, the level of education was negatively correlated with anxious, obsessive-compulsive, and depression aspects (E/OBS r = -0.81; E/DEP r = -0.67; E/ANX r = -0.59).

Correlations by the Pearson coefficient between results of the BAASIS, SF-36 (physical and mental index score), and demographic characteristics of the sample are shown in Figure 2. Regarding gender, women (female sex, FS) appeared to be less adherent to therapy in our study (FS/BT r = 0.46), while years of transplantation adversely affected the proper pharmacological assumption (YT/BT r = 0.34). In addition, as the index of subjective physical well-being increases, compliant behavior increases as well (PIS/BT r = -0.47), especially with regards to the treatment assumption of correct timing (PIS/TM r = -0.27). Adherence to therapy was not significantly correlated with the mental health index (MIS/BT r = -0.01).

Correlations by the Pearson coefficient between the results of the TEMPS-A, BAASIS of the sample are shown in Figure 3. The temperament variables measured with the TEMPS-A were correlated with treatment adherence. Specifically, the cyclothymic, irritable, and depression personality adversely affected adherent behavior (BT/CT

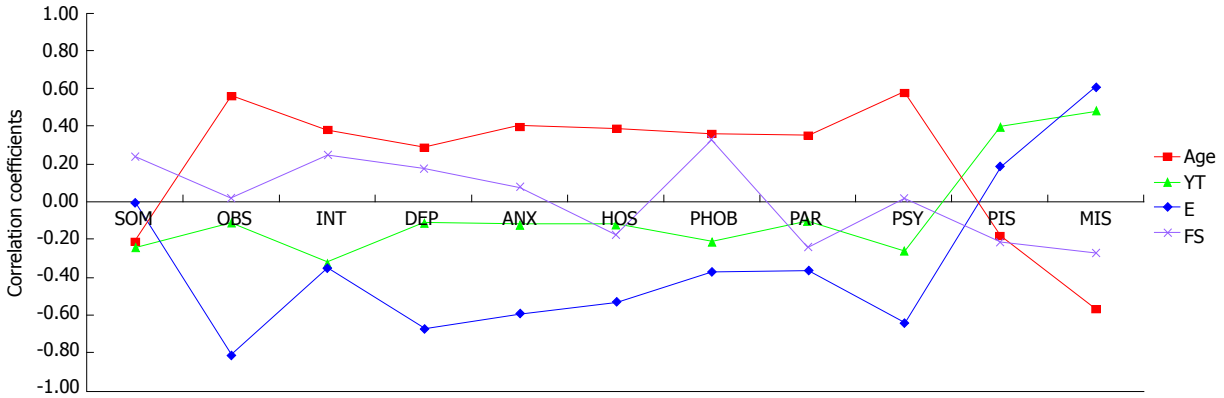

Figure 1 Correlations between symptom Checklist-90-R, short form health survey, and demographic characteristics. SF-36: Short form health survey; SOM: Somatization; OBS: Obsessive-compulsive; INT: Interpersonal sensitivity; DEP: Depression; ANX: Anxiety; HOS: Hostility; PHOB: Phobic anxiety; PAR: Paranoid ideation; PSY: Psychoticism; PIS: Physical index score of SF-36; MIS: Mental index score of SF-36; YT: Years since transplant procedure; E: Education; FS: Female sex. Correlation coefficients (r) < 0.3 indicate weak correlation, ≤ 0.7 moderate correlation, > 0.7 strong correlation.

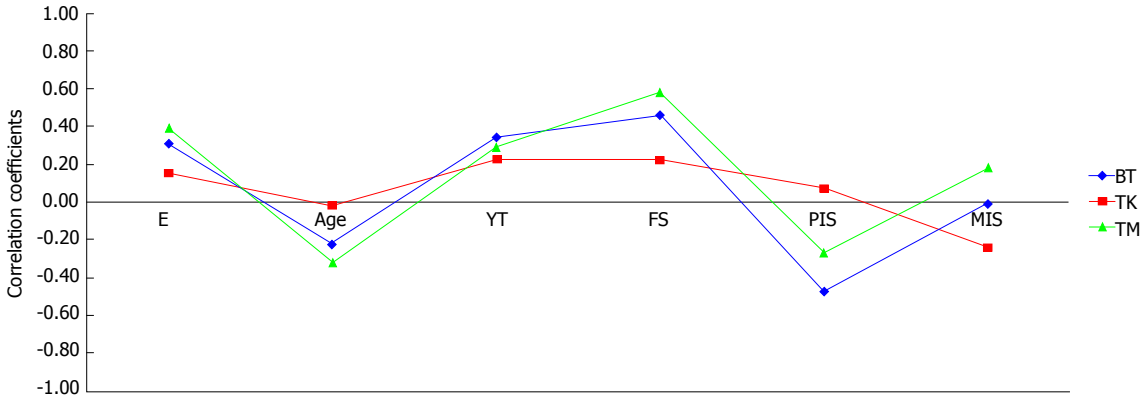

Figure 2 Correlations between basel assessment of adherence to immunosuppressive medication instrument, short form health survey, and demographic characteristics. BAASIS: Basel assessment of adherence to immunosuppressive medication instrument; BT: BAASIS total score; TK: BAASIS taking dimension; TM: BAASIS timing dimension; SF-36: Short form health survey; PIS: Physical index score of SF-36; MIS: Mental index score of SF-36; E: Education; YT: Years since transplant procedure; FS: Female sex. Correlation coefficients (r) < 0.3 indicate weak correlation, ≤ 0.7 moderate correlation, > 0.7 strong correlation.

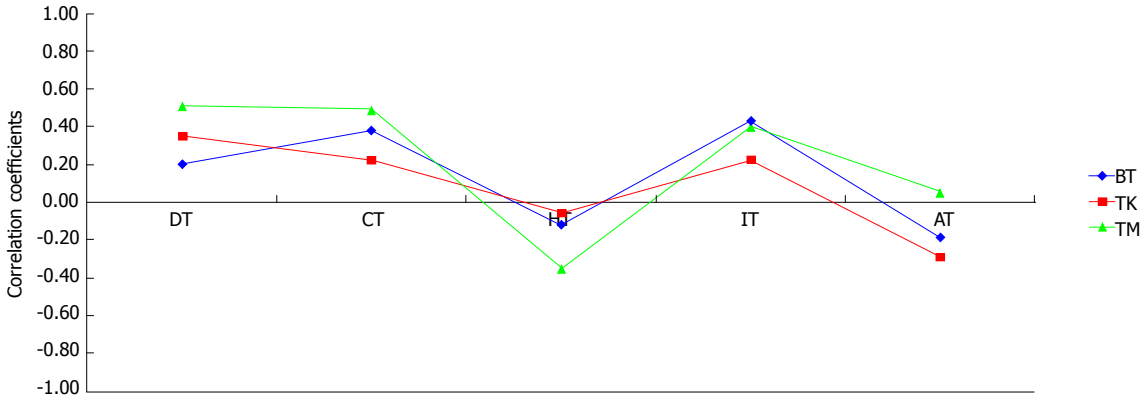

Figure 3 Correlations between temperament evaluation of memphis, pisa and san diego autoquestionnaire and basel assessment of adherence to immunosuppressive medication instrument. BAASIS: Basel assessment of adherence to immunosuppressive medication instrument; DT: Depressive temperament; CT: Cyclothymic temperament; HT: Hyperthymic temperament; IT: Irritable temperament; AT: Anxious temperament; BT: BAASIS total score; TK: BAASIS taking dimension; TM: BAASIS timing dimension. Correlation coefficients (r) < 0.3 indicate weak correlation, ≤ 0.7 moderate correlation, > 0.7, strong correlation.

r = 0.39; BT/IT r = 0.44; BT/DT r = 0.21); however, a moderate positive correlation was found between the timing scale of the BAASIS and depressive temperament variable (TM/DT r = 0.52), suggesting time management difficulties for patients with a depressive personality.

Multivariate linear regression analysis showed high

Table 2 Linear model of predictors sex, age, and years since transplant on basel assessment of adherence to immunosuppressive medication instrument total score

	B	SE B (SE)	β	*P*
Constant	-0.98 (-3.44 to 1.47)	1.13	0.00	0.39
Sex	1.53 (0.33 to 2.74)	0.55	0.75	0.01
Age	0.01 (-0.03 to 0.05)	0.02	0.14	0.58
YT	0.15 (0.03 to 0.27)	0.06	0.58	0.01

YT: Years since transplant procedure; Linear model with 95% bias corrected and accelerated confidence intervals (in parentheses).

Table 3 Linear model of temperament predictors on basel assessment of adherence to immunosuppressive medication instrument total score

	B	SE B (SE)	β	*P*
Constant	0.87 (-0.14 to 1.88)	0.39	0.00	0.06
CT	0.51 (0.10 to 0.93)	0.16	1.92	0.02
IT	-0.17 (-0.58 to 0.23)	0.16	-0.48	0.32
AT	-0.28 (-0.46 to -0.09)	0.07	-1.44	0.01

CT: Cyclothymic temperament; IT: Irritable temperament; AT: Anxious temperament; Linear model with 95% bias corrected and accelerated confidence intervals (in parentheses).

associations between predictor variables (sex and years since transplant procedure, cyclothymic temperament, and anxious temperament) and outcome variable (BAA SIS total score), whereas no consistent associations between other predictor variables (age, irritable temperament, IT) and outcome variable (BAASIS total score) were detected (Tables 2 and 3).

DISCUSSION

Similar studies on the subject have revealed significant psychological and behavioral differences between adherent and non-adherent transplanted patients, differences that express a greater vulnerability of the latter and which lead to consider that, next to drug therapy, psychological therapy is required[5,31]. Still not considered in other studies on the transplant topic is the temperament. "Temperament has been contemporarily defined as a biologically determined, hereditary core of the personality, being stable and relatively unchangeable throughout life, which determines the basic level of reactivity, mood and energy of given individual"[24].

Based on these assumptions, this study has allowed us to analyze different temperament styles and suggested that different temperaments can influence in different ways the treatment compliance and quality of life of transplant recipients.

In this study, the biopsychosocial illness model (BIM) provided a conceptual frame of reference within which biological, psychological, and social aspects took on the same importance in explaining the adherence to treatment protocols[32]. For good treatment management, it is necessary to understand the patients' personal experiences, their beliefs about the disease, health status perception, and mood, and to identify any "barriers" that could make them noncompliant. The analysis of the variables that are responsible for the behavior of not adhering to the treatment regimen should provide suggestions for psychological support and psychiatric treatment. Treatment adherence towards the prescribed medication is critical for the safe and successful delivery of efficacious interventions, especially for complex tasks such as the management of transplant patients[33,34].

The study revealed that years of transplantation positively affected mental health, but on the other hand, adversely affected therapeutic adherence, while the level of education was positively correlated with good mental balance. Studies examining the non-pharmacological risk factors that influence therapeutic adherence thus need further confirmation[5,35]. Another finding that requires careful reflection concerns the long-term negative impact that the cyclothymic and anxious personalities could have on adherent behavior, while the presence of post-transplant psychological symptoms (mental health index) did not affect treatment adherence[36-39]. This finding could be related to the notion that while people with mental problems feel the need to be cared for and are more willing to properly follow the treatment protocol, patients with mood swings (cyclothymic temperament) and those in an alert and apprehensive state (anxious temperament) are not prepared to calmly accept the rigorous therapeutic protocol and require constant attention by healthcare staff[40]. Thus, the quality of care is not just about the correct prescription but also about the patients' active participation through an assessment of their expectations and preferences. Patients adopt adherent behavior when they accept the type of care in terms of the therapeutic project[41]. Helpful in this regard is cognitive behavioral therapy aimed at increasing transplant recipients' awareness[42].

Immediately after transplant, patients must be assisted to increase the capacity for self-efficacy and resilience necessary to achieve the correct lifestyle for maintaining the graft. In a next step, it would be desirable to establish a cognitive and psychosocial rehabilitation plan to improve coping strategies and strengthen the patients' resources in order to positively influence the final outcome of the transplant process. These efforts therefore must operate simultaneously at several levels on the basis of an integrated strategy that organizes and coordinates the various types of treatment-medication, psychotherapy, assistance-and the operators' different functions, in an intervention program formulated on the basis of the characteristics and needs of each individual patient[43,44].

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COMMENTS

Background

Adherence to immunosuppressive therapy after transplantation is of fundamental importance for the patients' well-being both short- and long-term and assumes a set of adaptations to a new lifestyle. I pazienti trapiantati devono assumere quotidianamente farmaci immunosoppressori per la prevenzione del rigetto acuto e cronico (infezioni, complicanze secondarie). The treatment effectiveness and transplant success not only depend on the correct choice of immunosuppressive drugs, but also on the patients' active participation in the therapeutic program that often includes psychological support and appropriate motivation. Adequate adherence to doctor's orders is a resource for both patients and the health care system and society, as it reduces the costs for therapies, for minor complications associated with the disease, the health care interventions, morbidity and mortality. However various social, cultural, financial and psychological aspects affect adherence to immunosuppressive therapy.

Research frontiers

Although most research has focused on adherence to drug treatment, the concept of adherence must include other behaviors related to health protection involving the doctor-patient relationship, the service delivery system and change their living habits. The communication characteristics of the doctor, the kind of language used and the setting are essential to strengthen the motivation and awareness of the need for a cure. Future programs should provide the ability to support the transplanted in transplant experience, helping him to properly follow treatment, help him to learn cognitive and behavioral strategies of self-regulation.

Innovations and breakthroughs

Studies of the Italian population have revealed significant differences in psychological and personality traits among transplant patients adherent and non-adherent to therapy, differences that express a greater vulnerability of the latter and which lead to consider that, next to drug therapy, you are required psychological therapy. This study also allows to analyze different temperament styles, yet not studied in other research on transplantation and suggests that different temperaments influence in different ways the treatment compliance.

Applications

The data in this study suggested that psychological and psycho-educational support to the transplanted patient could yield favorable outcomes about adherence to immunosuppressive therapy. Furthermore, this study also provided readers with important informations about psychological problems that could highlight on transplanted subject.

Terminology

TA is the patient's ability to be able to follow precisely the prescribed cure. Specifically, the concept of adherence to therapy includes the compliance and persistence: Compliance reflects the acceptance of the patient to medical prescription (number of daily dose), the persistence instead indicates the time period between the start and the interruption of the treatment. BIM provided a conceptual frame of reference within which biological, psychological, and social aspects took on the same importance in explaining the adherence to treatment protocols. You must operate simultaneously at several levels in an intervention program formulated on the basis of the characteristics and needs of each individual patient.

Peer-review

Studies concerning the influence of temperament to the therapeutic adherence are scarce. The authors in this study analyzed the psychological characteristics of a sample of transplant recipients followed as outpatients at a transplant center. The results showed that the transplant experience change behaviors and quality of life based on the personality and temperament characteristics.

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ORIGINAL ARTICLE

Observational Study

Time spent in hospital after liver transplantation: Effects of primary liver disease and comorbidity

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Institutional review board statement: This study is exempt United Kingdom National Research Ethics Service approval as it involved analysis of existing databases of anonymised data for service evaluation. Approval for the use of Hospital Episode Statistics data was obtained as the standard Hospital Episodes Statistics approval process.

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Abstract

AIM

To explore the effect of primary liver disease and comorbidities on transplant length of stay (TLOS) and LOS in later admissions in the first two years after liver transplantation (LLOS).

METHODS

A linked United Kingdom Liver Transplant Audit - Hospital Episode Statistics database of patients who received a first adult liver transplant between 1997 and 2010 in England

was analysed. Patients who died within the first two years were excluded from the primary analysis, but a sensitivity analysis was also performed including all patients. Multivariable linear regression was used to evaluate the impact of primary liver disease and comorbidities on TLOS and LLOS.

RESULTS

In 3772 patients, the mean (95%CI) TLOS was 24.8 (24.2 to 25.5) d, and the mean LLOS was 24.2 (22.9 to 25.5) d. Compared to patients with cancer, we found that the largest difference in TLOS was seen for acute hepatic failure group (6.1 d; 2.8 to 9.4) and the largest increase in LLOS was seen for other liver disease group (14.8 d; 8.1 to 21.5). Patients with cardiovascular disease had 8.5 d (5.7 to 11.3) longer TLOS and 6.0 d (0.2 to 11.9) longer LLOS, compare to those without. Patients with congestive cardiac failure had 7.6 d longer TLOS than those without. Other comorbidities did not significantly increase TLOS nor LLOS.

CONCLUSION

The time patients spent in hospital varied according to their primary liver disease and some comorbidities. Time spent in hospital of patients with cancer was relatively short compared to most other indications. Cardiovascular disease and congestive cardiac failure were the comorbidities with a strong impact on increased LOS.

Key words: Length of stay; Hospital stay; Comorbidity; Liver transplantation

Core tip: Time patients spent in hospital in transplant admission and in later admissions after liver transplantation may reflect the success of liver transplantation. By analysing a linked United Kingdom Liver Transplant Audit - Hospital Episode Statistics database between 1997 and 2010, we found that average transplant length of stay (LOS) was 24.8 d, and mean LOS of all admissions in the first two years after transplantation was 24.2 d. Primary liver disease and comorbidities had a significant impact on LOS. Patients transplanted for cancer has shorter LOS compared to other indications. Cardiovascular disease and congestive cardiac failure were associated with increased LOS.

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INTRODUCTION

The short-term and long-term survival after liver transplantation has been improving over the last few decades. However, length of stay (LOS) is another outcome that reflects the success of liver transplantation. Transplant LOS (TLOS) has also been one of the main outcomes in investigating health care resource use in organ transplantation. Identifying variables predicting longer LOS will help clinicians plan hospital resources in advance, and maximise the resource utilisation. LOS in transplant admission can also reliably reflect the cost of liver transplant admission[1].

There are several studies report risk factors predicting longer transplant LOS[2-9]. Recipient factors (*e.g.*, age, sex, liver disease severity, retransplantation, pre-transplant nutritional status, pre-transplant renal support), donor factors (*e.g.*, age, weight, non-local donor centre) and early post-transplant complications and graft dysfunction have been shown to be associated with prolonged transplant LOS. LOS also varies between liver transplant centres[4,5]. Nevertheless, primary liver disease and comorbidities have rarely been investigated in terms of their effects on LOS. Moreover, the previous studies were based on only single or few centres, and the cohort sizes were often limited. In this study, we used a national clinical database linked to an administrative hospital database in England to investigate LOS in a larger national cohort of liver transplant patients.

LOS in later admissions after liver transplantation may reflect quality of life after liver transplantation and represent the success of liver transplantation. Moreover, it can reflect the healthcare resource use in the maintenance period after liver transplantation. To the best of our knowledge, there has not been any study in this topic.

In this study, we aim to investigate the effect of primary liver disease and pre-transplant comorbidities on TLOS as these two important factors have rarely been studied. The secondary aim is the effect of these factors on LOS in later admissions (LLOS) in the first two years after liver transplantation.

MATERIALS AND METHODS

The linked UKLTA-HES database

Records from the United Kingdom Liver Transplant Audit (UKLTA) database linked at a patient level to Hospital Episode Statistics (HES) records were used in this study. The UKLTA database prospectively collects liver transplant-specific data for all patients undergoing liver transplantation in the United Kingdom for audit purposes[10]. The HES database is an administrative hospital database of all admissions to National Health Service (NHS) hospitals in England[11]. A HES record contains the tenth revision of the International Classification of Diseases (ICD-10) diagnosis codes[12], procedure codes, admission method as well as length of hospital stay based on date of admission and date of discharge. The linkage process was based on hierarchical deterministic linkage criteria, including NHS number, sex, date of birth, postcode, date of transplant and a

procedure code for liver transplantation or a diagnosis code relevant to liver disease. A detailed description of the linkage process has been published elsewhere[13].

This linked database contained records of patients receiving a first liver transplant in England between 1st April 1997 and 31st March 2010. We excluded linked records of paediatric liver transplantation (younger than 17 years), multi-organ transplantation, living-donor liver transplantation and domino liver transplantation. To avoid the interference from the short LOS in patients who died early after transplantation, the patients who died within the first two years after liver transplantation (718 patients) were also excluded from the primary analysis. However, a sensitivity analysis for the whole cohort was also performed. At least two years follow-up was available for all included patients.

LOS information

LOS information was obtained from the HES database. TLOS was calculated from date of transplant to date of discharge, while LOS of a later admission was calculated from date of admission to date of discharge. LOS of all later admissions in the first two years after transplantation (LLOS) was defined as a sum of LOS of every admission in any NHS hospital in England that had an admission date within the first two years from the date of transplant.

Statistical analysis

Unadjusted TLOS and LLOS of patients in each primary liver disease group and of patients with each comorbidity were compared using unpaired two-tailed Student's *t*-test and analysis of variance (ANOVA) as appropriate. Primary liver diseases were categorised into ten indication groups according to Roberts *et al*[14], including one group with less common indications grouped together as the other liver diseases group. Eight comorbidities were identified from ICD-10 diagnosis codes in HES based on the adaptation of the Royal College of Surgeons Charlson Score for liver transplantation[15]. Cardiovascular comorbidity comprises of a history of myocardial infarction, peripheral vascular disease and cerebrovascular disease.

Multivariable linear regression analysis was performed to determine the effects of the individual variables on TLOS and LLOS, taking into account other baseline characteristics, severity of liver disease and transplant centres. Variables included in the model were 10-group primary liver disease, all eight comorbidities, recipient age, sex, serum bilirubin, creatinine, sodium and international normalised ratio (INR) of prothrombin time (factors reflecting the severity of liver disease), liver transplant centre and time period of liver transplantation. Serum bilirubin and creatinine were log-transformed before inputting into the model to improve the linearity of the relationship between these factors and LOS. The comorbidities and sex were included as binary variables, while primary liver disease groups, transplant centre and time period of liver transplantation were entered as categorical variables. The remaining variables were included as continuous variables.

LOS may not only depend on disease and patient factors, such as type and severity of liver disease and comorbidities, but also on hospital policy that may change over time. Therefore, we included the information about individual transplant centre and time period of liver transplantation in the models.

The ten primary liver disease groups were mutually exclusive. In the multivariable models, coefficients of primary liver disease groups were compared to cancer group as a reference group because it was one of the most common and shortest LOS groups. To make the comparison easier to interpret, we also presented adjusted mean LOS, which reflects LOS for these groups after taking other variables into account. The adjusted mean TLOS and LLOS for patients in each of the primary liver disease groups were calculated based on the prediction from multivariable linear regression models and presented along with their 95% confidence intervals (CI). This represents the LOS according to primary liver disease groups with an average case-mix profile of other variables in the model.

To ensure that patients with missing values were not excluded from the analyses, missing values were imputed with ten plausible data sets using multiple imputation with chain equations technique[16]. The ten completed data sets were individually analysed, and estimates were then pooled to give final estimates using Rubin's rules[17]. However, all of the variables in the model had missing values for less than 5% of the patients. All statistical analyses were performed using Stata version 11.2 (StataCorp, College Station, TX, United States). A *P*-value of less than 0.05 was considered statistically significant.

Sensitivity analysis

The primary analysis included only patients who survived the first two years after transplantation as the patients who died early after transplantation would shorten the average of LOS. A sensitivity analysis for TLOS was performed using the whole cohort including patients who died in the first two years after transplantation (4490 patients).

RESULTS

Demographic characteristics

The data used in this study were from 3772 adult patients who had a first liver transplant in England from April 1997 to March 2010 and survived the first two years after liver transplantation. The median (interquartile range: IQR) age was 52 (42 to 59) years, and 58.7% of the patients were male. The most common indication for liver transplantation was alcoholic cirrhosis (20.0%), followed by cancer (13.9%) and primary biliary cirrhosis (13.4%). The most common comorbidity was diabetes mellitus with a prevalence of 20.8%, followed by chronic

Table 1 Demographic characteristics of the adult recipients of a first liver transplant in England who survived the first two years after transplantation

Characteristic	Value	Missing (*n*)
Number	3772	
Age (yr)	52 (42-59)	0
Sex (%)		0
Male	2214 (58.7)	
Female	1558 (41.3)	
Primary liver disease (%)		0
Cancer	525 (13.9)	
Acute hepatic failure	455 (12.1)	
Hepatitis C cirrhosis	392 (10.4)	
Primary sclerosing cholangitis	354 (9.4)	
Hepatitis B cirrhosis	98 (2.6)	
Primary biliary cirrhosis	507 (13.4)	
Alcoholic cirrhosis	753 (20.0)	
Autoimmune and cryptogenic cirrhosis	348 (9.2)	
Metabolic liver disease	107 (2.8)	
Other liver disease	233 (6.2)	
Comorbidities (%)		
Cardiovascular disease	200 (5.3)	0
Congestive cardiac failure	82 (2.2)	0
Connective tissue disease	134 (3.6)	0
Dementia	159 (4.2)	0
Diabetes mellitus	784 (20.8)	0
Non-hepatic malignancy	40 (1.1)	0
Chronic pulmonary disease	344 (9.1)	0
Chronic renal disease	247 (6.6)	0
Era of liver transplantation (%)		0
April 1997 - September 2000	841 (22.3)	
October 2000 - September 2003	899 (23.8)	
October 2003 - September 2006	897 (23.8)	
October 2006 - March 2010	1135 (30.1)	
Bilirubin (μmol/L)	54 (27-124)	20
Creatinine (μmol/L)	89 (74-109)	2
INR	1.4 (1.2-1.8)	162
Sodium (mmol/L)	137 (134-140)	8
UKELD score	55 (51-59)	184

Results are numbers (percentages) or medians (interquartile ranges). INR: International normalised ratio; UKELD: United Kingdom End-stage Liver Disease.

pulmonary disease (9.1%) and chronic renal disease (6.6%) (Table 1). In terms of pre-transplant status, 74.0% of the patients were at home, while 16.0% were in hospital but not ventilated, and 10.0% were hospitalised and ventilated at the time of transplantation. Some 4.7% of the patients received a liver graft from donation after cardiac death.

Overall LOS

Overall, patients spent an average of 24.8 d (95%CI: 24.2 to 25.5) in hospital during their transplant admission, and 24.2 d (22.9 to 25.5) in later admissions in the first two years after transplantation (Table 2).

LOS according to primary liver diseases

Primary liver disease groups were significant predictors of both TLOS and LLOS (Table 2). Using cancer group as a baseline, the multivariable analysis demonstrated that patients in acute hepatic failure (6.1 d longer), other liver disease (5.9 d longer), metabolic and non-alcoholic fatty liver disease (4.3 d longer) and hepatitis *C* cirrhosis (3.9 d longer) groups had significantly longer TLOS than the baseline. As for LLOS, other liver disease (14.8 d longer) and primary sclerosing cholangitis (8.4 d longer) were significantly associated with longer LLOS than the baseline (Table 3).

Figure 1 presents the same above results using adjusted means for TLOS and LLOS, and further illustrates that patients with acute hepatic failure, hepatitis C cirrhosis, metabolic and non-alcoholic fatty liver disease and other liver disease had longer than average TLOS and LLOS, although not all significantly so. Whereas, patients with liver diagnosis of cancer, primary biliary cirrhosis, alcoholic cirrhosis and autoimmune hepatitis and cryptogenic cirrhosis groups had shorter than average TLOS and LLOS. Of note, primary sclerosing cholangitis was associated with shorter TLOS, but significantly longer LLOS (Figure 1).

LOS according to comorbidities

Patients with atherosclerotic cardiovascular disease had the longest unadjusted TLOS at 33 d, and those with chronic renal disease had the longest unadjusted LLOS at 32 d (Table 2). The multivariable linear regression analysis demonstrated that cardiovascular disease and congestive cardiac failure were significantly associated with longer TLOS. Patients with cardiovascular disease spent an average of 8.5 d longer in transplant admission than those without the comorbidity, and those with a history of congestive cardiac failure spent 7.6 d longer than those without, confirming what previously observed in unadjusted LOS (Table 3).

Patients with cardiovascular disease spent significantly longer time in hospital in the first two years after transplantation than those without the comorbidity (6.0 d longer). Those with chronic renal disease and chronic pulmonary disease spent 4.8 d and 4.3 d longer than those without the comorbidities, respectively, albeit not statistically significant (Table 3).

Sensitivity analysis of TLOS including patients who died in the first two years after liver transplantation

After including 718 patients who died within the first two years after transplantation, the sensitivity analysis of 4490 patients found that primary liver disease, cardiovascular disease and congestive cardiac failure remained statistically significant in predicting TLOS. In terms of primary liver disease groups, patients in other liver disease group had the longest TLOS, followed by acute hepatic failure group. Cardiovascular disease were associated with 8.7 d longer TLOS than those without the comorbidity, while patients with congestive cardiac failure had 7.7 d longer TLOS than those without, which were similar to those in the primary analysis (Table 4).

DISCUSSION

Liver transplant recipients spent in total 49 d in hospital during the first two years after transplantation,

Table 2 Unadjusted transplant length of stay and length of stay in later admissions in the first two years after liver transplantation regarding primary liver disease and comorbidities

Variable	*n* (%)	Unadjusted TLOS			Unadjusted LLOS		
		(d)	95%CI	*P*-value	(d)	95%CI	*P*-value
Overall average	3772	25	24-26	N/A	24	23-26	N/A
Primary liver disease groups							
Cancer	525 (13.9)	22	20-24	< 0.001	22	18-25	< 0.001
Acute hepatic failure	455 (12.1)	33	32-35		26	22-30	
Hepatitis C cirrhosis	392 (10.4)	27	25-29		26	22-30	
Primary sclerosing cholangitis	354 (9.4)	23	20-25		27	23-31	
Hepatitis B cirrhosis	98 (2.6)	22	18-26		21	13-29	
Primary biliary cirrhosis	507 (13.4)	21	20-23		20	16-23	
Alcoholic cirrhosis	753 (20.0)	24	23-26		22	19-25	
Autoimmune and cryptogenic	348 (9.2)	22	20-24		23	18-27	
Metabolic liver disease	107 (2.8)	26	22-30		29	21-37	
Other liver disease	233 (6.2)	28	26-31		37	32-42	
Comorbidities							
Cardiovascular disease	200 (5.3)	33	28-39	< 0.001	31	24-38	0.02
Congestive cardiac failure	82 (2.2)	32	24-39	0.003	18	13-24	0.18
Connective tissue disease	134 (3.6)	22	20-25	0.13	25	18-32	0.91
Dementia	159 (4.2)	25	21-28	0.87	26	21-32	0.54
Diabetes mellitus	784 (20.8)	24	23-26	0.42	26	23-29	0.18
Non-hepatic malignancy	40 (1.1)	23	17-29	0.65	24	10-37	0.91
Chronic pulmonary disease	344 (9.1)	27	25-30	0.04	28	24-33	0.06
Chronic renal disease	247 (6.6)	27	23-30	0.17	32	25-38	0.003

TLOS: Transplant length of stay; LLOS: Later length of stay; N/A: Not applicable.

Table 3 Multivariable linear regression analysis for transplant length of stay and later length of stay in the first two years after liver transplantation

Variable value	Coefficient for TLOS			Coefficient for LLOS		
	(d)	95%CI	*P*-value	(d)	95%CI	*P*-value
Primary liver disease groups[1]						
Cancer	0	Reference	< 0.001	0	Reference	< 0.001
Acute hepatic failure	6.1	2.8, 9.4		4	-3.3, 11.3	
Hepatitis C cirrhosis	3.9	1.3, 6.4		5.4	-0.1, 10.9	
Primary sclerosing cholangitis	0.2	-2.5, 3.0		8.4	2.6, 14.3	
Hepatitis B cirrhosis	1.9	-2.3, 6.1		1	-7.9, 9.9	
Primary biliary cirrhosis	-0.5	-3.2, 2.1		0.2	-5.6, 5.9	
Alcoholic cirrhosis	0.6	-1.6, 2.8		0.6	-4.1, 5.4	
Autoimmune and cryptogenic	0.1	-2.6, 2.9		1.4	-4.4, 7.2	
Metabolic liver disease	4.3	0.3, 8.4		7.1	-1.5, 15.7	
Other liver disease	5.9	2.8, 9.1		14.8	8.1, 21.5	
Comorbidities[2]						
Cardiovascular disease	8.5	5.7, 11.3	< 0.001	6	0.2, 11.9	0.04
Congestive cardiac failure	7.6	3.4, 11.8	< 0.001	-5	-14.0, 3.9	0.27
Connective tissue disease	-1.4	-4.8, 2.0	0.42	2.3	-4.9, 9.5	0.54
Dementia	0.2	-2.9, 3.4	0.87	3.6	-3.1, 10.4	0.29
Diabetes mellitus	1	-0.6, 2.6	0.22	2.6	-0.8, 6.0	0.13
Non-hepatic malignancy	-0.7	-6.7, 5.3	0.82	-0.5	-13.2, 12.2	0.94
Chronic pulmonary disease	1.6	-0.5, 3.8	0.14	4.3	-0.2, 8.9	0.06
Chronic renal disease	1	-1.6, 3.5	0.47	4.8	-0.7, 10.3	0.09

[1]A coefficient for primary liver disease groups represents a difference in days between LOS of patients in the primary liver disease group and the reference group (cancer); [2]A coefficient for comorbidities represents a difference in days between LOS of patients with and without the comorbidity. The multivariable models were adjusted for primary liver disease group, comorbidities, recipient age, sex, log bilirubin, log creatinine, INR, sodium, transplant centre and time of transplantation. TLOS: Transplant length of stay; LLOS: Later length of stay.

approximately half in the transplant admission and the other half in subsequent admissions. However, the LOS was affected by primary liver disease and some comorbidities. Patients with liver diagnosis in acute hepatic failure, hepatitis C cirrhosis and other liver disease groups had a longer TLOS than those in any other groups, while patients in primary sclerosing cholangitis and other liver disease groups had longer LLOS. Patients with

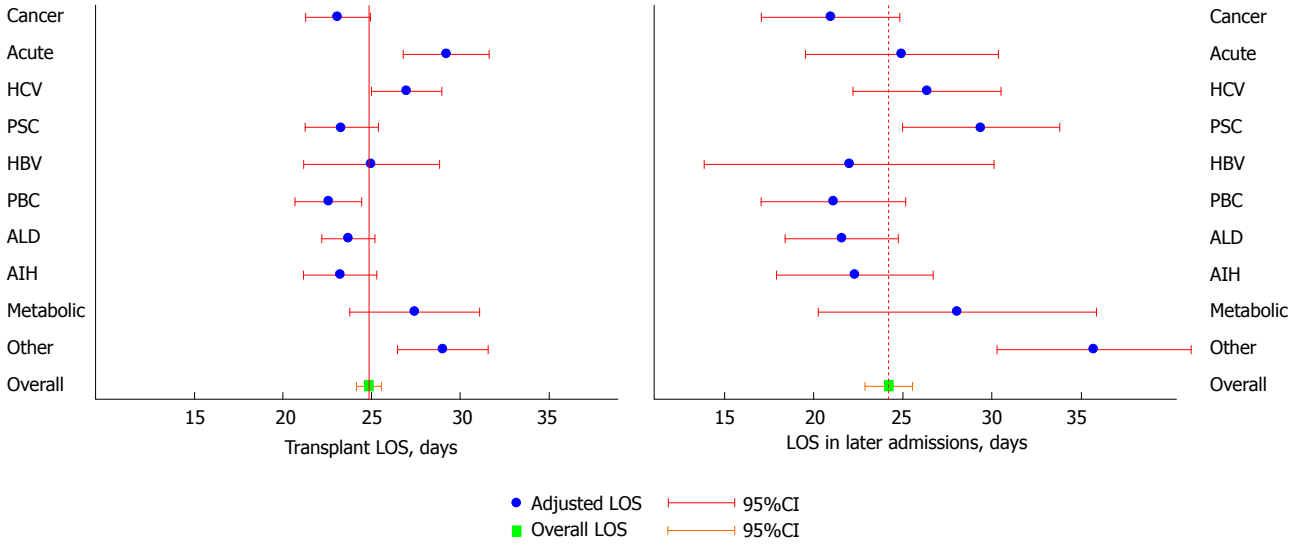

Figure 1 Adjusted transplant length of stay and length of stay in later admissions in the first 2 years after liver transplantation according to primary liver disease. HCV: Hepatitis C cirrhosis; PSC: Primary sclerosing cholangitis; HBV: Hepatitis B cirrhosis; PBC: Primary biliary cirrhosis; ALD: Alcoholic liver disease; AIH: Autoimmune hepatitis and cryptogenic cirrhosis; LOS: Length of stay.

Table 4 Sensitivity analysis of the multivariable linear regression for transplant length of stay including patients who died within the first two years after liver transplantation (4490 patients)

Variable	Coefficient for TLOS		
	(d)	95%CI	*P*-value
Primary liver disease groups[1]			
Cancer	0.0	Reference	0.001
Acute hepatic failure	3.7	0.2, 7.2	
Hepatitis C cirrhosis	3.3	0.6, 6.1	
Primary sclerosing cholangitis	-0.3	-3.2, 2.7	
Hepatitis B cirrhosis	0.6	-4.0, 5.2	
Primary biliary cirrhosis	-1.5	-4.4, 1.4	
Alcoholic cirrhosis	1.1	-1.3, 3.5	
Autoimmune and cryptogenic	0.5	-2.5, 3.4	
Metabolic liver disease	3.3	-0.9, 7.6	
Other liver disease	5.9	2.5, 9.2	
Comorbidities[2]			
Cardiovascular disease	8.7	5.8, 11.5	< 0.001
Congestive cardiac failure	7.7	3.6, 11.8	< 0.001
Connective tissue disease	-0.3	-4.0, 3.3	0.86
Dementia	1.1	-2.4, 4.6	0.54
Diabetes mellitus	1.1	-0.6, 2.8	0.21
Non-hepatic malignancy	0.9	-4.6, 6.3	0.76
Chronic pulmonary disease	1.2	-1.1, 3.5	0.3
Chronic renal disease	1.7	-1.0, 4.4	0.22

[1]A coefficient for primary liver disease groups represents a difference in days between LOS in the primary liver disease group and the reference group (cancer); [2]A coefficient for comorbidities represents a difference in days between LOS of patients with and without the comorbidity. The multivariable model was adjusted for primary liver disease group, comorbidities, recipient age, sex, log bilirubin, log creatinine, INR, sodium, transplant centre and time period of transplantation. TLOS: Transplant length of stay; INR: International normalised ratio.

cardiovascular disease and cardiac failure also had longer TLOS than those without these comorbidities, and patients with cardiovascular disease spent longer time in later admissions than those without.

In terms of primary liver disease, acute hepatic failure, hepatitis C cirrhosis and other liver disease were associated with longer TLOS. Patients with acute hepatic failure are mostly intubated and ventilated and on renal replacement therapy[18]. Therefore, it is not unexpected that they required more time to recover from the liver transplant operation. Hepatitis C cirrhosis may be related to more complications after liver transplantation, and this is probably the reason why patients transplanted for this indication spent longer time in transplant admission. The group of patients with other liver disease is the most heterogeneous group of patients with a wide range of liver diagnoses, consisting mainly of Budd-Chiari syndrome, secondary biliary cirrhosis and polycystic liver disease. Thus, the reason why these patients had longer LOS needs further investigation.

Patients with primary sclerosing cholangitis had relatively shorter TLOS, but relatively longer LLOS. These discrepancies may be a result of a higher rate of recurrent disease, vascular complications or conditions related to ulcerative colitis that need admissions for interventions or procedures[19]. Nevertheless, the reasons for readmissions can be either transplant-related or non-transplant-related, and they were not explored in this study.

With respect to comorbidities, common comorbidities, such as diabetes, chronic pulmonary and renal disease, showed no impact on TLOS, while less common comorbidities, such as cardiovascular disease and congestive cardiac failure, were found to have an impact on TLOS. Cardiovascular disease, which is the grouping of three comorbid conditions in the same disease spectrum (myocardial infarction, peripheral vascular disease and cerebrovascular disease), was significantly associated with longer TLOS as was congestive cardiac failure. We have shown elsewhere that a previous history of cardiovascular disease and cardiac failure were also associated with

higher 90-d mortality[15]. This study further showed that these groups of high-risk patients also used more health resources during their transplant admission. This is probably because of a higher risk of cardiac complications following a hemodynamically stressful liver transplant operation in these already compromised patients.

A previous single-centre study carried out in the United States with only 83 patients found that multi-vessel coronary artery disease is associated with higher mortality, increased LOS and post-operative vasopressor requirements[20], which is in line with the results found in our national cohort in England. In addition, we demonstrated that the LOS in later admissions in the first two years in patients with atherosclerotic cardiovascular disease was also longer, particularly in those who survived the first two years. This reflects that these patients with cardiovascular comorbidity have a higher mortality risk and require more healthcare resources during transplant admissions as well as the early period after liver transplantation.

Our results have a number of implications for clinical practice. First, LOS of the transplant admission and of later admissions can be an alternative marker of outcomes after liver transplantation, especially in the era that graft and patient survival after liver transplantation have been excellent. LOS in later admissions after liver transplantation may also reflect the quality of life and functional status of a patient after transplantation. A successful liver transplantation should return a patient back to the healthy status with as few admissions after transplantation as possible. Second, the ability to estimate LOS may be beneficial to the pre-transplant counselling process as it can help to inform patients and their relatives what to expect after liver transplantation. Third, another benefit of estimating LOS is that it would help clinicians and hospitals plan their resource utilisation and bed management. For example, patients transplanted for cancer spent, on average, a total of 44 d in the first two years after transplantation, whereas patients who were transplanted with an indication in other liver disease group, such as Budd-Chiari syndrome, had a total LOS that was 50% longer (66 d) (Figure 1). Fourth, our results can improve economic evaluations of liver transplantation as it provides more accurate estimates of LOS for patients with comorbidities.

We note a number of limitations of this study. Firstly, we have not explored the reasons for later admissions. It may be beneficial to understand the indication for readmissions in particular groups of patients, and this may warrant further research. Secondly, this study includes only patients who had a first liver transplant. It is known that the outcomes of retransplantation are much different to those of first liver transplantation[21]. Retransplantation has also been shown to be associated with longer transplant LOS[4].

Conclusion

We have shown that the time patients spent in hospital after liver transplantation is linked to primary liver disease and comorbidities. LOS was relative short for patient who had a liver transplant for cancer whereas the opposite was true for patients with atherosclerotic cardiovascular disease and congestive cardiac failure.

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COMMENTS

Background

Time patients spent in hospital after transplantation is directly related with health care resource use and partly reflects the success of liver transplantation. Identifying factors predicting longer length of stay will help clinicians and hospitals to plan and maximise the resource utilisation.

Research frontiers

Several recipient and donor factors have been found to be associated with prolonged transplant length of stay. However, primary liver disease and comorbidities have rarely been investigated in this issue. Length of stay in later admissions after transplantation also reflects quality of life after liver transplantation and can represent the success of liver transplantation. This has never been investigated in liver transplantation.

Innovations and breakthroughs

Based on the United Kingdom national liver transplant cohort, the authors demonstrated that transplant length of stay was affected by primary liver disease and comorbidities. Patients with acute hepatic failure, hepatitis C cirrhosis, atherosclerotic cardiovascular disease and a history of congestive cardiac failure stayed longer in hospital in their transplant admissions, while patients with primary sclerosing cholangitis spent more time in subsequent admissions in the first two years after liver transplantation.

Applications

Estimating length of stay will help clinicians and hospitals plan their health care resource utilisation including bed management. Moreover, knowing the estimated length of stay will be beneficial to the pre-transplant counselling process. It can help inform patients and relatives what they expect after liver transplantation. Finally, in the era that graft and patient survival after liver transplantation have been excellent, length of stay of the transplant admission and of later admissions can be a surrogate of outcomes after liver transplantation. Length of stay in later admissions after liver transplantation also specifically reflects the quality of life of patients after transplantation and the success of liver transplantation as it should return a patient back to the healthy status with as few admissions after transplantation as possible.

Terminology

Transplant length of stay (TLOS) was calculated from date of transplant to date of discharge, not including time patients spent in hospital in the pre-

transplant period. Length of stay in later admissions was a sum of length of stay of every admission in any National Health Service hospital in England that had an admission date within the first two years from the date of transplant. Cardiovascular comorbidity comprises of a history of myocardial infarction, peripheral vascular disease and cerebrovascular disease coded in the administrative hospital database in any previous admission in the preceding year before the transplant. Congestive cardiac failure is defined by a history of congestive cardiac failure coded in the administrative hospital database in any previous admission in the preceding year before the transplant. Other liver disease is a group of indications for liver transplantation that is consisted of less common indications grouped together. It is the most heterogenous group of indications, including mainly Budd-Chiari syndrome, secondary biliary cirrhosis and polycystic liver disease.

Peer-review

This study investigated the time after surgery after liver transplantation. The aim was clear, and methods were appropriate.

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ORIGINAL ARTICLE

Prospective Study

Magnetic resonance imaging of the transplanted pediatric heart as a potential predictor of rejection

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Abstract

AIM

To evaluate cardiac magnetic resonance imaging (CMR) as a non-invasive tool to detect acute cellular rejection (ACR) in children after heart transplant (HT).

METHODS

Thirty pediatric HT recipients underwent CMR at the time of surveillance endomyocardial biopsy (EMB) and results were compared to 14 non-transplant controls. Biventricular volumes, ejection fractions (EFs), T2-weighted signal intensities, native T1 times, extracellular volumes (ECVs) and presence of late gadolinium enhancement (LGE) were compared between patients and controls and between

patients with International Society of Heart and Lung Transplantation (ISHLT) grade ⩾ 2R rejection and those with grade 0/1R. Heart rate (HR) and brain natriuretic peptide (BNP) were assessed as potential biomarkers.

RESULTS

Significant ACR (ISHLT grade ⩾ 2R) was an infrequent event in our population (5/30, 17%). Ventricular volumes, EFs, LGE prevalence, ECVs, native T1 times, T2 signal intensity ratios, HR and BNP were not associated with the presence of ⩾ 2R ACR.

CONCLUSION

In this pilot study CMR did not reliably identify ACR-related changes in pediatric HT patients.

Key words: Heart; Pediatric; Transplantation; Magnetic resonance imaging; Rejection

Core tip: After heart transplantation the diagnosis of significant acute cellular rejection (ACR) changes management and is associated with adverse outcome. Endomyocardial biopsy is the gold standard for the detection of ACR but has important limitations. This prospective trial examined the use of cardiac magnetic resonance imaging (CMR) for the diagnosis of ACR in pediatric heart transplant recipients. Significant rejection was a rare event in our cohort and was not associated with changes in CMR parameters in this pilot study.

Greenway SC, Dallaire F, Kantor PF, Dipchand AI, Chaturvedi RR, Warade M, Riesenkampff E, Yoo SJ, Grosse-Wortmann L. Magnetic resonance imaging of the transplanted pediatric heart as a potential predictor of rejection. *World J Transplant* 2016; 6(4): 751-758 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i4/751.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i4.751

INTRODUCTION

Acute cellular rejection (ACR) is an immune-mediated process leading to allograft damage and decreased graft survival. It is a serious and potentially lethal complication after heart transplant (HT). The gold standard for the detection of rejection is an endomyocardial biopsy (EMB). However, EMB is an invasive procedure, exposes the patient to ionizing radiation and carries a small but important risk of serious complications[1-4].

Cardiac magnetic resonance imaging (CMR) has been proposed as a non-invasive method for the detection of rejection in adults after HT. However, CMR measurements used in adults for the detection of rejection or myocardial inflammation, including T2-weighted imaging[5,6], native T1 times and extracellular volume fractions (ECVs) derived from T1 mapping[7], myocardial thickness, ventricular volumes and ejection fraction (EF)[8,9] have not been systematically evaluated in pediatric HT recipients with EMB-proven ACR.

In this pilot study we sought to assess the utility of parameters of ventricular function and myocardial tissue characterization for the non-invasive detection of ACR in children and adolescents after HT.

MATERIALS AND METHODS

Patients and study design

This single center, prospective, cross-sectional study was approved by the institutional research ethics board and included pediatric (age < 18 years) HT patients who were scheduled for a clinically-indicated EMB between April 2010 and March 2011. All consecutive and eligible patients without contraindications to contrast-enhanced CMR during the study period were invited to participate. In patients who underwent more than one CMR/EMB procedure during the study period only the first set of investigations was analyzed for this study. Following written informed consent, CMR was performed immediately prior to cardiac catheterization and EMB. Control subjects were asymptomatic relatives of patients diagnosed with arrhythmogenic right ventricular cardiomyopathy (ARVC) who had normal echocardiograms, electrocardiograms (ECGs), signal-averaged ECGs as well as CMR scans and who were negative for ARVC-associated mutations if testing had been performed. Control subjects did not receive gadolinium as part of their CMR study. Heart rate (HR) was obtained from the average HR during the short axis CMR cine acquisition for ventricular volumetry.

Standardized immunosuppression post-transplantation for all patients included the use of thymoglobulin for induction (1-5 doses depending on risk factors), tacrolimus and mycophenolate mofetil. Perioperative steroids were discontinued 6 mo post-HT until 2007 and thereafter were discontinued at 5 d post-HT. Routine surveillance for rejection included serial echocardiograms, ECGs and cardiac catheterization with decreasing frequency over time post-transplantation.

EMB

At the authors' institution right ventricular EMBs are obtained at 1, 6 and 12 mo and then annually up to 5 years post-HT; thereafter only if there is clinical or echocardiographic suspicion for rejection. During the EMB five or six tissue samples were obtained from the right ventricular surface of the interventricular septum, stained with hematoxylin and eosin and evaluated using light microscopy. Samples were graded by a hospital pathologist who was blinded to the CMR and biochemistry results (below) and reported according to the International Society of Heart and Lung Transplantation (ISHLT) Standardized Cardiac Biopsy Grading Criteria[10]. Congruent with clinical practice grades 2R and 3R were classified as significant ACR and grades 0R and 1R as

non-significant ACR. Tissue samples were also evaluated for the presence of antibody-mediated rejection (AMR) by C4d immunohistochemical staining.

CMR

CMR was performed using a 1.5 Tesla scanner (Magnetom Avanto, Siemens AG Healthcare Sector, Erlangen, Germany) and a phased-array multi-channel surface receiver coil.

Ventricular volumetry and late gadolinium enhancement

A stack of multiphase short axis slices was acquired using the steady state free precession technique for left and right ventricular volumes, as described previously[11,12]. Ventricular volumes were extracted from the cine short axis stack in end-diastole and end-systole in the routine clinical fashion using commercially available software (QMass, version 7.2, Medis, Leiden, The Netherlands). Ventricular volumes were reported as indexed to recipient body surface area. EFs for both ventricles were calculated using end-diastolic and end-systolic volumes. The presence of late gadolinium enhancement (LGE) was determined qualitatively on standard long-axis (4-chamber, 2-chamber and 3-chamber) and short-axis slices using phase-sensitive inversion-recovery acquisitions > 10 min after the administration of 0.2 mmol/kg gadopentetate dimeglumine (Magnevist®, Bayer, Leverkusen, Germany).

T1 mapping and extracellular volumes

We previously described our T1 mapping approach for these patients in detail[13]. In short, a modified Look-Locker inversion recovery sequence (MOLLI) with inversion pulses of 100 msec and 150 msec, respectively, as well as 3 and 5 single-shot images after these inversion pulses was used to measure native and post-contrast longitudinal relaxation T1 times of myocardium and blood. Images were acquired in diastole at a single mid-ventricular short axis slice orientation before and > 10 min after administration of contrast (same injection as described above for LGE). Breathholds were used in cooperative patients and all other patients were scanned during free breathing. Longitudinal relaxation times (T1 times) were measured using commercially available software (CVI42, Circle Cardiovascular Imaging, Calgary, AB, Canada). Contours were drawn in the interventricular septum, the left ventricular (LV) free wall and in a region encompassing the entire LV myocardium. T1 times in the blood pool were measured in the LV cavity. The ECV was calculated using pre- and post-gadolinium T1 times of blood and myocardium as well as the patient's hematocrit, obtained at the time of the scan[14].

T2-weighted imaging

An ECG-gated turbo spin-echo readout sequence without fat saturation pulse preceded by a double inversion recovery dark-blood preparation and the following parameters was obtained in a single midventricular short axis slice[15]: Inplane spatial resolution 1.6 mm, slice thickness 6-10 mm, TE 59 ms. Imaging was performed in diastole during every other or every third heartbeat, depending on the HR, to achieve a TR of at least 1000 ms. The scanner's body coil was used for a homogeneous signal reception within the field of view. Myocardial signal intensity was measured around the circumference of the short axis slice and normalized to that of skeletal muscle using a dedicated module within the CVI42 software[16].

Brain natriuretic peptide levels

A blood sample was drawn upon insertion of the peripheral intravenous cannula needed for the CMR and analyzed for brain natriuretic peptide (BNP) levels (Modular Analytics, Roche Diagnostics, Laval, QC, Canada).

Statistical analysis

CMR data from transplant patients were stratified according to the presence (grade $\geqslant$ 2R) or absence (grade 0R or 1R) of significant ACR. Most variables were not normally distributed and results are thus presented as medians, 10th and 90th percentiles. Medians between groups were compared using a non-parametric Wilcoxon two-sample test or the Kruskal-Wallis test where appropriate. A *P*-value < 0.05 was considered statistically significant. All analyses were performed using SAS for Windows 9.4 (SAS Institute Inc., Cary, NC, United States). Statistical review of the study was performed by a biomedical statistician (FD).

RESULTS

Patient demographics and non-imaging biomarkers of rejection

The CMR studies from 14 non-transplant pediatric controls and 30 pediatric HT recipients were included in this study. The EMBs from 25 HT patients (83%) showed no significant ACR (ISHLT grades 0R or 1R) while 5 (17%) demonstrated significant rejection (ISHLT 2R). No patient had ISHLT grade 3R ACR. None of our HT patients were identified as having AMR. There were no statistically significant differences between the transplant groups with < 2R and $\geqslant$ 2R ISHLT rejection with respect to age at CMR or for time since transplant (Table 1). Patients with grade 2R rejection were younger than the controls and "no rejection" groups at the time of CMR but this difference was not statistically significant. HR and BNP were significantly increased in both groups of HT patients compared to controls but there were no statistically significant differences between the "no rejection" and "rejection" HT groups.

Ventricular function, volumes and mass

Biventricular end-diastolic volumes were decreased in the rejection group compared to the controls but not in the group without rejection (Figure 1). LVEF was decreased and LV mass increased only in the "no rejection" group compared to controls (Table 2). However, no significant

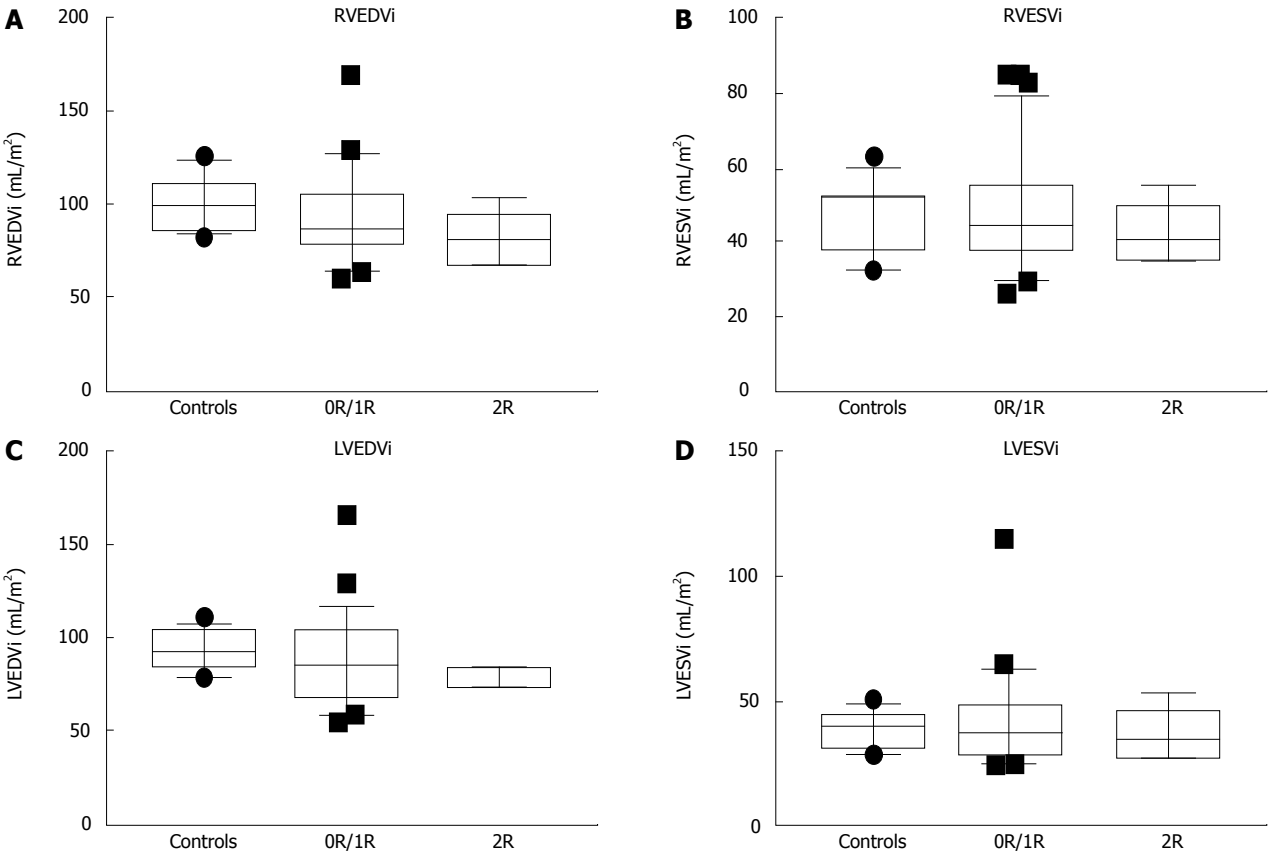

Figure 1 Box and whiskers plots for end-diastolic and end-systolic ventricular volumes of controls and transplant patients without (0R/1R) and with (2R) significant rejection. A: Right ventricular end-diastolic volume indexed to body surface area (RVEDVi); B: Right ventricular end-systolic volume indexed to body surface area (RVESVi); C: Left ventricular end-diastolic volume indexed to body surface area (LVEDVi); D: Left ventricular end-systolic volume indexed to body surface area (LVESVi).

Table 1 Patient characteristics

	Controls	No rejection (0R/1R)	Rejection (2R)
Number	14	25	5
Female (%)	8 (57)	10 (40)	1 (20)
Days post-transplant	-	702 (78, 1797)	160 (12, 800)
Age at CMR (yr)	12.8 (9.2, 15.3)	13.6 (2.2, 17)	7.7 (1.6, 17.5)
HR	71 (57, 84)	96[b] (82, 126)	108[d] (101, 130)
BNP	9.2 (5, 12.9)	38.7[f] (5, 81.6)	59.9[g] (14.9, 202)

Data shown as number (percentage) or median (10^{th}, 90^{th} percentiles). Significantly different compared to controls, [b]P = 5.76E-05, [d]P = 0.005, [f]P = 0.005, [g]P = 0.03. There were no significant differences between the transplanted rejection groups. HR: Heart rate in beats per minute; BNP: Brain natriuretic peptide in ng/L; CMR: Cardiac magnetic resonance imaging.

differences between HT patients with and without clinically important rejection were observed with regards to ventricular volumes, ejection fractions, LV mass or LV mass/volume ratio. The absence of a significant change in ventricular volumes with rejection may be confounded by the increase in ventricular size with age (Figure 2). There was no significant association between BNP and CMR parameters.

Native T1 times, myocardial extracellular volume fraction and LGE

The MOLLI sequence for T1 mapping became available to us after study enrollment had begun and therefore this data was available only in a subgroup of patients (Table 3). With regards to patient demographics there were no significant differences between this patient subset and the entire cohort. There were no significant differences in native T1 times and ECV fraction between patients with < 2R and ⩾ 2R ISHLT rejection. LGE was not observed in any of the HT patients. Native T1 times and ECV were not quantified and LGE imaging was not obtained in controls who did not receive contrast.

Myocardial T2-weighted imaging

The global ratios of myocardial:skeletal muscle T2 signal intensities on a mid-ventricular short axis slice were similar between groups and did not differ between controls (median 1.37, 10^{th} percentile 1.29, 90^{th} percentile 1.67) and transplant patients with no rejection (median 1.3, 10^{th} percentile 1.02, 90^{th} percentile 1.6) or with rejection (median 1.3, 10^{th} percentile 1.12, 90^{th} percentile 1.47). There were no significant differences between transplant patients with < 2R and ⩾ 2R ACR rejection.

Table 2 Ventricular volumes, function and mass

	Controls	No rejection (0R/1R)	Rejection (2R)
Number	14	25	5
RVEDVi (mL/m^2)	98.5 (85.7, 120.4)	86.6 (64.1, 124.4)	80.6[a] (68.1, 102.7)
RVESVi (mL/m^2)	50.5 (33.9, 56.1)	44.1 (30.7, 77)	41 (34.9, 55.4)
RVEF (%)	53.4 (48, 60)	50 (41, 57)	47.4 (40, 56)
LVEDVi (mL/m^2)	93 (79.2, 104)	85.2 (58.9, 112)	74.1[c] (73.7, 85)
LVESVi (mL/m^2)	40.3 (29.8, 45)	37.5 (24.6, 60.9)	33.8 (27.2, 52)
LVEF (%)	58.8 (53.2, 63)	54[e] (46, 64)	56 (36, 63)
LV mass (g/m^2)	53.5 (45.8, 61)	61.5[h] (50, 84.6)	66.1 (48, 80)

Data shown as median (10th, 90th percentiles) except for number. Significantly different compared to controls, [a]P = 0.03, [c]P = 0.02, [e]P = 0.05, [h]P = 0.003. There were no significant differences between the transplant rejection groups. RVEDVi: Right ventricular end-diastolic volume indexed to recipient body surface area; RVESVi: Right ventricular end-systolic volume indexed to recipient body surface area; RVEF: Right ventricular ejection fraction; LVEDVi: Left ventricular end-diastolic volume indexed to recipient body surface area; LVESVi: Left ventricular end-systolic volume indexed to recipient body surface area; LVEF: Left ventricular ejection fraction; LV mass: Left ventricular mass indexed to recipient body surface area; CMR: Cardiac magnetic resonance imaging.

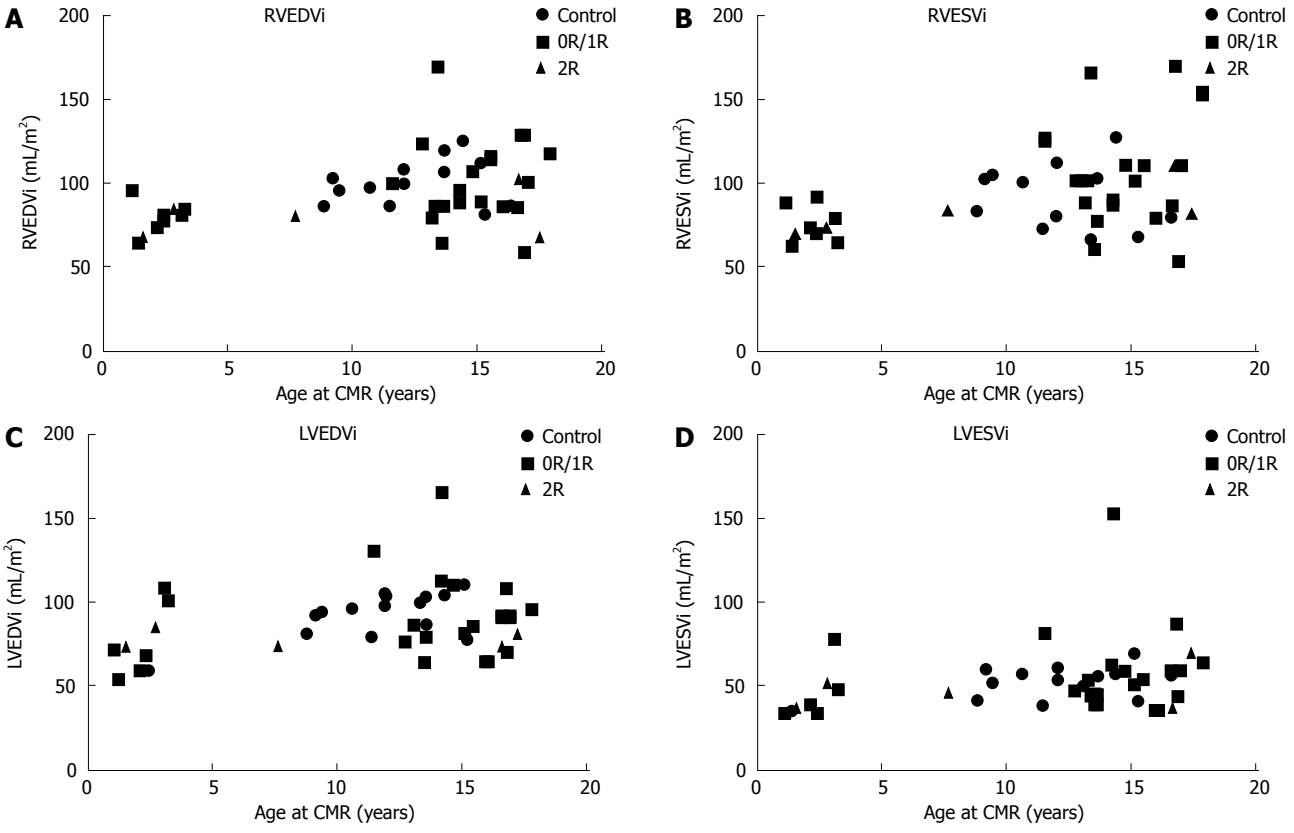

Figure 2 Ventricular volumes plotted as a function of age for controls and transplant patients without (0R/1R) and with (2R) significant rejection. A: Right ventricular end-diastolic volume indexed to body surface area (RVEDVi); B: Right ventricular end-systolic volume indexed to body surface area (RVESVi); C: Left ventricular end-diastolic volume indexed to body surface area (LVEDVi); D: Left ventricular end-systolic volume indexed to body surface area (LVESVi).

DISCUSSION

Despite a growing body of evidence in adult HT patients and important information from animal experiments the role of CMR for the detection of ACR in children has not been explored[17-19]. CMR tissue characterization overcomes important limitations of EMB such as the potential of containing scar from a previous EMB in the histological sample and the fact that specimens are collected from the RV surface of the interventricular septum and may not be representative of the remainder of the myocardium[17]. The current study compared descriptors of myocardial edema, expansion of the myocardial extracellular space, presence of patchy myocardial scarring as well as ventricular size and function between controls and HT recipients as well

Table 3 T1 and extracellular volume data for heart transplant patients

	No rejection (0R/1R)	Rejection (2R)
Number	18	4
Female (%)	8 (44)	1 (25)
Days post-transplant	485 (13, 1818)	142 (12, 800)
Age at CMR (yr)	13.2 (1.4, 16.9)	5.3 (1.6, 16.8)
Native T1 (ms)		
IVS	1008 (963, 1067)	976 (967, 1026)
LV free wall	988 (903, 1018)	978 (924, 1016)
Entire LV	991 (930, 1031)	978 (944, 1020)
Hematocrit	0.37 (0.26, 0.44)	0.35 (0.29, 0.38)
ECV		
IVS	0.3 (0.26, 0.34)	0.29 (0.26, 0.33)
LV free wall	0.27 (0.24, 0.34)	0.28 (0.25, 0.31)
Entire LV	0.29 (0.26, 0.33)	0.29 (0.27, 0.32)

Data shown as number (percentage) or median (10^{th}, 90^{th} percentiles). There were no significant differences between the groups. ECV: Extracellular volume; IVS: Interventricular septum; LV: Left ventricle.

as between HT patients with < 2R and ⩾ 2R ISHLT rejection. However, in contrast to the experience in adult HT populations for several of these parameters, we were unable to demonstrate an association of any of them with ACR in pediatric HT recipients[17,19].

None of the 30 patients in our study displayed patchy myocardial scarring as evidenced by LGE. This finding is in contrast to studies in adult HT recipients which found myocardial scarring on LGE imaging in a sizeable proportion (although this was not correlated with rejection)[19,20]. The reason for this discrepancy remains unclear, but may be related to the younger age of the donor hearts used for pediatric HT[21,22]. While LGE reflects patchy myocardial scarring of a certain size native T1 and ECV are regarded as measures of expansion of the extracellular matrix. Both are elevated in states of increased myocardial fibrosis or edema. Acute rejection is characterized histologically by inflammation of the myocardium while chronic or repeated episodes of rejection have been associated with fibrotic remodeling[7,23,24]. Native T1 and ECV have been explored as markers of ACR in a pilot study in adults after HT but an association with rejection has yet to be demonstrated[8]. In the current study, albeit in a limited number of patients, ECV and native T1 times did not distinguish between < 2R and ⩾ 2R ISHLT rejection. T2-weighted imaging is an established approach to detect tissue edema in inflammatory conditions and in the heart it is used as a marker for myocardial edema in myocarditis[6]. Studies that employed T2 signal intensity for the non-invasive detection of rejection have yielded mixed results in adult HT patients[5,9,25]. Our early results did not reveal increased signal intensity on T2-weighted imaging in patients with ACR. T2 mapping is another approach to myocardial edema which has yielded promising results in adult ACR[19,26-28], but this technique was not available to us at the time of the study. When discussing the lack of agreement between CMR markers and histological indicators of ACR, important shortcomings of EMB as the gold standard for the detection of ACR must be considered. Marie *et al*[26] found T2 mapping CMR to be "positive" for significant rejection several weeks before a follow-up EMB confirmed it suggesting a lack of sensitivity for EMB.

Ventricular size, LV myocardial mass, and function did not distinguish between patients with < 2R and ⩾ 2R ISHLT rejection in our study. An increase in indexed right ventricular end-diastolic volume has emerged as a potential predictor of rejection in adults[19], but the trend in our patients was in the opposite direction for both right and left ventricular end-diastolic volumes. The use of ventricular volumes as a biological marker is potentially problematic for two reasons: Firstly, there is often a size mismatch between the donor and the recipient which can be up to two-fold in children. This mismatch is fairly random and quite possibly obscures any association between ventricular size and the presence of rejection. Secondly, indexing to body surface area, although standard practice, is a crude strategy for normalizing ventricular volumes in children. Z-scores are more reliable in ensuring comparability across a spectrum of ages, body sizes and genders, but universally accepted Z-scores for CMR volumes are missing.

Another potential sign of inflammation is myocardial swelling as evidenced by increased LV "mass". Studies in adults have shown an increase in LV wall thickness during episodes of rejection[28,29]. However, an increase in LV mass in HT patients also occurs unrelated to rejection due to myocardial hypertrophy either as an adverse effect of medications[30], myocardial TNF-α expression[31] or hypertension. In our study there was no significant difference between HT patients with and without ⩾ 2R ACR with regards to LV mass.

With regards to non-CMR parameters, higher HRs were noted in the HT recipients as compared to controls due to denervation during the transplant operation. However, in our small cohort HR did not differ significantly between patients with and without significant rejection. An elevated BNP has also been proposed as a marker for rejection in pediatric cardiac transplant patients[32] and, although elevated in the transplant patients, there was no significant difference between the transplant rejection groups.

The most important limitation of this pilot study is the small number of patients with ⩾ 2R rejection which may have obscured associations of EMB with CMR parameters. The number of patients with available T1 mapping data, in particular, was very small. The small numbers may have also augmented the effects of potential confounders, for example donor:recipient size mismatch in HT patients, and thereby affected the comparability of ventricular volumes. The relatively low prevalence of ACR in the current era is related to improved immunosuppression regimes and, consistent with contemporary outcomes[33], none of the patients in our study had severe grade 3R rejection. The incidence of moderate (grade 2R) ACR (17%) was similar

to the 13%-23% found by others[8,19,20]. T1 relaxometry and T2-weighted imaging were based exclusively on measurements in a single mid-ventricular short axis slice. Many experts now recommend a wider representation of all regions of the LV in tissue characterization. Since many of the measures we assessed are associated with intramyocardial edema, which is rare in 2R rejection, it is perhaps unsurprising that the studied CMR parameters were unchanged. It is possible that, rather than detecting acute rejection, CMR may have a greater role in identifying long-term changes in the myocardium perhaps associated with cardiac allograft vasculopathy.

Studies in adults have produced mixed results with regards to the use of CMR as a screening tool for rejection and our pilot study did not identify CMR parameters altered by the presence of 2R rejection. However, myocardial tissue characterization by CMR is undergoing continuous refinement. Given the conceptual association between ACR and myocardial inflammation and the multiple disadvantages of EMB, CMR should continue to be evaluated for its ability to non-invasively detect rejection. Larger trials producing sizable cohorts of patients with clinically-significant rejection episodes and including T2 relaxometry are recommended.

COMMENTS

Background

Cardiac magnetic resonance imaging (CMR) has been proposed as a non-invasive method for the detection of rejection in adults after heart transplant (HT). However, CMR measurements used in adults for the detection of rejection or myocardial inflammation have not been systematically evaluated in pediatric HT recipients with biopsy-proven acute cellular rejection (ACR). In this pilot study, the authors sought to assess the utility of parameters of ventricular function and myocardial tissue characterization for the non-invasive detection of ACR in children and adolescents after HT.

Research frontiers

CMR tissue characterization overcomes important limitations of endomyocardial biopsy (EMB) such as the potential of containing scar from a previous EMB in the histological sample and the fact that specimens are collected from the RV surface of the interventricular septum and may not be representative of the remainder of the myocardium.

Innovations and breakthroughs

CMR has shown potential utility in adult heart transplant recipients. However, in this pilot study CMR did not reliably identify ACR-related changes in pediatric heart transplant patients.

Applications

Given the multiple disadvantages of EMB, CMR should continue to be evaluated for its ability to non-invasively detect rejection. Larger trials producing sizable cohorts of patients with clinically-significant rejection episodes and including T2 imaging are recommended.

Terminology

EMB: Invasive procedure used to sample the endomyocardium of the right ventricle to diagnose rejection; ACR: Damage created by T-cell mediated immune response directed by the recipient against the transplanted organ; T1- and T2-weighted imaging: MRI sequences that are used to differentiate tissues based mainly on their composition of fat and water.

Peer-review

The authors have produced an interesting study evaluating the use of CMR scanning as a means to diagnose acute cellular rejection in paediatric HT recipients.

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Corticosteroid minimization in renal transplantation: Careful patient selection enables feasibility

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Abstract

AIM

To explore the benefits and harms of corticosteroid (CS) minimization following renal transplantation.

METHODS

CS minimization attempts to improve cardiovascular risk factors (hypertension, diabetes, dyslipidemia), to enhance growth in children, to ameliorate bone disease and to lead to better compliance with immunosuppressive agents. Nevertheless, any benefit must be carefully weighed against the reduction in net immunosuppression and the potential harm to renal allograft function and survival.

RESULTS

Complete CS avoidance or very early withdrawal (*i.e.*, no CS after post-transplant day 7) seems to be associated with better outcomes in comparison with later withdrawal. However, an increased incidence of CS-sensitive acute rejection has been observed with all CS minimization strategies. Among the prerequisites for the safe application of CS minimization protocols are the administration of induction immunosuppression and the inclusion of calcineurin inhibitors in maintenance immunosuppression regimens.

CONCLUSION

Transplant recipients at low immunological risk (primary transplant, low panel reactive antibodies) are

thought as optimal candidates for CS minimization. CS avoidance may also be undesirable in patients at risk for glomerulonephritis recurrence or with severe delayed graft function and prolonged cold ischemia time. Thus, CS minimization is not yet ready for implementation in the majority of transplant recipients.

Key words: Acute rejection; Corticosteroid withdrawal; Corticosteroid minimization; Corticosteroid avoidance; Immunosuppression; Renal transplantation

Core tip: Although corticosteroids have been traditional components of immunosuppressive regimens in renal transplantation, corticosteroid minimization strategies are developed in an attempt to mitigate their many side-effects. The benefit from this approach must be balanced against the risk of acute rejection due to insufficient immunosuppression and the potential harm to allograft survival. We present an overview of these strategies and their impact on clinical outcomes analyzing the key clinical trials performed. Furthermore, we focus on patient selection according to the immunological risk and the induction immunosuppression, the principal factors that determine the success of corticosteroid withdrawal and avoidance protocols.

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INTRODUCTION

Corticosteroids (CS) have been ubiquitously included in immunosuppressive regimens since the early days of renal transplantation (Tx). They have significantly contributed to the successful transformation of a highly experimental intervention into a universally adopted clinical treatment. However, their use is associated with a plethora of adverse events due to their non-specific mode of action. The negative impact of CS on cardiovascular disease risk factors such as hypertension, diabetes mellitus, and dyslipidemia is well known. Non-cardiovascular adverse events such as growth retardation, impaired wound healing, subcapsular cataract, bone problems (osteoporosis, fractures, avascular necrosis) and cosmetic effects leading to patient non-compliance are equally established[1-4]. An increasing interest in minimizing the exposure to CS in transplant recipients with stable allograft function has been manifested by renal transplant clinicians to reduce the morbidity burden associated with their use. In the United States, CS avoidance regimens were administered to 23% of all first renal transplant recipients in 2004[5]. Among the remaining 77% who were discharged on CS, roughly 10% had CS withdrawn during the first post-transplant year. Nonetheless, this policy has to be carefully balanced against the risk of acute rejection due to insufficient immunosuppression and should not jeopardize renal allograft function and survival.

MATERIALS AND METHODS

Definitions

Strategies for CS minimization can be categorized as: (1) CS avoidance; and (2) CS withdrawal following a period after Tx. The latter can be further divided as early withdrawal (weeks or months after Tx, usually 3-6 mo after Tx) or late withdrawal (at least 6 mo after Tx). Overlapping between these categories has been reported in the literature leading to a degree of uncertainty over the exact terminology. For instance, very early withdrawal (< 2 wk) has been classified under both CS avoidance and CS withdrawal strategies. For the purpose of this manuscript, we will include very early withdrawal under the CS avoidance strategy. The overall efficacy of CS minimization regimens depends on the extent to which the rest of the immunosuppressive agents can suppress the alloimmune response and on the immunological risk stratification. In general, induction immunosuppression is required for the safe application of CS minimization as well as the inclusion of calcineurin inhibitors in maintenance immunosuppression. Patients at low immunological risk (first transplant, non-sensitized) are considered as ideal candidates for the implementation of CS minimization[6].

Data from studies on CS minimization have produced conflicting results regarding benefit *vs* harm. Clinical heterogeneity across these studies is moderate to high, especially regarding the spectrum of induction and maintenance immunosuppression agents used. Some studies have reported reductions in cardiovascular risk factors such as dyslipidemia[7], but there is no clearly proven reduction of the burden of cardiovascular disease. On the other hand, although CS avoidance or withdrawal studies resulted in increased rates of acute rejection, the impact on allograft survival appears to be neutral. Given the current dilemma over the efficacy *vs* safety profile of CS minimization strategies, our institution has continued on the traditional strategy of rapid CS tapering after Tx to the lowest possible dose. We reserve CS avoidance or withdrawal for highly selected cases at low immunological risk who present compelling contraindications to CS such as severe osteoporosis. We will try to elaborate on the potential advantages and disadvantages of our protocol focusing on the comparison with the CS minimization practices mentioned above. Our goal is to identify the optimal management strategy, which will allow for the maximum benefit of different patient subsets without compromising safety and will likely improve Tx outcomes.

Table 1 Characteristics of the multi-center, randomized Astellas Corticosteroid Withdrawal Study with a follow-up time of 5 years[8]

	CS withdrawal at day 7 arm (n = 191)	Standard CS arm (n = 195)	P-value
Baseline demographic, immunological risk and immunosuppressive therapy data			
Age (mean ± SD, yr)	46.6 ± 12.2	46.2 ± 12.7	NS
Female gender (%)	30.9	36.4	NS
African American (%)	17.8	21.5	NS
Deceased donor (%)	43.5	42.6	NS
Cold ischemic time (mean ± SD, h)	18.4 ± 5.7	17.2 ± 7.3	NS
HLA mismatch (mean)	3.5	3.5	NS
Current PRA (mean ± SD)	1.6 ± 5.3	1.8 ± 5.5	NS
Induction immunosuppression (%)			NS
Thymoglobulin	65.4	69.7	
Basiliximab	31.4	27.2	
Daclizumab	3.1	3.1	
Maintenance immunosuppression	TAC, MMF	TAC, MMF	
Main outcomes			
Biopsy-proven acute rejection (%)	17.8	10.8	0.04 (with Kaplan-Meier analysis)
Allograft survival (%)	94.2	93.3	NS
Patient survival (%)	94.2	96.4	NS
Creatinine clearance (Cockroft-Gault equation, mean ± SD, mL/min)	58.6 ± 19.7	59.8 ± 20.5	NS

CS: Corticosteroid; HLA: Human leukocyte antigens; MMF: Mycophenolate mofetil; NS: Not significant; PRA: Panel-reactive antibodies; SD: Standard deviation; TAC: Tacrolimus.

RESULTS

Low CS dose as maintenance therapy

We advocate the immunosuppressive protocol, which involves the administration of three daily intravenous pulses of 500, 250 and 250 mg methylprednisolone intraoperatively and on postoperative days 1 and 2 respectively. We attempt to rapidly taper CS dose to 20 mg oral methylprednisolone per day by 2-4 wk following Tx. Thereafter, we further reduce CS dose with the aim of 4 mg methylprednisolone per day at 3 mo in the absence of acute rejection. This dose is continued indefinitely. Data from randomized clinical trials (RCT) argue that maintenance CS treatment has still a dominant place in the management of renal transplant recipients. In the RCT with the longest follow-up to date (Astellas Corticosteroid Withdrawal Study), Woodle *et al*[8] assigned 386 renal transplant recipients with PRA (panel reactive antibodies) ⩽ 25% to either very early CS withdrawal at one week post-transplant or CS continuation tapered to 5 mg prednisolone per day at 6 mo (Table 1). All patients received induction immunosuppression; 68% of them with the lymphocyte-depleting agent anti-thymocyte globulin (ATG) and 32% with anti-interleukin-2 receptor monoclonal antibodies. Maintenance immunosuppressive regimen consisted of tacrolimus and mycophenolate mofetil (MMF). After a follow-up of 5 years, no difference was found in the rate of patient death, death-censored allograft loss and moderate/severe acute rejection. Total biopsy-confirmed acute rejection was lower in the CS continuation arm (10.8% *vs* 17.8%, P = 0.04). This result was driven by the increased rates of mild, CS-sensitive acute rejection in the very early CS withdrawal arm. It is interesting that biopsy-proven acute rejection rates were numerically lower with ATG induction than with anti-interleukin-2 receptor monoclonal antibodies in very early CS withdrawal patients, but that did not reach statistical significance (14.4% *vs* 24.2%, P = 0.09). Serum creatinine and creatinine clearance estimated by the Cockroft-Gault equation were similar between the two arms at 5 years. However, chronic allograft nephropathy (CAN) incidence at 5 years was more than double (9.9% *vs* 4.1%, P = 0.028) with very early CS withdrawal compared to a continuation. This finding raises an important concern. Although very early CS withdrawal seems to be non-inferior to CS continuation at 5 years concerning patient and allograft survival, it is unknown if the increased incidence of CAN would negatively influence those outcomes beyond that time-point. Clinical trials with extended follow-up time to 10 years are needed to resolve this issue. The effect of very early CS withdrawal on cardiovascular risk factors was mixed. No significant difference was found in hypertension, new-onset diabetes mellitus, total cholesterol and low-density lipoprotein (LDL) levels; very early CS withdrawal led only to improvement in serum triglycerides. As far as it concerns non-cardiovascular adverse events, very early CS withdrawal reduced bone fractures and avascular necrosis but it was paradoxically associated with more frequent subcapsular cataract.

A meta-analysis of 34 studies, which included 5637 patients, produced broadly similar results[9]. It was found that acute rejection risk was significantly increased with CS avoidance or withdrawal regimens compared to maintenance CS (relative risk: 1.56, 95%CI: 1.31-1.87, P = 0.0001). No statistically significant differences

were found for patient or allograft survival, but allograft function was modestly better with maintenance CS (weighted mean difference in creatinine clearance: 3.05 mL/min, 95%CI: 1.45-4.66). In contrast to the abovementioned RCT, occurrence of hypertension, new onset diabetes mellitus and hypercholesterolemia was reduced with CS avoidance or withdrawal regimens. However, the effect on hard cardiovascular endpoints cannot be estimated because included studies underreported cardiovascular events. In conclusion, acute rejection rates are constantly lower when CS maintenance regimens are used. Patient and allograft survival seems not to be influenced by CS minimization, but it is unknown if this remains the same with longer follow-up. Although CS minimization may permit some improvement in cardiovascular risk factors, data are not consistent about it.

CS avoidance

The rationale behind CS avoidance or very early withdrawal is that acute rejection may be triggered more easily with CS withdrawal within weeks or months after Tx. However, it invariably requires the use of potent induction immunosuppression and the selection of low immunological risk recipients. Attempts to use CS avoidance regimens in the absence of induction immunosuppression resulted in unacceptably high acute rejection rates[10]. In a three-arm multicenter RCT, which included 336 renal transplant recipients with PRA $\leqslant$ 20%, Vincenti *et al*[11] used basiliximab as an induction agent and compared no CS at all *vs* CS withdrawal by day 7 *vs* standard CS. Maintenance immunosuppression consisted of cyclosporine and enteric-coated mycophenolate sodium. Biopsy-proven acute rejection rates were significantly higher with complete CS avoidance and very early CS withdrawal regimens (31.5% *vs* 26.1% *vs* 14.7%) at a follow-up of 12 mo. No difference was found for patient and allograft survival as well as for median 12-mo estimated glomerular filtration rate. A prospective RCT, which included 300 patients, compared very early CS withdrawal at day 2 with standard CS[12]. It also used basiliximab for induction, but maintenance was a calcineurin inhibitor and mycophenolate mofetil or sirolimus. It found absolutely no difference in patient and allograft survival, acute rejection, incidence of CAN and allograft function between the two arms at 3 years. A lower frequency of new-onset diabetes mellitus was noted in the very early CS withdrawal group.

Induction with a lymphocyte-depleting agent (rabbit anti-lymphocyte globulin, rALG) was explored in the clinical context of CS avoidance for the first time by Laftavi *et al*[13]. They randomized 60 renal transplant recipients to either very early CS withdrawal at day 7 or CS continuation. Maintenance immunosuppression involved tacrolimus and MMF. No difference in acute rejection and allograft function was demonstrated with very early CS withdrawal at a follow-up time of 12 mo. However, increased interstitial fibrosis was found in protocol biopsies at 12 mo in the group of very early CS withdrawal. In a case series of 1241 renal transplant recipients with an impressive follow-up time of 10 years, the results of CS withdrawal at day 5 were reported[14]. All patients received induction immunosuppression with Thymoglobulin while maintenance immunosuppression comprised of a calcineurin inhibitor (tacrolimus or cyclosporine) and a secondary agent (MMF or sirolimus). Despite acute rejection rates of 25% for cadaveric donor Tx and 31% for living donor Tx at 10 years, patient and allograft survival was comparable to that reported in national registry databases. A beneficial effect of very early CS withdrawal was shown for new-onset diabetes mellitus, subcapsular cataract, and avascular necrosis. Till now, induction with lymphocyte-depleting agents seems to be the optimal option for consolidating the benefits of CS avoidance strategies without putting renal allografts at risk of acute rejection. It is not surprising that approximately 90% of United States renal transplant recipients with a steroid-free regimen on discharge have received induction with a lymphocyte-depleting agent[15]. Anti-interleukin-2 receptor monoclonal antibodies have been used in the remaining 10% of the patients.

The monoclonal lymphocyte-depleting antibody alemtuzumab has lately emerged as a promising CS-sparing agent. In a comparative, multicenter RCT, 852 unselected (both low and high immunological risk) renal transplant recipients were administered either induction with alemtuzumab (followed by reduced-dose tacrolimus and MMF without CS) or with basiliximab (followed by standard-dose tacrolimus, MMF, and CS)[16]. According to the preliminary results, alemtuzumab halved biopsy-proven acute rejection at 6 mo. Patient and allograft survival were not different between the two groups. Long-term follow-up results of this study are eagerly awaited. In a direct comparison of alemtuzumab with basiliximab (both arms were subjected to CS withdrawal by day 5) in a cohort of 335 low-risk patients, the rate of biopsy-confirmed acute rejection was lower with alemtuzumab (10% *vs* 22%, P = 0.003) at 3 years[17]. The major studies on CS avoidance are summarized in Table 2. Lastly, an important question is whether patients on CS avoidance regimens should be put in CS maintenance after treatment of an acute rejection episode. A retrospective study found that allograft survival is not affected by the introduction of CS maintenance or not but the lack of CS maintenance is a risk factor for a subsequent second acute rejection[18].

CS withdrawal

Early CS withdrawal: Initial attempts to apply early CS withdrawal under cyclosporine-based maintenance immunosuppressive regimens did not meet success[19,20]. The advent of more potent maintenance immunosuppressants like tacrolimus and MMF renewed researchers' interest in assessing the feasibility of early CS withdrawal (Table 3). Vanrenterghem *et al*[21] studied CS withdrawal 3 mo after Tx in 556 low immunological risk patients enrolled

Table 2 Characteristics of major randomized corticosteroid avoidance trials (the trial by Woodle *et al*[8] is described separately in table 1); $P > 0.05$ for all comparisons unless otherwise stated

Ref.	Patient number	Immunological risk	Timing of CS withdrawal	Induction immunosuppression	Maintenance immunosuppression	Biopsy-proven acute rejection (%)	Allograf/ patient survival (%)	Follow-up (mo)
Vítko *et al*[10]	151 147	Low/moderate (PRA < 50%, first transplant)	Day 1 Standard CS	No	TAC, MMF	30.5[f] 8.2[f]	97/99 96/100	6
Laftavi *et al*[13]	30 30	Low (PRA < 30%, first transplant)	Day 7 Standard CS	rALG	TAC, MMF	13 11	NR	12
Kumar *et al*[12]	150 150	Low (PRA < 10%)	Day 2 Standard CS	Basiliximab	TAC or CsA, MMF or sirolimus	16 14	78/91 79/89	36
Vincenti *et al*[11]	112 115 109	Low (PRA < 20%, first transplant)	No CS Day 7 Standard CS	Basiliximab	CsA, EC-MPS	31.5[a] 26.1[b] 14.7[b,a]	96/95 98/98 97/98	12
Hanaway *et al*[17]	164 171	Low (PRA < 20%, first transplant)	Day 5 Day 5	Alemtuzumab Basiliximab	TAC, MMF	10[d] 22[d]	93/95 92/98	36
Haynes *et al*[16]	426 426	Unselected patients	No CS Standard CS	Alemtuzumab Basiliximab	Low-dose TAC-MMF/ Standard TAC-MMF	7[h] 16[h]	96/97 97/99	6

[a]$P = 0.046$, [b]$P = 0.004$, [d]$P = 0.003$, [f]$P < 0.001$, [h]$P = 0.0001$. CS: Corticosteroids; CsA: Cyclosporine; EC-MPS: Enteric-coated mycophenolate sodium; MMF: Mycophenolate mofetil; NR: Not reported; PRA: Panel-reactive antibodies; rALG: Rabbit antilymphocyte globulin; TAC: Tacrolimus.

in a multicenter RCT. Maintenance immunosuppression consisted of tacrolimus and MMF. In the follow-up time of only 6 mo, it was shown that acute rejection rates were higher in the CS withdrawal arm during months 3-6. Mean total cholesterol and LDL were reduced in the CS withdrawal arm at the same period. Pascual *et al*[22] summarized RCTs in CS withdrawal between 3 and 6 mo in a systematic review including 9 studies with 1820 patients. They concluded that patient and allograft survival is not affected by early Cs withdrawal up to 3 years after Tx. Total acute rejection rates were higher with early CS withdrawal in cyclosporine-treated patients. Although reduction of total cholesterol levels was observed with early CS withdrawal, no significant difference was found for any of the other cardiovascular or non-cardiovascular adverse events. It is worth mentioning that induction immunosuppression was not used in any of the included studies. Overall, evidence about the benefit-risk ratio of early CS withdrawal is weaker than that of CS avoidance and follow-up times are shorter. It is unknown if induction with lymphocyte depleting agents or anti-interleukin-2 receptor monoclonal antibodies were used in any of the studies, it would have any meaningful impact on the results.

Late CS withdrawal: It appears that late CS withdrawal (more than 6 mo and possibly years after Tx) represents the least favorable method of the CS minimization strategies. It is apparent that certain CS-related complications would already have been established by that time. For instance, it is well known that a rapid deterioration in osteoporosis occurs within the first post-transplant year[23]. Moreover, acute rejection risk is clearly increased upon late withdrawal of immunosuppressants as dictated by cases of non-compliant patients[3]. In a single-center RCT, Smak Gregoor *et al*[24] examined the effect of CS withdrawal at 6 mo after Tx in 212 renal transplant recipients. Biopsy-proven acute rejection was manifested in 4% of CS withdrawal patients *vs* 1.4% of controls ($P > 0.05$). Patient and allograft survival was not different after a follow-up of 2 years. Allograft function was also not different. CS withdrawal resulted in reduced mean blood pressure but had no effect on other metabolic risk factors. Interestingly enough, a prospective, observational study from the Collaborative Transplant Study group reported that in renal transplant recipients with CS withdrawal more than 6 mo from Tx, patient and allograft survival was better than retrospectively matched controls over a follow-up time of 7 years with no difference in acute rejection rates[25]. The reduction was also noted in the incidence of cardiovascular parameters. However, the lack of randomized design remains a significant limitation of this study.

DISCUSSION

Challenges and opportunities of CS minimization strategies

The beneficial effects of CS minimization in selected, low-risk patients have prompted researchers to attempt CS minimization in renal transplant recipients at higher immunological risk. However, available data are sparse (Table 4). In a small RCT, 21 patients with PRA > 20% or retransplantation were assigned to either alemtuzumab and tacrolimus monotherapy without CS or Thymoglobulin with standard tacrolimus, MMF and very early CS withdrawal at day 5[26]. Biopsy-proven acute rejection rates were quite high at one year; 18.2% with alemtuzumab *vs* 37.5% with Thymoglobulin. In a more recent, head to head comparison of alemtuzumab with ATG (both

Table 3 Characteristics of major randomized corticosteroid withdrawal trials

Ref.	Patient number	Immunological risk	Timing of CS withdrawal	Induction immunosuppression	Maintenance immunosuppression	Biopsy-proven acute rejection (%)	Allograft/ patient survival (%)	Follow-up (mo)
Vanrenterghem *et al*[20]	252	Low	At month 3	No	CsA, MMF	23[b]	95/99	12
	248		Standard CS			14[b]	96/98	
Smak Gregoor *et al*[24]	76	Low	After month 6	No	CsA, MMF	4.0[a]	98/97	24
	73		Standard CS			1.4	97/97	
Vanrenterghem *et al*[21]	279	Low	After month 3	No	TAC, MMF	5.9[a,d]	93/99	6
	277		Standard CS			0.9[d]	94/98	

$P > 0.05$ for all comparisons unless otherwise stated. [a]After CS discontinuation, [b]$P = 0.008$, [d]$P = 0.004$. CS: Corticosteroids; CsA: Cyclosporine; MMF: Mycophenolate mofetil; TAC: Tacrolimus.

Table 4 Characteristics of corticosteroid avoidance/withdrawal trials in immunologically high-risk and in pediatric patients

Ref.	Patient number	Immunological risk	Timing of CS withdrawal	Induction immunosuppression	Maintenance immunosuppression	Acute rejection (%)	Allograft/ patient survival (%)	Follow-up (mo)
Immunologically high-risk patients								
Thomas *et al*[26]	11	PRA > 20%, or repeat transplant	No CS	Alemtuzumab	TAC	18.2	86/100	12
	10		Day 5	ATG	TAC, MMF	37.5	88/88	
Hanaway *et al*[17]	164	PRA > 20%, or black race, or repeat transplant	Day 5	Alemtuzumab	TAC, MMF	18	91/99	36
	171		Day 5	ATG		15	84/91	
Pediatric patients								
Grenda *et al*[28]	98	Low/ moderate (PRA < 50%)	Day 4	Daclizumab	TAC, MMF	10.2	97/99	6
	98		Standard CS	No induction		7.1	97/100	
Höcker *et al*[29]	23	Moderate/ high (PRA < 80%)	After year 1	No	CsA, MMF	4	100/100	24
	19		Standard CS			10	100/100	

ATG: Antithymocyte globulin; CS: Corticosteroids; CsA: Cyclosporine; MMF: Mycophenolate mofetil; PRA: Panel-reactive antibodies; TAC: Tacrolimus.

arms underwent CS withdrawal by day 5) in a cohort of 139 high-risk patients, there was no difference in biopsy-proven acute rejection at 3 years (18% *vs* 15%, $P = 0.63$)[17]. The inference is that CS minimization is not yet ready for prime time in immunologically high-risk patients. It has been hypothesized that CS minimization may increase post-transplant glomerulonephritis recurrence. In a major retrospective study, it was found that recurrence rate was indeed higher with rapid CS discontinuation compared to CS maintenance for all glomerulonephritis types (hazard ratio 4.86, 95%CI: 2.34-10.07, $P < 0.0001$)[27]. The analysis also showed no difference in patient, allograft, and death-censored allograft survival. Pediatric patients are a subgroup in which CS minimization may be of special interest due to growth retardation that is associated with chronic CS use (Table 4). In a multicenter RCT, Grenda *et al*[28] assessed the effect of CS withdrawal at day 4 (together with daclizumab induction and tacrolimus, MMF) *vs* standard tacrolimus, MMF, and CS in a cohort of 196 children. Growth was significantly enhanced at 6 mo by CS withdrawal. Patient survival, allograft survival and allograft function were not different. The effect of CS withdrawal on total cholesterol and triglycerides was positive. Similar results were obtained by Höcker *et al*[29] who evaluated CS withdrawal $\geqslant$ 1 year after Tx in 42 moderate- to high-risk children (maintenance immunosuppression was cyclosporine and MMF).

In contrast to the perceived benefits of CS minimization in younger transplant recipients, this strategy may not be suitable for elderly patients. Although acute rejection rates may be lower in the elderly, it has been suggested that acute rejection may be more severe and lead to a compromised death-censored allograft survival[30]. Furthermore, the potentially beneficial effect of CS minimization in cardiovascular disease risk factors in the elderly may not be relevant due to their limited lifespan. For these reasons, it seems that CS minimization in the elderly may result in poor outcomes and should not be exercised except with extreme caution. Finally, CS minimization may not also be suitable for transplant recipients with delayed graft function (DGF) and prolonged cold ischemia time. The ischemic injury in these allografts is strongly associated with the development of acute rejection[31]. Therefore, it is

prudent to avoid CS minimization in this patient subgroup if possible.

Based on current evidence, we believe that the majority of renal transplant recipients should continue to receive indefinite CS maintenance immunosuppression. However, selected patients can be good candidates for CS minimization protocols. The optimal patient phenotype to undergo CS minimization is that of a young transplant recipient (including children) who has no prior transplants and is unsensitized to HLA alloantigens. A primary disease that caused end-stage renal disease should not be glomerulonephritis. Any severe perioperative ischemic insult to the allograft should discourage the application of CS minimization. As such, CS minimization may be contraindicated with DGF, prolonged cold ischemia time, and donation after cardiac death. Available data indicate that the preferred CS minimization strategy is probably either complete CS avoidance or very early CS withdrawal. ATG (or alemtuzumab) may be preferable to anti-interleukin-2 receptor monoclonal antibodies as induction agents in this clinical scenario whereas maintenance immunosuppression should better contain the calcineurin inhibitor tacrolimus instead of cyclosporine.

In conclusion, CS maintain their position as important components of the therapeutic armamentarium in renal transplantation. A movement towards CS elimination from induction and maintenance immunosuppression regimens has developed to reduce the myriad side effects associated with chronic CS use. CS minimization strategies have resulted in an increased incidence of acute rejection compared to CS continuation. However, these acute rejection episodes are considered mild and amenable to treatment. Moreover, they do not seem to have detrimental effects on patient survival, allograft survival or allograft function at a follow-up until 5 years. Nonetheless, an observed trend towards increased fibrosis is alarming and calls for the conduction of RCTs with longer follow-up to determine the true consequences of CS minimization. Although CS minimization protocols have been associated with a reduction of adverse effects (especially improvement of dyslipidemia), these results are not always reproducible, and it is unclear if they could clinically translate to less cardiovascular events. At present, the implementation of CS minimization cannot be universally recommended to renal transplant recipients.

COMMENTS

Background

Due to their immunosuppressive properties, corticosteroids (CSs) have been extensively used for the prevention and treatment of rejection in solid organ transplantation. However, myriad side-effects have been associated with CS. Recent research attempts to minimize CS use in renal transplantation in an effort to reduce the burden of their side-effects without compromising allograft and patient survival.

Research frontiers

The choice of the induction immunosuppression agent - and of maintenance immunosuppression to a lesser degree - is an extremely important aspect of CS minimization strategies and the focus of many studies. Studies with follow-up times of more than 5 years and with data on interstitial tissue fibrosis are needed.

Innovations and breakthroughs

Alemtuzumab appears to be a very promising induction agent potentially allowing the combination of CS avoidance with lower doses of tacrolimus and mycophenolate mofetil. Long term results of the 3C study may provide valuable insights on this topic.

Applications

In the United States, CS avoidance regimens were administered to 23% of all first renal transplant recipients in 2004. Although they cannot yet be recommended to the majority of renal transplant recipients, selected patient groups such as primarily those with low immunological risk and low risk for glomerulonephritis recurrence may benefit more from CS minimization.

Terminology

CS avoidance: Either no CS use at all or CS use only until day 7 after transplantation (Tx). CS withdrawal: CS tapering following a period after Tx. It is divided as early withdrawal (weeks or months after Tx, usually 3-6 mo after Tx) or late withdrawal (at least 6 mo after Tx).

Peer-review

This review paper is a well written paper of the impact of corticosteroid minimisation on kidney transplant and has valuable information.

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Systematic review of the negative pressure wound therapy in kidney transplant recipients

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Abstract

AIM

To review negative pressure wound therapy (NPWT) as an important addition to the conventional methods of wound management.

METHODS

A systematic review, performed by searching the PubMed, EMBASE and Cochrane Library databases, showed 11 case reports comprising a total of 22 kidney transplantation (KT) patients (range, 1 to 9), who were treated with NPWT. Application of NPWT was associated with successful healing of wounds, leg ulcer, lymphocele and urine leak from ileal conduit.

RESULTS

No complications related to NPWT were reported. However, there was paucity of robust data on the effectiveness of NPWT in KT recipients; therefore, prospective studies assessing its safety and efficacy of NPWT and randomised trials comparing the effectiveness of NPWT with alternative modalities of wound management in KT recipients is recommended.

CONCLUSION

Negative pressure incision management system, NPWT with instillation and endoscopic vacuum-assisted closure system are in investigational stage.

Key words: Negative pressure; Wound therapy; Kidney transplantation; Wound infection; Wound dehiscence

Core tip: Systematic review of the safety and efficacy of negative pressure wound therapy (NPWT) in kidney transplant (KT) recipients revealed 11 case reports, which have shown the effective role NPWT in the management of wound dehiscence, lymphocele, urine leak from ileal conduits and leg ulcers. Because of the lack of robust evidence on the safety and efficacy of NPWT in KT patients, prospective multicentre studies recruiting large number of patients is recommended to examine the role

of NPWT in the treatment of wound-related complications in KT recipients. The efficacy of negative pressure incision management system, NPWT with instillation and endoscopic vacuum-assisted closure system remain in investigational stage.

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INTRODUCTION

Kidney transplantation (KT) represents the best treatment modality for patients with end-stage renal disease, providing the best outcomes for survival, quality of life and cost-effectiveness[1]. Immunosuppressive agents administered to prevent rejection and prolong transplant survival, not only increase susceptibility to infections, but also delay wound healing. Post-operative wound infection leading to cavitation and dehiscence continue to remain serious problems resulting in extended hospital stay, readmissions, repeated surgical interventions and protracted recovery, thereby imposing extra cost to the healthcare delivery system[2]. The wound complication rate after KT ranges between 2% to 47%. The risk factors for these complications are advancing age, diabetes mellitus, body mass index, kidney failure, type of surgical incision, re-operation, operator's experience, and immunosuppressive drugs including sirolimus and steroids[3-6]. The wound-related complications can present as superficial infection, haematomas, lymphocele, and partial or full-thickness wound dehiscence leading to incisional hernias[7].

Negative pressure wound therapy (NPWT), also referred to as, vacuum-assisted closure therapy (VACT), topical negative pressure therapy or microdeformational wound therapy has been evaluated over last two decades and is considered as an useful adjunct to the management of diverse range of lesions including open abdominal wounds, open fractures, post-traumatic wounds, split-thickness skin grafts and after clean surgery in obese patients[8-13]. Application of any new form of treatment in KT patients is associated with concerns on the part of clinicians, particularly when robust evidence supporting their safety and efficacy are lacking. A systematic review of the published literature was carried out to evaluate the effectiveness and safety of NPWT in KT recipients presenting with wound-related complications.

MATERIALS AND METHODS

Literature search

A systematic electronic literature search was performed in PubMed, EMBASE and Cochrane Library databases from inception to March 2016. The search terms "renal transplantation", "kidney transplantation", "negative pressure wound therapy", "vacuum-assisted closure", "wound", and "topical negative pressure therapy" were used. EndNote software (Version X7.5, BLD 9325; Thomson Reuters, Philadelphia, PA, United States) was used to compile pertinent references.

Renal transplantation technique

KT is performed by using classical Gibson's muscle-cutting incision, where the iliac vessels and urinary bladder are accessed extraperitoneally. The renal vessels are anastomosed to the iliac vessels and the ureter to the bladder by the techniques described previously[14]. Wound infection leading to muscular dehiscence exposes the kidney to the external environment, which predisposes to infection around the kidney, haemorrhage from mycotic aneurysms of the vascular anastomoses, lymph leak, urine leak, and dehiscence of muscle layers leading to incisional herniation.

Principles of NPWT

The beneficial effect of negative sub-atmospheric pressure on the wound results in gradual closure of wound edges by micro- and macrodeformation of the wound surface, and by suction of infectious material and interstitial fluid, reduces tissue oedema. Decompression of tissue increases blood flow and tissue oxygenation, thereby accelerates the wound healing cascade including, angiogenesis, neurogenesis, granulation tissue formation, cellular proliferation, differentiation and migration of appropriate cellular components at the site of healing[15-20].

Glass *et al*[21] in a systematic review, evaluated the molecular basis for the promotion of wound healing by NPWT and observed an increase in the expression of cytokines, chemokines and growth factors, which reflected mechanoreceptor and chemoreceptor transduction in response to stress and hypoxia. There was reduction of expression of matrix metalloproteinase-1, -2, -9 and -13, with no changes on the activity of tissue inhibitor of metalloproteinase-1[21].

The NPWT device comprises of black polyurethane ether foam dressing or white polyvinyl alcohol foam, which is tailored to fit into the dimension of the wound. A tube with multiple perforations is placed within the foam for the evacuation of the wound discharge. The tube together with the foam is then covered with an occlusive drape, which helps to maintain uniform negative pressure. The effluent of the wound is collected in a canister, which is attached to the vacuum pump with an adjustable negative pressure, ranging from 50 and 125 mmHg (Figure 1A and B). At the interval 48-72 h, the soiled dressing is replaced with fresh dressing at the bedside, when progress of healing is assessed. The device can be used in preparation for secondary suture, a skin graft, flap or until full closure of the wound has taken place[22,23]. The oldest and most popular device in clinical practice is the vacuum-assisted

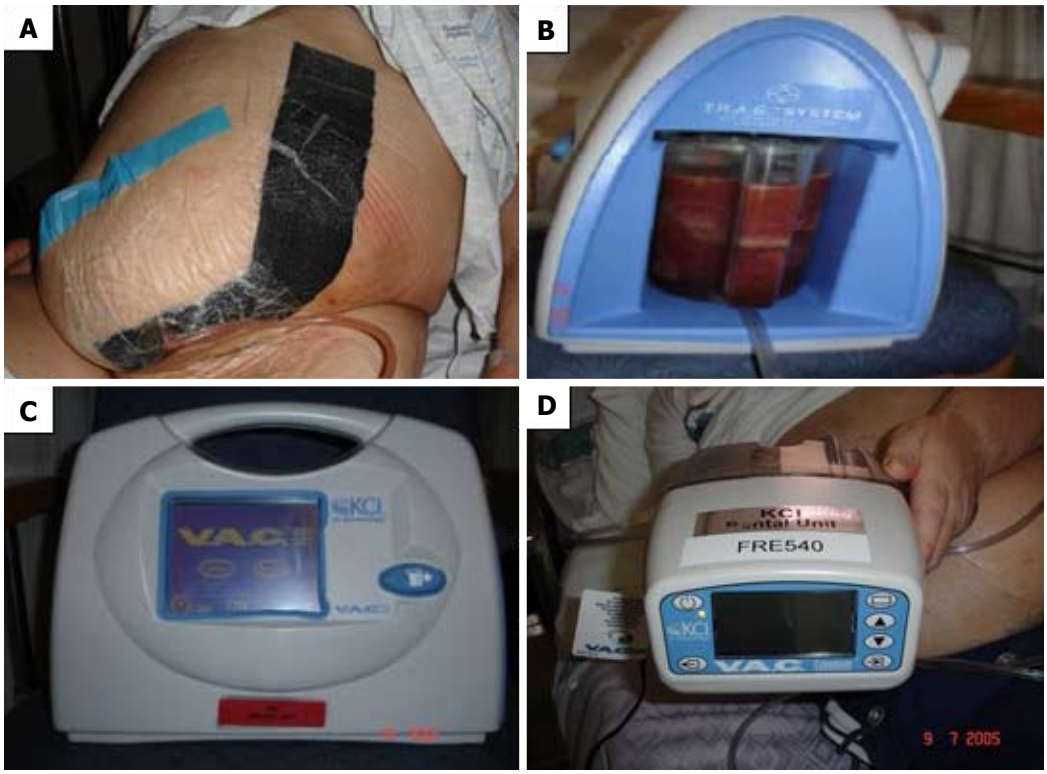

Figure 1 Negative pressure wound therapy device and its components. A: Black polyurethane foam dressing and tubing in the wound; B: Canister containing exudates; C: Standard suction device; D: Portable suction device.

closure (VAC® KCI, San Antonio, Texas) system (Figure 1C and D)[24].

The contra-indications for the applications of NPWT are excessive pain with NPWT, presence of pus or excessive bleeding and intolerance. The success of NPWT is assessed by the reduction in wound size by at least 10% per week or 50% improvement over 4 wk period, which indicates high probability of success of the therapy[25].

RESULTS

The literature search identified 11 case reports comprising of a total of 22 KT patients (range, 1 to 9) (Table 1), who were treated with NPWT[26-36]. Comparison between NPWT and other methods of wound treatment in KT patients has not been reported in any study.

In 2003, Hodzic *et al*[32], for the first time, reported successful outcome of application of NPWT for 15 d in 2 KT patients prior to secondary suture of wounds. Successful treatment of an infected and dehisced laparotomy wound following liver and KT in a patient by employing NPWT was reported by Zanus *et al*[26], where associated complications included acute pancreatitis, abdominal compartment syndrome and wound infection by multi-drug resistant organisms. The patient required 14 successive laparotomies and NPWT for 6 mo for complete closure of the wound[26]. Similarly, Markić *et al*[34] have described successful treatment with application of NPWT in 2 KT patients who developed infected and dehisced wounds. NPWT was applied for 2 and 3 wk, respectively, which was followed by secondary sutures[34].

The occurrence of ureteric complications significantly delays the recovery following KT and the incidence of such complication ranges between 1.2% and 8.9%[37]. Urinary leak rate of 11% requiring re-implantation was reported by Surange *et al*[38] in a series of KT into ileal conduits. Two cases of urine leak and wound dehiscence following KT into ileal conduits were managed successfully by Heap *et al*[28] with the application of NPWT. Secondary suture of the wounds was carried out after two and three months in these patients. The renal function was restored in both patients leading to 141 μmol/L and 75 μmol/L of serum creatinine, respectively, at the end of 3 mo[28]. On the other hand, Ortiz *et al*[27], had negative experience of NPWT in a KT recipient with peri-renal collection and wound infection. They concluded that NPWT had encouraged and prolonged urine leak, which had healed after 5 d of discontinuation of NPWT[27]. Iesari *et al*[36] had applied VAC device in a KT patient who had developed spontaneous rupture of urinary bladder due to gangrenous cystitis and extensive wound dehiscence associated with multidrug resistant *Acinetobacter baumanni* infection. There was significant urine leak following VAC therapy, hence this was discontinued and topical homologous platelet-rich gel was used resulting in complete wound healing[36].

Infection caused by virulent organisms after skin grafts and reconstructive surgery in KT recipients not only lead to failure of treatment, but also can be life-

Table 1 Characteristics of studies

Ref.	Year	Country	No. of cases	No. of NPWT days	Indications
Iesari *et al*[36]	2015	Italy	1	Not described	Wound dehiscence
Bozkurt *et al*[33]	2015	Turkey	1	5	Primary surgery
Markic *et al*[34]	2014	Croatia	2	14, 21	Wound dehiscence
Franchin *et al*[35]	2014	Italy	1	45	Infected lymphocele
Zanus *et al*[26]	2011	Italy	1	180	Wound dehiscence, pancreatitis
Ortiz *et al*[27]	2011	United States	1	15	Wound infection
Heap *et al*[28]	2010	United Kingdom	2	Not described	Wound dehiscence, Urine leak
Thodis *et al*[29]	2009	Greece	1	Not described	Vibrio infection of leg
Devries *et al*[31]	2009	United States	1	Not described	Leg wound
Shrestha *et al*[30]	2007	United Kingdom	9	3, 5, 5, 5, 8, 10, 10, 15, 30	Wound dehiscence, infection
Hodzic *et al*[32]	2003	Germany	2	15	Wound dehiscence

NPWT: Negative pressure wound therapy.

threatening. Thodis *et al*[29] treated soft tissue infection caused by Vibrio vulnificus with NPWT, which involved the leg in a KT recipient. Autologous platelet concentrate spray further enhanced granulation tissue formation leading to complete epithelialization of the wound after 4 wk[29]. In a similar situation, Devries *et al*[31] were unsuccessful in treating soft tissue infection on the leg of a KT recipient, that culminated in amputation. As the patient was on sirolimus, wound healing could have been compromised by the same drug[5,31,39].

Lymphocele following KT can cause significant morbidity due to infection and compression of ureter and blood vessels. The reported incidence of lymphocele ranges between 0.6% to 49%[40]. Franchin *et al*[35] have described successful management of a large deep-seated lymphocele infected with *Staphylococcus haemolyticus*, *Escherichia coli* and *Enterococcus faecal,* with the application of NPWT. Following surgical drainage, the wound had completely dehisced and transplanted kidney exposed. The cavity was packed with foam dressing and device was applied. A negative pressure of 80 mmHg was maintained. The dressing was changed every 5 d. After 45 d, the lymphocele had sealed and skin closed[35].

In a prospective study reported by Shrestha *et al*[30], 9 KT patients had developed wound infection with cavitation and wound dehiscence. This was associated with significant amount of discharge from the wound, which failed to respond to standard method of treatment. Treatment with NPWT for a median of 9 (range 3-30) d led to cessation of discharge from the wound. Of the 9 patients, 4 patients were managed on an outpatient with portable NPWT device, where the treatment was discontinued after a median of 5.5 (range 3-7) d. The median hospital stay since the employment of NPWT was significantly shorter (5, range 2-12 d) compared to the standard method of treatment prior to application of NPWT (11 d, range, 5-20 d; P = 0.003). The wound healed completely in all 9 cases after the therapy[30].

Recently, Bozkurt *et al*[33], for the first time, employed Prevena incision management system (Kinetic Concept Inc. San Antonio, Texas, United States) to the clean closed surgical wound for 5 d after a KT and observed complete healing of the wound with no skin or device-related problems.

DISCUSSION

All infected wounds with associated collections require surgical drainage for early healing. Fleischmann *et al*[41] from Germany, in 1993, for the first time described the benefit of exposing wounds to sub-atmospheric pressure, which promoted wound debridement and healing. He applied this method in 15 patients with compound fractures and observed enhanced proliferation of the granulation tissue with no associated bone infection leading to complete healing of fractures[41]. In 1997, Louis Argenta and Michael Morykwas introduced NPWT therapy, for the first time, in the treatment of bed sores and slow healing wounds. Since then, NPWT has been extended to treat various types of wounds resulting from surgery, trauma, infection, congenital deformities and tumours[42-44]. The experience of NPWT gained over the past two decades has encouraged clinicians to treat patients globally in both hospital and domiciliary environments[44-46].

This systematic review has confirmed the available evidence on the safety and efficacy of the application of NPWT in KT recipients limited to case reports. On the other hand, the reported experiences do support NPWT in the management of complex wounds following KT, including urine leak from KT in ileal conduits and lymphoceles. The theoretical risk of haemorrhage and urine leak from transmission of suction pressure on the vascular and ureteric anastomoses cannot be ignored. Prolonged urine leak had occurred in two reported cases after KT where NPWT was applied. Discontinuation of NPWT had led to resolution of urine leak. In author's single KT patient with a urine leak from the ureterovesical junction, treatment with NPWT led to persistence of urine leak for 1 wk. Resolution of the urine leak occurred 2 d after discontinuation of NPWT therapy. Successful outcomes of NPWT in the management of wound infections in cardiac and liver transplant recipients have been described previously[47,48].

Development of enterocutaneous fistula during the course of NPWT is always a concern, which is particularly

applicable in in deep wounds after KT, where thin layer of peritoneum lies between the bowel and the foam dressing. Occurrence of enterocutaneous fistula has been observed after NPWT in open abdominal wound. However, the evidence in support of the occurrence of this complication after NPWT is weak[49-51].

Shrestha *et al*[30], in their largest reported series of 9 patients, observed benefit of NPWT on wound healing, reduced hospital stays and convenience of wound management. The management of 4 patients on an outpatient basis with the NPWT device *in situ*, was convenient to the patient and saved hospital cost significantly[30].

Comparison of NPWT with standard treatment modalities

There is no data available comparing the safety and efficacy of NPWT over conventional methods of wound management in KT recipients. However, there are several randomised trials and meta-analyses, which have assessed the effectiveness of NPWT for skin grafts and surgical wound healing by primary and secondary intentions and in chronic wounds compared with several conventional treatment methods. With regards to healing of surgical wound by primary intention, the evidence for the effect of NPWT for reducing surgical site infections, time to complete healing and wound dehiscence remains unclear[52]. A Cochrane Database Systematic Review assessed the effect of NPWT for the treatment of chronic wounds in comparison with five different comparators, which did not show that NPWT significantly increased the healing rate. The trials did have methodical flaws, therefore need for better quality research was recommended[53]. Similarly, a recent Cochrane review did not show clinical effectiveness of NPWT over alginate dressings in the treatment of open infected groin wounds and a silicone dressing in the treatment of excised pilonidal sinus when they were allowed to heal by secondary intention[54].

NPWT with instillation

NPWT with instillation (NPWTi) is a recent advancement, which is being assessed in the management of complicated surgical wounds. The wound is covered with normal saline (0.9%) and left for 10-20 min for diffusion to take place. Then, 2-4 h of negative pressure at -125 mmHg is applied. A panel of experts in the first International Consensus Guidelines for NPWTi have recommended its use in high risk patients with multiple comorbidities including diabetes, contaminated traumatic wounds, and wounds complicated by invasive infection or extensive biofilm. Available evidence suggest achievement of better outcomes with the addition of NPWTi to standard of care in properly selected cases, compared to standard care alone[55,56]. As majority of KT recipients often have associated co-morbidities, NPWTi may be an option in this group of patients.

Negative pressure incision management

Colli *et al*[57] employed the negative pressure incision management system in clean closed incisions, for the first time, in 10 patients after cardiac surgery and observed normal wound healing in patients where complications were expected after surgery. Bozkurt *et al*[33], have reported their experience of using Prevena incision management device in a KT recipient. A recent meta-analysis of NPWT for closed surgical incisions, (including 10 studies, 1311 incisions in 1089 patients) showed significant reduction in wound infection (RR = 0.54) and seroma formation (RR = 0.48), when NPWT was compared with standard care. The reduction in wound dehiscence was not significant. The numbers needed to treat were 3 (seroma), 17 (dehiscence) and 25 (infection). Due to heterogeneity between the included studies, no general recommendations could be made yet[58]. However, this device has a potential for its use in immunosuppressed and obese patients undergoing KT.

Endoscopic vacuum-assisted closure system

Endoscopic vacuum-assisted closure system (E-VAC) has developed as an important alternative in patients with upper gastrointestinal leaks not responding to standard endoscopic or surgical treatment procedures. Leak from oesophageal and gastric anastomosis sites and perforations resulting from endoscopic procedures were successfully closed using the E-VAC therapy[59,60]. Application of this device in KT recipients remains to be explored.

This systematic review has shown successful healing of wounds, leg ulcer, lymphocele and urine leak from ileal conduit following application of NPWT in KT recipients and there was no report of complications associated with NPWT. However, there is lack of robust evidence on safety and efficacy of NPWT in KT patients. Based on available evidence on the application of NPWT in KT recipients, NPWT can be considered as a valuable adjunct in the management of infected and dehisced wounds following KT. The safety and efficacy of NPWT, negative pressure incision management system, NPWT with instillation and E-VAC system, and efficacy of NPWT in comparison with standard methods of wound management, need to be examined prospectively by including large number patients in multicentre studies.

COMMENTS

Background

Negative pressure wound therapy (NPWT) is a useful adjunct to the conventional methods of management of infected wounds with deep cavitation in the kidney transplant (KT) recipients. A systematic review was performed to assess the safety and efficacy of NPWT in KT recipients, which showed 11 case reports including 22 KT recipients who were treated with NPWT showing beneficial outcomes.

Research frontiers

There are no randomised trials comparing the safety and efficacy of NPWT with alternative modalities of wound management, hence multicentre prospective study by including large number of patients is recommended.

Innovations and breakthroughs

The negative pressure incision management, NPWT with instillation, and

endoscopic vacuum-assisted closure system are the new developments in this field, which need to be applied and examined in the RT recipients.

Applications

NPWT has been applied in the treatment of abdominal wounds, leg ulcers, lymphoceles and urine leak from ileal conduit in RT recipients successfully.

Terminology

Negative pressure wound therapy (NPWT), also referred to as, vacuum-assisted closure therapy (VACT), topical negative pressure therapy (TNPT) or microdeformational wound therapy.

Peer-review

The authors made a comprehensive review on NPWT on KTx recipients. It provides useful information for clinicians.

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SYSTEMATIC REVIEWS

Outcomes in randomized controlled trials of exercise interventions in solid organ transplant

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Abstract

AIM

To identify the outcome measures that have been used in randomized controlled trials (RCTs) of exercise training in solid organ transplant (SOT) recipients and to link these outcomes to the International Classification of Functioning, Disability and Health (ICF) framework.

METHODS

Electronic literature searches of MEDLINE, EMBASE, CINAHL, Cochrane, Scopus, and Web of Science were performed. We sought RCTs that investigated the effect of exercise training in SOT recipients. Reference lists of all eligible publications were searched for other appropriate studies not identified by the electronic search. A complete list of outcome measures used in the RCTs was generated and each of these was linked to an ICF category.

RESULTS

Four hundred and thirteen articles were retrieved, of which 35 met our inclusion criteria. The studies included were designed to compare the effects of exercise training programs to usual care or to another exercise training program and reported on recipients of heart (n = 21), kidney (n = 9), lung (n = 3) or liver (n = 2) transplant. Of the 126 outcome measures identified, 62 were used as primary outcome measures. The most commonly occurring primary outcomes were aerobic capacity using the peak VO_2 (n = 13), quality of life using the short-form-36 (n = 8), and muscle strength (n = 7). These

outcome measures were linked to 113 ICF categories and the majority of outcomes fall into the body function domain (n = 93).

CONCLUSION

There is little standardization in outcome measures used in RCTs of exercise interventions in SOT recipients. The ICF framework can be used to select a core set of outcomes that cross all domains of ICF and that would be appropriate to all SOT recipients.

Key words: Solid organ transplantation; Systematic review; Rehabilitation; Exercise; Outcome measures; International Classification of Functioning, Disability and Health

Core tip: Over 30 randomized controlled trials (RCTs) have been conducted to examine the effectiveness of exercise training on outcomes in solid organ transplant recipients. However, the synthesis of findings across studies has been limited by the lack of similar outcomes. We identified 126 unique outcomes used in RCTs of exercise training and categorized them according to the International Classification of Functioning, Disability and Health framework. Most commonly, outcomes fell into the domains of body structure and body function, whereas there were a limited number of outcomes examining activities and participation. This review highlights the need for a core set of outcomes for RCTs in exercise training for this population.

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INTRODUCTION

As the acute morbidity and mortality associated with solid organ transplantation continues to improve, interventions that improve quality of life and long-term health outcomes are needed. Exercise training has several important health benefits for solid organ transplant (SOT) recipients, such as improving maximal aerobic capacity (VO_2 peak), body composition and quality of life[1]. Exercise and physical activity also have potential effects for mitigating long-term complications post-transplant and side-effects of immunosuppressant medication such as reducing blood pressure, controlling blood glucose[2], managing weight gain[3], improving muscle[4] and bone strength[5], and reducing fatigue[6-8]. A limitation of the current literature on exercise for SOT is the inability to combine outcomes from studies due to the wide range of reported outcomes. In a systematic review of exercise training in SOT recipients conducted in 2012 by Didsbury *et al*[1], the authors included 15 randomized controlled trials (RCTs) with 28 unique outcomes. The majority of outcomes were related to cardiovascular parameters (VO_2 peak, blood pressure, cholesterol), with fewer studies examining body composition, frailty indicators or quality of life. The authors were therefore hampered in their ability to conduct meta-analyses, which limited the conclusions of their comprehensive review.

The inability to synthesize data from studies in the field of SOT is of particular concern, as this is a small population and studies on exercise training are often conducted at single transplant centres with relatively small sample sizes. In order to gain greater statistical power to draw conclusions, studies need to be combined using knowledge synthesis approaches, which require common outcomes. Inconsistencies in the reporting of outcomes can affect the conclusions of systematic reviews and may contribute to reporting bias[9]. Therefore, in order to facilitate standard reporting of key outcomes across studies, the development of core outcomes sets for clinical trials is gaining more attention[10,11].

The International Classification of Functioning, Disability and Health (ICF) is an established framework developed by the World Health Organization and is commonly used in rehabilitation. The ICF is designed to describe health and health-related status from biological, personal and societal perspectives[12]. The framework classifies human function into four domains: Body functions; body structures; activities and participation; and environmental factors[12]. These domains match well with the goals of exercise training and physical rehabilitation programs; specifically to identify, measure and treat physical impairments (body function and structure); to reverse or normalize activity limitations; and to enhance participation in all settings[13]. Using the ICF to map the outcomes of the current literature on exercise training in SOT recipients will assist in classifying the breadth of outcomes that have been used in the studies to date and also in identifying any domains that are understudied in this population. This information can provide a starting point for developing a core set of standard outcomes[10] for clinical trials of exercise and physical rehabilitation in SOT recipients.

The objectives of this systematic review were to identify the outcome measures that have been used in RCTs of exercise training in SOT recipients and to link these outcomes to the ICF framework.

MATERIALS AND METHODS

Data sources and search strategy

This systematic review is in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement[14]. A librarian designed and performed electronic literature searches of Medline from inception until May 2016. The search was then adapted for EMBASE, CINAHL, Cochrane, Scopus, and Web of Science and run on these databases.

Table 1 Electronic search strategy used in MEDLINE

Search #	Keywords and number of records identified
Search #1	Organ transplantation (110179)
Search #2	Transplantation conditioning (7738)
Search #3	Transplant recipients (195)
Search #4	"Transplant recipient$" (27594)
Search #5	1 or 2 or 3 or 4 (122169)
Search #6	Exercise/or Exercise Therapy/or exercise$ (192344)
Search #7	Rehab$/or rehabilitation (151761)
Search #8	Resistance training/or "physical education and training"/or training (181282)
Search #9	"Physical activity" (47446)
Search #10	Physical exertion (11451)
Search #11	6 or 7 or 8 or 9 or 10 (474657)
Search #12	5 and 11 (2399)
Search #13	Heart or lung or kidney or pancreas or liver (1433618)
Search #14	12 and 13 (2200)
Search #15	Limit 14 to humans (2156)
Search #16	Limit 14 to animals (76)
Search #17	15 not 16 (2121)
Search #18	Limit 17 to randomized controlled trial (60)

Search terms included organ transplantation, transplant recipients, graft recipient, heart, lung, kidney, pancreas, liver, exercise, exercise therapy, rehab, rehabilitation, resistance training, physical education, training, physical activity, and physical exertion (Table 1). The searches were limited to RCTs, published in English, and in humans. One investigator (Stacey Konidis) also conducted hand searches of the reference lists of all the studies that met the inclusion criteria to identify additional relevant articles.

Criteria for including studies in the review

We selected all RCTs that investigated the effect of exercise training in SOT recipients. We included trials that compared the effects of exercise training programs to standard care as well as trials that compared two or more different exercise training programs in SOT recipients. In the case of multiple publications of the same study, we considered all of them if the outcomes measures were different. We excluded studies that did not have an isolated exercise intervention group (*i.e.*, those that examined the effect of a drug combined with exercise). We also excluded non-English articles and conference abstracts. One investigator (Stacey Konidis) reviewed the study titles and abstracts to determine potential study eligibility. When this investigator was uncertain, a second reviewer (Tania Janaudis-Ferreira) was consulted. Two investigators independently reviewed the full texts of the articles to determine eligibility (Stacey Konidis and Tania Janaudis-Ferreira).

Data extraction and synthesis

Two reviewers (Stacey Konidis and Cecile Beaurepaire) performed the data extraction and tabulation. A third reviewer (Tania Janaudis-Ferreira) double-checked the extracted data. Outcome measures were abstracted using a standard form and imported into a spreadsheet, sorted into primary and secondary outcomes and classified according to four domains of the ICF (body functions, body structures, activities and participation, and environmental factors). Information about the exercise interventions and patient populations were also retrieved. Considering the purpose of this review, study quality or risk of bias assessments of the included studies were not deemed to be necessary.

RESULTS

Literature search

The electronic and hand searches led to the identification of 522 articles. After excluding 109 duplicates, there were 413 articles left for title and abstract screening. Following the study title and abstract screening, 366 were considered to be unrelated to the objectives of the review. Of the 47 articles that remained for full-text analysis, 12 were excluded. This left a total of 35[2-5,15-45] articles for inclusion in this review. The study flow and reasons for exclusion are shown in Figure 1.

Review of studies and outcome domains assessed

The studies included were designed to compare the effects of exercise training programs to usual care or to another exercise training program and reported on transplantation of heart (n = 21), kidney (n = 9), lung (n = 3), and liver (n = 2). A total of 1313 patients were randomized in the 35 studies. Description of the exercise programs and other details about the studies is presented in Table 2.

Table 3 outlines the outcome measures that were used in each study. In total, there were 126 outcome measures. Of the 126 outcome measures, 62 were used as primary outcome measures in at least one study. The most commonly occurring primary outcomes were peak VO_2 (n = 13), SF-36 (n = 8), and muscle strength (n = 7).

Each outcome measure was linked to an ICF

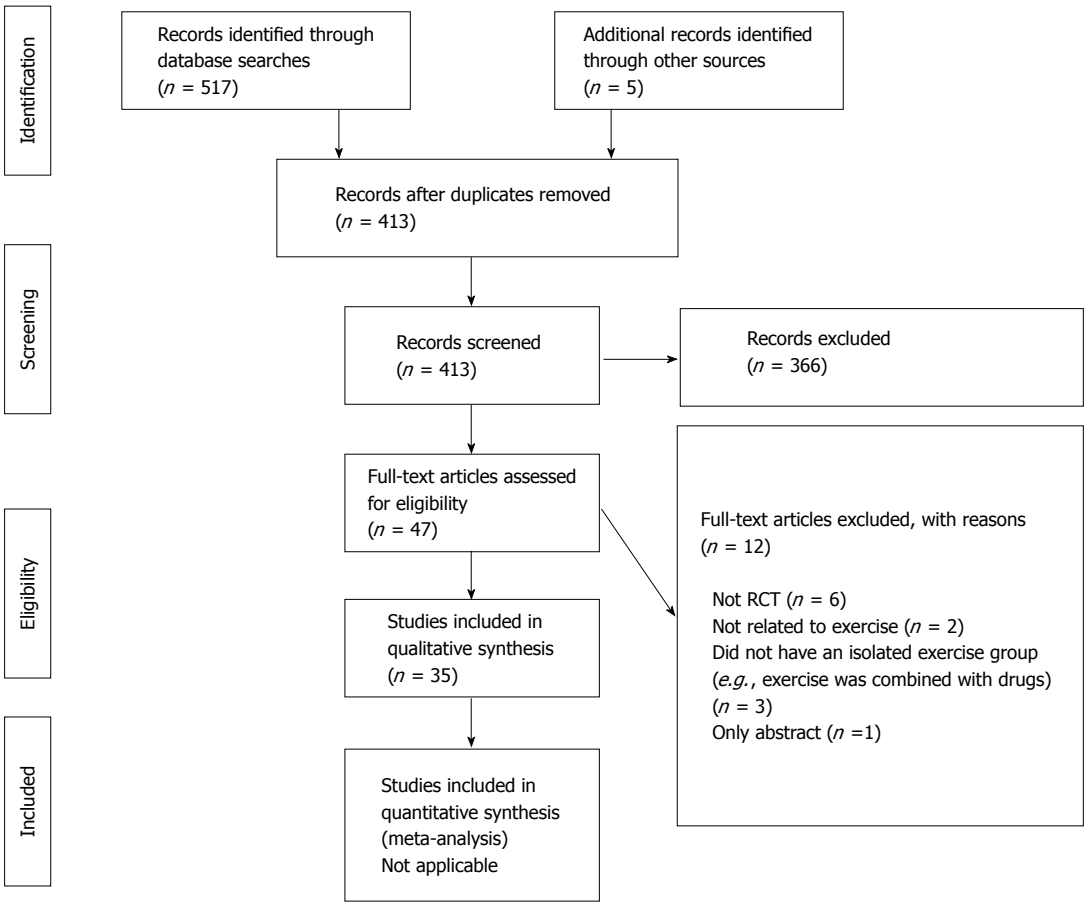

Figure 1 PRISMA 2009 flow diagram. From: Moher *et al*[14]. For more information, visit www.prisma-statement.org.

Table 2 Description of studies

Ref.	Country	Year	Organ	Time-post transplant (wk)	Treatment duration (wk)	Randomized patients[1]	Exercise intervention	Comparison
Braith *et al*[5]	United States	1996	Heart	> 8	24	16	Lumbar extension 1 d/wk; variable resistance exercises 2 d/wk	Usual care
Braith *et al*[4]	United States	1998	Heart	> 8	24	16[2]	Lumbar extension 1 d/wk; variable resistance exercises 2 d/wk	Usual care
Kobashigawa *et al*[15]	United States	1999	Heart	> 2	26	27	Individualized cardiac rehabilitation (strengthening, flexibility, and moderate aerobic exercises) 1-3 d/wk	Usual care (unstructured therapy at home)
Painter *et al*[16]	United States	2002	Kidney	4-8	48	167	Independent home-based exercise 4 d/wk	Usual care
Mitchell *et al*[17]	United States	2003	Lung	> 8	26	16	Lumbar extension resistance exercise 1 d/wk and walking program	Usual care (walking program)
Painter *et al*[18]	United States	2003	Kidney	> 4	48	96	Independent home-based exercise 4 d/wk	Usual care
Braith *et al*[19]	United States	2005	Heart	> 8	24	15	Variable resistance exercises 2 d/wk	Usual care
Juskowa *et al*[20]	Poland	2006	Kidney	> 0.5	4-5	69	Strength exercise training 7 d/wk	Usual care
Krasnoff *et al*[3]	United States	2006	Liver	> 8	40	151	Cardiovascular exercise training 3 d/wk	Usual care
Bernardi *et al*[21]	Italy	2007	Heart	> 24	24	26	Stationary bicycle; 30 min/5 d per week	Usual care
Karapolat *et al*[22]	Turkey	2007	Heart	Mean 14-17	8	38	Hospital-based exercise program (flexibility, stretching, aerobic, strengthening, breathing, relaxation) 3 d/wk	Home-based exercise program (flexibility, stretching, aerobic, strengthening, breathing, relaxation) 3 d/wk
Braith *et al*[23]	United States	2008	Heart	> 8	12	20	Aerobic treadmill exercise	Usual care

Karopola *et al*[24]	Turkey	2008	Heart	Mean 14-17	8	38[3]	Hospital-based exercise program (flexibility, stretching, aerobic, strengthening, breathing, relaxation) 3 d/wk	Home-based exercise program (flexibility, stretching, aerobic, strengthening, breathing, relaxation) 3 d/wk
Pierce *et al*[25]	United States	2008	Heart	> 8	12	20	Aerobic exercise training	Usual care
Wu *et al*[26]	Taiwan	2008	Heart	> 52	8	37	Resistance and aerobic training 3 d/wk	Usual care
Haykowsky *et al*[27]	Canada	2009	Heart	> 26	12	23	Aerobic 5 d/wk and strength training 2 d/wk	Usual care
Mandel *et al*[28]	United States	2009	Liver	6-12	12	50	Targeted lower body resistance strengthening exercise 3-4 d/wk	Usual care (walking program)
Hermann *et al*[29]	Denmark	2011	Heart	> 52	8	27	Aerobic interval training program 3 d/wk	Usual care
Ihle *et al*[30]	Germany	2011	Lung	> 52	4	60	Inpatient rehabilitation (exercise training 4 d/wk and aerobic session 5 d/wk)	Outpatient physiotherapy
Christensen *et al*[31]	Denmark	2012	Heart	Mean 84	8	[4]	High-intensity aerobic interval training 3 d/wk	Usual care
Langer *et al*[2]	Belgium	2012	Lung	1-6	12	40	Aerobic and resistance training 3 d/wk	Usual care
Nytrøen *et al*[32]	Norway	2012	Heart	52-416	52	52	High-intensity aerobic interval training 3 d/wk	Usual care
Rustad *et al*[33]	Norway	2012	Heart	52-416	12	52	High-intensity aerobic interval training 3 d/wk	Usual care
Kawauchi *et al*[34]	Brazil	2013	Heart	< 1	to hospital discharge	22	10-phase incremental exercise program (breathing, active resistance exercises, aerobic exercises, stretching)	Institution exercise routine (breathing, stretching walking) 5 d/wk
Kouidi *et al*[35]	Greece	2013	Kidney	> 52	26	24	Aerobic exercise and strength training 4 d/wk	Usual care
Nytrøen *et al*[36]	Norway	2013	Heart	52-416	52	52[5]	High-intensity aerobic interval training 3 d/wk	Usual care
Dall *et al*[37]	Denmark	2014	Heart	> 52	12 (5 mo washout)	17	High-intensity aerobic interval training 3 d/wk	Moderate biking exercise 3 d/wk
Monk-Hansen *et al*[38]	Denmark	2014	Heart	> 52	8	30	High intensity training 3 d/wk	Usual care
Pascoalino *et al*[39]	Brazil	2015	Heart	> 52	12	42	Endurance exercise training 3 d/wk	Usual care
Pooranfar *et al*[40]	Iran	2013	Kidney	104-156	10	44	Aerobic and resistance training 3 d/wk	Usual care
Riess *et al*[41]	Canada	2013	Kidney	> 26	12	31	Endurance and strength training 2 d/wk	Usual care
Tzvetanov *et al*[42]	United States	2014	Kidney	> 4	52	17	Resistance exercise training 2 d/wk (as well as behaviour and nutrition)	Usual care
Dall *et al*[43]	Denmark	2015	Heart	> 52	12 (5 mo washout)	17[6]	High-intensity aerobic interval training 3 d/wk	Moderate biking exercise 3 d/wk
Greenwood *et al*[44]	England	2015	Kidney	< 52	12	60	Home-based aerobic training and resistance training 3 d/wk	Usual care
Karelis *et al*[45]	Canada	2015	Kidney	6-8	16	24	Resistance training 3 d/wk (once a week in hospital and 2 × /week at home)	Usual care (no exercise)

[1]Does not add to 1313 since some patients included in more than one study; [2]Same patients as Braith 1996; [3]Same patients as Karolopat 2007; [4]Same patients as Hermann 2011; [5]Same patients as Nytrøen 2012; [6]Same patients as Dall 2014. BMD: Bone mineral density; HR: Heart rate; BP: Blood pressure; HRQOL: Health-related quality-of-life; CVD: Cardiovascular disease; BMI: Body mass index; METs: Metabolic unit of task; HRRe: Heart rate reserve; HRR1: Heart rate recovery; CRI: Chronotropic response index; CRP: C-reactive protein; IL-6: Interleukin-6; TNF-a: Tumor necrosis factor-alpha; sICAM-1: Intercellular adhesion molecule-1; 6MWD: 6 minute walk distance; FVC: Forced vital capacity; HRV: Heart rate variability; BRS: Baroreflex sensitivity.

domain and the list is shown in Table 4. The majority of outcomes fell into the body function domain (n = 93). Fourteen outcome measures were linked to the activities and participation, 5 to body structures, 2 to environmental factors and 2 described outcomes were unclassified in the ICF. Frailty indicators such as grip strength (n = 1), fatigue (n = 0) or gait speed (6-minute-walk) (n = 3) were rarely used. Ten multi-dimensional questionnaires were used in the studies reviewed.

DISCUSSION

Physical rehabilitation in SOT patients strives to minimize the impairments associated with prolonged chronic illness, allowing individuals to improve their ability to carry out daily tasks and activities and to participate in life roles. When selecting outcome measures to use in clinical trials

Table 3 List of outcome measures by study

Ref.	Year	Organ group	Primary outcome measures	Secondary outcome measures
Braith *et al*[5]	1996	Heart	Bone mineral density (body and regional: Femur neck, lumbar vertebra)	Bone mineral content Total bone calcium Acute rejection episodes
Braith *et al*[4]	1998	Heart	Body mass Fat-free mass Fat mass Muscle strength (upper and lower body)	Percent body fat Acute rejection episodes
Kobashigawa *et al*[15]	1999	Heart	Blood pressure (peak and resting) Heart rate (peak and resting) Anaerobic threshold Exercise duration (to exhaustion) Peak ventilation Peak VO_2 Peak workload Ventilatory equivalent for carbon dioxide and oxygen	Muscle strength (lower limb)
Painter *et al*[16]	2002	Kidney	Body mass index Body weight Fat mass/body fat Lean tissue mass Percent body fat Blood pressure (peak) Muscle strength (quadriceps) Peak ventilation Peak VO_2 SF-36	Self-reported activity level (frequency, type, length, and intensity of exercise) Blood creatinine Blood urea nitrogen levels Hematocrit Hemoglobin Bone mineral density Peak workload Rating of perceived exertion (Borg) Peak respiratory exchange ratio Immunosuppression use (type, dose)
Mitchell *et al*[17]	2003	Lung	Bone mineral density (lumbar spine)	Acute rejection episodes Muscle strength (lumbar extensor)
Painter *et al*[18]	2003	Kidney	Cholesterol (TC, HDL) Body mass index Total CVD risk (Framingham) Blood pressure Peak workload (METs)	Blood lipids Incidence of diabetes Smoking status
Braith *et al*[19]	2005	Heart	Muscle composition (fiber types) Muscle metabolic enzyme activity	Muscle strength (upper and lower body)
Juskowa *et al*[20]	2006	Kidney	Blood lipids Cholesterol (TC, HDL, LDL) Body mass index	Blood calcium level Blood creatinine Blood electrolytes Blood glucose Blood phosphorus Blood protein levels (albumin, fibrinogen, total protein level) Enzyme levels (alanine transferase, alkaline phosphatase, aspartate aminotransferase) Folate concentrations Hemoglobin Interleukin-18 Total-homocysteine Vitamin B12 Blood pressure Muscle strength (upper limbs) Peak expiratory flow
Krasnoff *et al*[3]	2006	Liver	Body mass index Body weight Bone mineral content Bone mineral density Fat mass/body fat Lean tissue mass Percent body fat Muscle strength (quadriceps) Peak VO_2 SF-36 Peak respiratory exchange ratio Nutritional intake (Block-95 - calories/day; protein, carb and fat calories)	Rating of perceived exertion (Borg)

Bernardi *et al*[21]	2007	Heart	Baroceptor control of blood pressure Baroceptor control of heart rate	Blood pressure; Heart rate Neck pressure RR interval Anaerobic threshold CO_2 production Exercise duration (to exhaustion) Peak ventilation Peak VO_2; Peak workload Ventilatory equivalent for CO_2 and oxygen
Karapolat *et al*[22]	2007	Heart	Peak VO_2 Beck depression inventory SF-36 State-trait anxiety inventory	
Braith *et al*[23]	2008	Heart	Endothelial function (flow-mediated dilation)	Blood glucose Blood lipids Cholesterol (TC, HDL, LDL) Oxidative stress-induced lipid peroxidation Plasma norepinephrine Serum metabolic and hematologic indicators Body mass Acute rejection episodes Blood pressure (resting and peak) Brachial artery diameter Exercise duration (to exhaustion) Peak VO_2
Karapolat *et al*[24]	2008	Heart	Chronotropic response index Heart rate recovery Heart rate reserve Peak VO_2	Duke Treadmill Score
Pierce *et al*[25]	2008	Heart	C-reactive protein Interleukin-6 Serum metabolic profile Soluble cell adhesion molecules (sICAM-1) Tumour necrosis factor-alpha Muscle vasodilation (forearm and calf)	Blood glucose Cholesterol (TC, HDL, LDL) Cytomegalovirus IgG status White blood cell levels Acute rejection episodes Blood pressure (resting) Heart rate (peak and resting) Exercise duration (to exhaustion) Rating of perceived exertion (Borg) Peak respiratory exchange ratio
Wu *et al*[26]	2008	Heart	Muscle endurance (quadriceps) Muscle strength (quadriceps) Peak VO_2 World Health Organization Questionnaire on Quality of Life - BREF	Daily physical activity Blood pressure Heart rate (resting and peak) Nutritional intake (caloric intake questionnaire) Peak ventilation Peak workload Rating of perceived exertion (Borg)
Haykowsky *et al*[27]	2009	Heart	Peak VO_2	Lean tissue mass (total and leg) Blood pressure (peak) Endothelial function (endothelial-dependent vasodilation, endothelial-independent vasodilation, reactive hyperemia index) Heart rate (peak) Left ventricular systolic function Muscle strength (upper and lower body) Peak power output Peak respiratory exchange ratio
Mandel *et al*[28]	2009	Liver	6MWD Muscle strength (lower body) Chronic liver disease questionnaire (CLDQ) SF-36 (physical function/limitations)	
Hermann *et al*[29]	2011	Heart	Peak VO_2	Blood creatinine Blood glucose; Blood lipids Blood protein levels (adiponectin, MR-proANP, NT-proBNP, provasopressin/copeptin) Cholesterol Hemoglobin High sensitive C-reactive protein Interleukin-6 Serum insulin Tumour necrosis factor-alpha Body mass index; Body weight

				Hip-waist ratio Blood pressure (resting) Brachial artery diameter Endothelial function (flow-mediated vasodilation, nitroglycerin-induced vasodilation) Heart rate (resting) Peak power output
Ihle *et al*[30]	2011	Lung	6MWD Peak VO_2 SF-36 St. George's Respiratory Questionnaire	Heart rate (peak and resting) Anaerobic threshold Oxygen uptake at anaerobic threshold Peak workload Peak respiratory exchange ratio Ventilatory reserve and capacity
Christensen *et al*[31]	2012	Heart	Hospital Anxiety and Depression Scale	Peak VO_2
Langer *et al*[2]	2012	Lung	SF-36 Daily walking time (time spend in different postures: sedentary, standing, walking)	Daily steps Movement intensity Time spent in moderate intense activities Blood lipids Body weight Bone mineral density Blood pressure 6MWD Muscle strength (quadriceps and handgrip) Peak workload Mood status SF-36 Forced expiratory volume Respiratory muscle force Incidence of morbidity (diabetes, hyperlipidemia, hypertension, osteoporosis)
Nytrøen *et al*[32]	2012	Heart	Peak VO_2	Blood lipids Blood protein levels (NT-proBNP) C-reactive protein Interleukin-6, 8 and 10 levels Body mass index; Body weight; % body fat Chronotropic response index Glycemic control parameters Blood pressure (peak and resting) Heart rate (peak and resting) Heart rate recovery and reserve Stroke volume (O_2 pulse; resting and peak) Anaerobic threshold Exercise duration (to exhaustion) Muscle strength (quadriceps and hamstrings) Peak ventilation Rating of perceived exertion (Borg) SF-36 Visual Analog Scale (subjective difference in HRQoL) Peak respiratory exchange ratio
Rustad *et al*[33]	2012	Heart	Echocardiographic parameters (rest and during exercise; systolic and diastolic parameters) Peak VO_2	Biochemical parameters Blood pressure Cardiac allograft vasculopathy (coronary angiography) Cardiac output Heart rate (resting and peak) Stroke volume Peak workload Peak respiratory exchange ratio
Kawauchi *et al*[34]	2013	Heart	6MWD Forced vital capacity Respiratory muscle force/strength	Muscle strength (upper and lower limbs) Maximum expiratory/inspiratory pressure
Kouidi *et al*[35]	2013	Kidney	Baroreflex sensitivity Heart rate variability parameters (SDNN, rMSSD, pNN50, LF, HF, LF/HF)	Baroreflex effectiveness index Blood pressure (peak and resting) Heart rate (peak and resting) Exercise duration (to exhaustion) Peak ventilation Peak VO_2
Nytrøen *et al*[36]	2013	Heart	Cardiac allograft vasculopathy (intravascular ultrasound and virtual histology)	Blood creatinine Blood glucose Blood lipids

				C-reactive protein Cholesterol (TC, HDL, LDL) Hemoglobin Interleukin-6, 8 and 10 levels Body mass index Body water (total) Body weight Bone mass Lean tissue mass Percent body fat Visceral fat scale Basal metabolic rate Glycemic control parameters Metabolic age Muscle strength (quadriceps and hamstrings)
Dall *et al*[37]	2014	Heart	Peak VO_2	Peak VO_2 Body weight Blood pressure Heart rate (peak and resting) Heart rate recovery Heart rate reserve CO_2 production Peak ventilation Peak workload Peak respiratory exchange ratio
Monk-Hansen *et al*[38]	2014	Heart	Echocardiography parameters (systolic and diastolic function)	Body mass index Blood pressure Heart rate (peak and resting) Peak VO_2 Peak workload
Pascoalino *et al*[39]	2015	Heart	Arterial stiffness (carotid-femoral pulse wave velocity) Blood pressure (ambulatory; peak and resting)	Plasma norepinephrine Heart rate (peak and resting) Anaerobic threshold CO_2 production Exercise duration (to exhaustion) Peak VO_2 Peak respiratory exchange ratio Respiratory compensation point
Pooranfar *et al*[40]	2013	Kidney	Blood lipids Cholesterol (TC, HDL, LDL) Sleep quality and quantity questionnaire (self-report; Pittsburgh Sleep Quality Index)	
Riess *et al*[41]	2013	Kidney	Peak VO_2	Cholesterol (TC, HDL) Lean tissue mass Total CVD risk (Framingham) Arterial pressure (mean) Arterial stiffness (pulse wave velocity) Arteriovenous oxygen difference (a-vO_2) Blood pressure (ambulatory; peak and resting) Cardiac output Heart rate (peak); Stroke volume Systemic vascular endurance Muscle strength (lower body) Peak workload SF-36 Peak respiratory exchange ratio
Tzvetanov *et al*[42]	2014	Kidney	Glomerular filtration rate SF-36 Adherence to training and follow-up Employment status	Blood creatinine; Blood glucose; Blood lipids Cholesterol (TC, HDL, LDL) Hemoglobin Body mass index Body weight Bone mineral content Lean tissue mass Percent body fat Arterial stiffness (carotid-femoral pulse wave velocity Blood pressure Carotid intima-media thickness Muscle strength

Dall *et al*[43]	2015	Heart	Blood glucose Blood protein levels (adiponectin, orosomucoid, YLK 40) Interleukin-6 Serum insulin Tumour necrosis factor-alpha Arterial stiffness (augmentation index) Endothelial function (reactive hyperemia index) Hospital Anxiety and Depression Scale SF-36	Body weight Homeostasis model assessment Heart rate (peak) Peak VO_2 Peak respiratory exchange ratio
Greenwood *et al*[44]	2015	Kidney	Muscle strength (quadriceps)	Arterial stiffness (pulse wave velocity) Blood pressure (peak and resting) Heart rate (peak and resting) STS-60 Peak VO_2 Body mass index; Body weight Waist girth Glomerular filtration rate high-sensitivity C-reactive protein interleukin-6 Fetuin A Tumor necrosis factor-alpha tumor necrosis factor receptors 1 and 2 SF-36 Duke Activity Status Index
Karelis *et al*[45]	2015	Kidney	World Health Organization-5 Well-Being Index Muscle strength index Adherence to training and follow-up (feasibility)	Body weight Body height Body mass index Waist girth Hip girth Fat mass/body fat Lean tissue mass Cholesterol (TC, HDL, LDL) Blood glucose Blood pressure Peak VO_2

SF-36: Short-form 36; TC: Total cholesterol; HDL: High-density lipoprotein fraction of cholesterol; LDL: Low-density lipoprotein fraction of cholesterol; RR-interval: Inter-beat interval (heart rate); BREF: A shorter version of the original; rMSSD: Root-mean-square of successive NN interval differences; pNN50: Percentage value of NN50 count; LF: Low-frequency components; HF: High-frequency components; CVD: Cardio-vascular disease; STS-60: Sit-to-stand 60.

Table 4 International Classification of Functioning, Disability and Health outcome classifications

ICF component	Domain	Category	Outcome measures	Count primary[1]	Organ group
Body Function	Global mental functions	b134	Sleep quality and quantity	1	Kidney
		b152	Mood status	0	Lung
	Functions of the cardiovascular system (heart functions)	b410	Cardiac output	0	Heart, kidney
		b410	Carotid intima-media thickness	0	Kidney
		b410	Echocardiographic parameters	2	Heart
		b410	Endothelial function	2	Heart
		b410	Left ventricular systolic function	0	Heart
		b410	RR interval	0	Heart
		b410	Stroke volume	0	Heart, kidney
		b410	Systemic vascular endurance	0	Kidney
	Functions of the cardiovascular system (heart rate)	b4100	Heart rate	1	Heart, kidney, lung
		b4100	Heart rate recovery	1	Heart
		b4100	Heart rate reserve	1	Heart
		b4100	Heart rate variability	1	Kidney
	Functions of the cardiovascular system	b410-429	Baroceptor control of blood pressure	1	Heart
		b410-429	Baroceptor control of heart rate	1	Heart
		b410-429	Baroflex effectiveness index	0	Kidney
		b410-429	Baroflex sensitivity	1	Kidney
		b410-429	Chronotropic response index	1	Heart
		b410-429	Total CVD risk	1	Kidney
		b410-429	Cardiac allograft vasculopathy	1	Heart
	Functions of the cardiovascular system (blood vessel functions)	b415	Arterial stiffness	3	Heart, kidney
		b415	Brachial artery diameter	0	Heart
	Functions of the cardiovascular system (blood pressure functions)	b420	Arterial pressure	0	Kidney
		b420	Blood pressure	4	Heart, kidney, lung

	b420	Neck pressure	0	Heart
Functions of the cardiovascular system (oxygen-carrying functions of the blood)	b4301	Arteriovenous oxygen difference	0	Kidney
Functions of the hematological and immunological	b430-439	Biochemical parameters	0	Heart
systems	b430-439	Blood calcium level	0	Kidney
	b430-439	Blood creatinine	0	Heart, kidney
	b430-439	Blood electrolytes	0	Kidney
	b430-439	Blood glucose	1	Heart, kidney
	b430-439	Blood lipids	2	Heart, kidney, lung
	b430-439	Blood phosphorus	0	Kidney
	b430-439	Blood protein levels	1	Heart, kidney
	b430-439	Blood urea nitrogen levels	0	Kidney
	b430-439	C-reactive protein	1	Heart
	b430-439	Cholesterol	3	Heart, kidney
	b430-439	Folate concentrations	0	Kidney
	b430-439	Hematocrit	0	Kidney
	b430-439	Hemoglobin	0	Heart, kidney
	b430-439	High sensitive C-reactive protein	0	Heart
	b430-439	Interleukin levels	2	Heart, kidney
	b430-439	Plasma norepinephrine	0	Heart
	b430-439	Soluble cell adhesion molecules	1	Heart
	b430-439	Total-homocysteine	0	Kidney
	b430-439	Tumour necrosis factor-alpha	2	Heart
	B430-439	Tumor necrosis factor receptor	0	Kidney
	b435	Cytomegalovirus IgG status	0	Heart
	b435	White blood cell levels	0	Heart
	b435	Acute rejection episodes	0	Heart, lung
Functions of the respiratory system (respiration functions)	b440	Forced expiratory volume	0	Lung
functions)	b440	Forced vital capacity	1	Heart
	b440	Maximum expiratory/inspiratory pressure	0	Heart
	b440	Peak expiratory flow	0	Kidney
	b440	Peak respiratory exchange ratio	1	Heart, kidney, liver, lung
	b440	Respiratory compensation point	0	Heart
	b440	Ventilatory reserve and capacity	0	Lung
Functions of the respiratory system (respiration rate)	b4400	CO_2 production	0	Heart
	b4400	Oxygen uptake at anaerobic threshold	0	Lung
	b4400	Peak ventilation	2	Heart, kidney
	b4400	Peak VO_2	13	Heart, kidney, liver, lung
	b4400	Ventilatory equivalent for carbon dioxide and oxygen	1	Heart
Functions of the respiratory system (respiratory muscle functions)	b445	Respiratory muscle force/strength	1	Heart, lung
Functions of the cardiovascular system (general physical endurance)	b4550	Rating of perceived exertion	0	Heart, kidney, liver
Functions related to the digestive, metabolism and the endocrine system	b530	Body mass index	4	Heart, kidney, liver
endocrine system	b530	Body weight/mass	3	Heart, kidney, liver, lung
	b530	Fat mass/body fat	3	Heart, kidney, liver
	b530	Fat-free mass	1	Heart
	b530	Hip girth	0	Kidney
	b530	Hip-waist ratio	0	Heart
	b530	Lean tissue mass	2	Heart, kidney, liver
	b530	Percent body fat	2	Heart, kidney, liver
	b530	Visceral fat scale	0	Heart
	b530	Waist girth	0	Kidney
General metabolic functions, unspecified	b5400	Basal metabolic rate	0	Heart
	b5400	Metabolic age	0	Heart
General metabolic functions, other, specified	B5408	Maximal metabolic units	1	Kidney
Functions related to metabolism and the endocrine system	b540-559	Enzyme levels	0	Kidney
system	b540-559	Fetuin A	0	Kidney
	b540-559	Oxidative stress-induced lipid peroxidation	0	Heart
	b540-559	Serum insulin	1	Heart

		b540-559	Serum metabolic and/or hematologic profile	1	Heart
		b540-559	Vitamin B_{12}	0	Kidney
		b540-559	Glycemic control parameters	0	Heart, kidney
		b540-559	Muscle metabolic enzyme activity	1	Heart
		b545	Body water	0	Heart
		b545	Homeostasis model assessment	0	Heart
	Functions of the genitourinary and reproductive functions (urinary functions)	b610-639	Glomerular filtration rate	1	Kidney
	Neuromusculoskeletal and movement-related functions (muscle power functions)	b730	Peak workload/power output	1	Heart, kidney, lung
		b730	Muscle strength	7	Heart, kidney, liver, lung
		b730-b749	Muscle vasodilation	1	Heart
		b740	Muscle endurance	1	Heart
Body structure	Structures related to movement - additional musculoskeletal structures related to movement (bones)	s7700	Bone mass	0	Heart
		s7700	Bone mineral content	1	Heart, kidney, liver
		s7700	Bone mineral density	3	Heart, kidney, liver, lung
		s7700	Total bone calcium	0	Heart
		s7702	Muscle composition (fibre types)	1	Heart
Activities and participation	Mobility - walking and moving	d410	STS-60	0	Kidney
participation	Mobility - walking and moving (walking)	d450	Daily steps	0	Lung
		d450	Daily walking time	1	Lung
		d450	6 Minute Walk Distance	3	Heart, liver, lung
		d450	Anaerobic threshold	1	Heart, lung
	Mobility - walking and moving	d450-469	Daily physical activity	0	Heart
		d450-469	Movement intensity	0	Lung
		d450-469	Self-reported activity level	0	Kidney
		d450-469	Time spent in moderate intense activities	0	Lung
		d450-469	Duke Treadmill Score	0	Heart
		d450-469	Exercise duration	1	Heart, kidney
	Managing diet and fitness	d5701	Caloric intake	0	Heart
		d5701	Nutritional intake	1	Liver
	Major life areas (work and employment)	d840-859	Employment status	1	Kidney
Environmental factors	Products or substances for personal consumption, other specified	e1108	Smoking status	0	Kidney
	Drugs	e1101	Immunosuppression use	0	Kidney
Questionnaires			DASI	0	Kidney
			Quality of Life Profile for Chronic Diseases Questionnaire	1	Lung
			SF-36	8	Heart, kidney, liver, lung
			St. George's Respiratory Questionnaire	1	Lung
			State-Trait Anxiety Inventory	1	Heart
			Beck Depression Inventory	1	Heart
			Hospital Anxiety and Depression Scale	2	Heart
			Visual Analog Scale (change in HRQoL)	0	Heart
			WHOQOL-BREF	2	Heart, kidney
Not covered by ICF			Chronic Liver Disease Questionnaire	1	Liver
			Incidence of morbidity	0	Kidney, lung
			Adherence to training and follow-up	2	Kidney

[1]Count Primary: Count of studies that used this measure as a primary measure. RR-interval: Inter-beat interval (heart rate); CVD: Cardio-vascular disease; STS-60: Sit-to-stand 60; SF-36: Short-form 36; HRQoL: Health-related quality of life; WHOQOL-BREF: A shorter version of the original World Health Organization Quality of Life Questionnaire; DASI: Duke Activity Status Index.

of SOT recipients, it is important to capture changes across all domains that are relevant to the primary goals of the physical rehabilitation intervention. We have used the ICF categories to classify the outcome measures used in RCTs of exercise interventions after SOT. From this systematic review, we have learned that the outcome measures used in these RCTs vary widely. This finding is in line with the results of similar systematic reviews conducted in other populations (*e.g.*, individuals with critical illness, post-surgery and stroke)[11] Some of the studies focused on multiple primary outcomes and others used just two or three. In total, 62 different primary outcomes were used with the most common being peak VO_2 (n = 13) and the SF-36 (n = 8). Most of the outcomes used fell into the body functions domain (n = 93) with very few in the activities and participation domain (n = 14). Few

studies included outcomes that are also considered frailty indicators. These are important outcomes as frailty is present in many SOT recipients and can have a negative impact on transplant outcomes[6-8].

As we did, Disdbury *et al*[1] found that the most commonly used outcome measure was VO_2 peak. However, this is an expensive test that requires complex equipment as well as expertise from a professional to interpret the results. Functional exercise capacity tests that are more relevant to patients' activities and participation in daily life and less costly to administer should be considered.

Disdbury *et al*[1] were unable to merge data on health-related quality-of-life (HRQoL) measures since so many different questionnaires were used. We found that 11 of the RCTs analyzed used multi-dimensional questionnaires as an outcome measure with several using more than one. These questionnaires each cover many different ICF categories. For instance, Cieza and Stucki[46] have linked individual questions from the short-form-36 (SF-36) questionnaire to ICF domains and found that this questionnaire incorporates at least 21 ICF codes. Linking individual items on HRQoL questionnaires could help researchers select a questionnaire that covers many ICF codes and that would be most suited to be part of the core set of outcome measures recommended, thus making it possible to meaningfully merge data from multiple studies.

A core set of outcome measures to be used in all of these populations would be helpful to minimize and standardize the number of outcomes used in this patient group. While it is important to conduct a comprehensive assessment, the use of a large number of outcome measures can be burdensome for both patients and evaluators. Ideally, the core set of variables should cover all four domains of the ICF, *i.e*., they need to cover all aspects of the health condition. Furthermore, the core set of variables needs to include outcomes that are common to all organ groups. Many of the issues that affect physical function and exercise capacity are common across the transplant types despite each SOT having its own unique characteristics and challenges[47]. Some of the pre-transplant issues that limit physical function are specific to the failing organ, but the physiological changes associated with severe chronic disease, deconditioning and nutritional depletion are common to all groups[48]. Post-transplant issues that limit physical function vary depending on the phase of recovery, but include things such as extended hospital and intensive care stay, prolonged sedentary time, immunosuppressant medications and episodes of organ rejection[48]. Outcome measures that relating to these commonalities and to increasing physical function would be suitable for inclusion in the core set of variables. However, there are some organ specific issues that may be important to address differently among the groups (*e.g*., the effects of exercise in the denervation of the heart after transplant or the effects of exercise on early onset of diabetes after kidney transplant) and researchers should be encouraged to include secondary outcomes to address them.

The selection of outcome measures should reflect the length of time since the transplant and whether the course of recovery has been complicated. For example, the main goal of physical rehabilitation for acute phase post-transplant is usually to improve basic mobility and activities of daily living while rehabilitation for long-term recipients is generally focused on improving their exercise capacity and levels of physical activity to prevent cardiovascular complications. When considering appropriate outcomes, is also important to take into account their psychometric properties[49]. Knowing the validity of the outcomes in the transplant population can help researchers with sample size calculations for interventional studies and justify the use of the selected primary outcomes.

None of the studies reviewed included an economic evaluation of the exercise programs and the potential cost savings if SOT recipients experience less long-term cardiovascular disease and fewer hospital readmission related to frailty and physical disability. Although robust economic studies can be challenging, they may be important to convince healthcare funders that exercise programs can be cost-effective and have a positive impact on transplant outcomes and survival. Exercise programs also need to be more readily available for transplant recipients as lack of availability of post-transplant exercise programs has been identified for example in Canada[50].

Limitations

A limitation of this systematic review is the inclusion of only RCTs. There are other studies on exercise training in SOT recipients that use different research designs, especially observational studies using pre-post designs that were not included. We chose this strategy because RCTs are of the highest quality of study design. We assumed that investigators conducting RCTs have chosen their outcomes carefully and that this group of studies is representative of all rehabilitation trials in transplant recipients. We have also limited our search to studies published in English, which may have reduced our sample size.

There is little standardization in outcome measures used in RCTs of exercise interventions in SOT recipients. Outcome measures for clinical trials should also be selected based on their psychometric properties, stage post transplantation and severity of impairments of the patient population. Further research is needed to develop consensus on a standardized core set of outcomes to measure the effectiveness of such interventions. The ICF framework can be used to select appropriate outcomes that cross all domains and that would be appropriate to all SOT recipients.

COMMENTS

Background

Over 30 randomized controlled trials (RCTs) have been conducted to examine

the effectiveness of exercise training on outcomes in solid organ transplant (SOT) recipients. However, the synthesis of findings across studies has been limited by the lack of similar outcomes across studies. The objectives of this systematic review were to identify the outcome measures that have been used in RCTs of exercise training in SOT recipients and to link these outcomes to the International Classification of Functioning, Disability and Health (ICF) framework.

Research frontiers

Between 1996 and 2015 more than 30 RCTs were published on the effects of exercise training in SOT recipients. Taken together, the results of these RCTs show that exercise training improves maximal aerobic capacity, muscle strength, body composition, cardiopulmonary variables and quality of life. There is little evidence for the effect of exercise in physical activity and participation in SOT recipients. In a systematic review of exercise training in SOT recipients conducted in 2012 by Didsbury *et al*, the authors included 15 RCTs with 28 unique outcomes. The majority of outcomes were related to cardiovascular parameters (VO_2 peak, blood pressure, cholesterol), with fewer studies examining body composition, frailty indicators or quality of life. The authors were therefore hampered in their ability to conduct meta-analyses, which limited the conclusions of their comprehensive review.

Innovations and breakthroughs

There are numerous studies examining the role of exercise training to improve outcomes following SOT. Exercise training has several important health benefits for SOT recipients, such as improving maximal aerobic capacity (VO_2 peak), body composition and quality of life. A limitation of the current literature on exercise for SOT is the inability to combine outcomes from studies due to the wide range of reported outcomes.

Applications

This systematic review suggests that there is a need to develop consensus on a standardized core set of outcomes to measure the effectiveness of exercise interventions in SOT. A standardized core set of outcomes would facilitate standard reporting of key outcomes across studies.

Terminology

The ICF is an established framework developed by the World Health Organization and is commonly used in rehabilitation. The ICF is designed to describe health and health-related status from biological, personal and societal perspectives. The framework classifies human function into four domains: body functions; body structures; activities and participation; and environmental factors. These domains match well with the goals of exercise training and physical rehabilitation programs; specifically to identify, measure and treat physical impairments (body function and structure); to reverse or normalize activity limitations; and to enhance participation in all settings.

Peer-review

It is a well written review concerning several domains to assess the function outcome of patients with organ transplants subjected to exercise training. It is very helpful for the readers.

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META-ANALYSIS

Incidence of kidney stones in kidney transplant recipients: A systematic review and meta-analysis

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Abstract

AIM

To evaluate the incidence and characteristics of kidney stones in kidney transplant recipients.

METHODS

A literature search was performed using MEDLINE, EMBASE, and Cochrane Database of Systematic Reviews from the inception of the databases through March 2016. Studies assessing the incidence of kidney stones in kidney transplant recipients were included. We applied a random-effects model to estimate the incidence of kidney stones.

RESULTS

Twenty one studies with 64416 kidney transplant patients were included in the analyses to assess the incidence of kidney stones after kidney transplantation. The estimated incidence of kidney stones was 1.0% (95%CI: 0.6%-1.4%). The mean duration to diagnosis of kidney stones after kidney transplantation was 28 ± 22 mo. The mean age of patients with kidney stones was 42 ± 7 years. Within reported studies, approximately 50% of kidney transplant recipients with kidney stones were males. 67% of kidney stones were calcium-based stones (30% mixed CaOx/CaP, 27%CaOx and 10%CaP), followed by struvite stones (20%) and uric acid stones (13%).

CONCLUSION

The estimated incidence of kidney stones in patients after kidney transplantation is 1.0%. Although calcium based stones are the most common kidney stones after

transplantation, struvite stones (also known as "infection stones") are not uncommon in kidney transplant recipients. These findings may impact the prevention and clinical management of kidney stones after kidney transplantation.

Key words: Nephrolithiasis; Incidence; Kidney stones; Kidney transplantation; Transplantation

Core tip: The authors performed this meta-analysis to assess the incidence and characteristics of kidney stones in kidney transplant recipients. The estimated incidence of kidney stones in patients after kidney transplantation is 1.0%. Calcium based stones (CaOx and CaP) are the most common kidney stones after transplantation following by struvite stones and uric acid stones. The findings from this study may impact the management of kidney stone prevention after kidney transplantation.

Cheungpasitporn W, Thongprayoon C, Mao MA, Kittanamongkolchai W, Jaffer Sathick IJ, Dhondup T, Erickson SB. Incidence of kidney stones in kidney transplant recipients: A systematic review and meta-analysis. *World J Transplant* 2016; 6(4): 790-797 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i4/790.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i4.790

INTRODUCTION

Kidney stones are one of the most common metabolic disorders and urological problems with a prevalence of 7.2%-7.7% in the adult population, and a ten-year recurrence rate of ⩾ 30%[1-4]. The incidence of kidney stones is increasing especially in industrialized countries with an estimated global prevalence between 10%-15%[5-8]. Approximately 13% of men and 7% women will have a kidney stone during their lifetime[5,8].

Previous studies have shown that stone recurrence rates may be lower, when glomerular filtration rate (GFR) reduced[9,10]. Thus, patients with advanced chronic kidney disease (CKD) or end-stage kidney disease (ESRD) may encounter less stone disease[10], reported being as low as 0.68%[11]. After successful kidney transplantation, ESRD patients subsequently have significant improvement in renal function resulting in urinary excretion of metabolites that increases risk of stone disease. Studies have identified kidney stones in allograft kidney as one of the serious problems in kidney transplant recipients[12-40]. However, unlike the general population, the incidence and characteristics of kidney stones in kidney transplant recipients are not well studied. The aim of this meta-analysis was to appraise the incidence and types of kidney stones after kidney transplantation.

MATERIALS AND METHODS

Cheungpasitporn W and Thongprayoon C individually examined published studies and conference abstracts indexed in MEDLINE, EMBASE, and Cochrane Database from the inception of the databases through March 2016. The search strategy used is detailed in the supplementary material (Item 1). Further pertinent studies were retrieved by conducting a manual search using references from the articles that were reclaimed from the search strategy noted above. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews and meta-analyses[41] and previously published guidelines[42,43].

The inclusion criteria were as follows: (1) randomized controlled trials or observational studies (case-control, cross-sectional, cohort studies, or case series); (2) patient population age > 18 years old; and (3) data on kidney stones in kidney transplant recipients were provided. The search was limited to English-language studies. Both published studies and conference abstracts were incorporated. Study eligibility was independently determined by the two investigators mentioned earlier. Differing decisions were settled by joint agreement.

A standardized information collection form was applied to derive the following data: The first author of each study, study design, year of publication, country where the study was conducted, number of kidney transplant recipients studied, number of patients with kidney stone, age and gender of patients with kidney stones, time of diagnosis after kidney transplantation, type of donor (Live or deceased donor), type and location of kidney stones, and period of follow-up.

Statistical analysis

MetaXL software (EpiGear International Pty Ltd)[44] was utilized for data analysis. The incidence rates (IRs) and 95%CIs of adverse effects were reported using a DerSimonian-Laird random-effects model[45]. A random-effects model was implemented due to the high likelihood of inter-study variances. The Cochran Q test was completed to assess statistical heterogeneity. The I^2 statistic was added to evaluate the degree of variation across studies related to heterogeneity instead of chance. An I^2 of 0%-25% represents insignificant heterogeneity, 26%-50% low heterogeneity, 51%-75% moderate heterogeneity and > 75% high heterogeneity[46]. To assess for publication bias funnel plots were used[47].

RESULTS

Our search strategy yielded 1554 articles. Of these, 1397 articles were excluded following the review of their title and abstract based on their relevance and the eligibility criteria. The remaining 157 articles underwent full-length review, and an additional 136 were excluded for failing to meet the criteria. Twenty one articles[12-29,36,38,40] met all inclusion criteria and were identified for the meta-analysis of kidney stones in kidney transplant recipients (Table 1). Supplementary Item 2 outlines our search methodology

Table 1 Main characteristics of the studies included in this meta-analysis

Ref.	Country	Year	Total number	No. of patients with kidney stone	Time of diagnosis	Sex of patients with stone	Age of patients with stone	Donors	Stone location	Stone composition	Mean follow-up time
Cho *et al*[12]	United States	1988	544	9	Mean 14.7 mo, Median 7 mo (range 3-42 mo)	6 male, 3 female	Mean 30 yr (range 8-65 yr)	6 living, 3 cadaveric	4 bladder, 3 kidney, 2 unknown	4 calcium oxalate/ calcium phosphate, 2 ammonium magnesium phosphate and carbonate appetite, 1 uric acid, 2 not studied	5 (range 1.5-15.5) yr
Hayes *et al*[13]	United States	1989	892	10	Mean 13 mo (range 4-49 mo)	7 male, 3 female	Mean 29 yr (range 17-53 yr)	3 living, 7 cadaveric	NR	NR	NR
Harper *et al*[38]	United Kingdom	1994	178	6	NR	4 male, 1 female	NR	4 living, 1 cadaveric	NR	1 uric acid, 2 calcium phosphate, 1 calcium oxalate, 1 Magnesium ammonium phosphate	NR
Shoskes *et al*[14]	United Kingdom	1995	812	2	Mean 3.5 yr (range 2-5 yr)	NR	Mean 40 yr	NR	2 ureter	NR	At least 1 yr
Benoit *et al*[36]	France	1996	1500	12	NR	7 male, 5 female	Mean 36 yr	2 living, 10 cadaveric	5 calyces, 6 ureter, 1 pyeloureteral junction	4 calcium oxalate and phosphate, 2 struvite	NR
Del Pizzo *et al*[15]	United States	1998	540 (445 renal transplant, 95 pancreas/ renal transplant)	4	NR	NR	NR	NR		NR	NR
Rhee *et al*[16]	United States	1999	1813 (1730 renal transplant, 83 pancreas/ renal transplant)	8	NR	4 male, 4 female	Mean 51 yr (range 34-60 yr)	2 living, 1 cadaveric, 5 pancreas/ renal	3 kidney, 1 ureter, 4 bladder	1 uric acid, 1 calcium oxalate, 1 calcium oxalate/ calcium phosphate, 1 calcium phosphate, 1 struvite stone, 3 unknown	Mean 68.6 mo (range 27-98 mo)
El-Mekresh *et al*[17]	Egypt	2001	1200	11	NR	NR	NR	NR	3 kidney, 4 ureter, 4 bladder	NR	NR
Kim *et al*[18]	United States	2001	849	15	Mean 17.8 mo (range 3-109 mo)	10 male, 5 female	Mean 41.5 yr (range 28-67 yr)	8 living, 7 cadaveric	11 bladder, 3 kidney, 1 multiple sites	5 mixed form of calcium oxalate and calcium phosphate, 1 calcium oxalate, 3 predominant calcium phosphate, 2 struvite, 2 mixed form of struvite and calcium phosphate, 2 not studied	Mean 58 mo (range 11-149 mo)

Klinger *et al*[19]	Austria	2002	1027	19 (4 diagnosis during transplant, 5 perioperative, 10 *de novo*)	For *de novo*: Mean 27.7 mo (range 13 to 48 mo)	8 male, 11 female	Mean 48.1 yr (range 26-72 yr)	1 living, 18 cadaveric	14 kidney, 3 infundibulum, 1 distal ureter, 1 staghorn	11 calcium oxalate, 2 uric acid, 1 calcium phosphate, 5 not studied	Mean 29 mo (range 14-48 mo)
Doehn *et al*[20]	Germany	2002	1500	11	NR	5 male, 6 female	Median 50 yr	11 cadaveric	NR	3 uric acid, 3 calcium oxalate, 2 magnesium ammonium stone, 3 not studied	Median 4 yr
Streeter *et al*[21]	United Kingdom	2002	1535	12	For renal calculi: Median 150 d (range 56-1280 d); For bladder calculi: Range 8 mo - 4 yr	NR	NR	NR	9 ureter, 3 bladder	NR	NR
Abbott *et al*[22]	United States	2003	42906	52	NR	NR	NR		35 kidney, 17 ureter	NR	1.89 ± 1.15 yr
Lipke *et al*[23]	United States	2004	500	7	9 mo (range 1.5-26 mo)	7 female	Mean 50 yr (range 8-73 yr)	4 living, 3 cadaveric	7 bladder	7 mixed between calcium oxalate and calcium phosphate	NR
Yigit *et al*[24]	Turkey	2004	125	5 (2 preoperative, 1 early posttransplant, 2 *de novo*)	For *de novo*: Mean 6.5 mo (range 6-7 mo)	3 male, 2 female	Mean 35.2 yr	NR	NR	2 calcium oxalate, 1 uric acid, 2 infectious	Mean 32.4 mo
Challacombe *et al*[25]	United Kingdom	2005	2085	21	3.7 (0.17-18) yr	8 male, 13 female	Mean 41 yr (range 15-64 yr)	3 living, 18 cadeveric	13 kidney, 7 ureter, 1 bladder	NR	NR
Ferreira Cassini *et al*[26]	Brazil	2012	1313	12 *de novo*	Range 6 mo to 13 yr	8 males, 9 females	Mean 45.6 yr (range 32-63 yr)	2 living, 15 cadaveric	6 calyces, 3 renal pelvis, 3 ureter	NR	NR
Stravodimos *et al*[27]	Greece	2012	1525	7	Mean 3.2 (2-7) yr	NR	NR	NR	5 kidney, 2 ureter	NR	Mean 8 yr
Cicerello *et al*[40]	Italy	2014	953	10	NR	4 male, 6 female	Mean 43 yr	NR	7 kidney, 3 ureter	NR	NR
Mamarelis *et al*[28]	Greece	2014	2045	9	Mean 3.1 yr (range 1-7 yr)	NR	NR	NR	6 kidney, 3 ureter	NR	6.6 yr (range 1-15 yr)
Rezaee-Zavereh *et al*[29]	Iran	2015	574	25	NR	NR	NR	NR	NR	NR	55 ± 53 mo

CaOx: Calcium oxalate; CaP: Calcium phosphate; NR: Not reported.

and selection process.

Incidence of kidney stones in kidney transplant recipients

The incidence of kidney stones after kidney transplantation within the 21 individual study ranged between 0.2% to 4.4% with an overall meta-analytical incidence of 1.0% (95%CI: 0.6%-1.4%)with evidence of a high level of heterogeneity (I^2 = 93%, P < 0.001; Figure 1).

We performed a sensitivity analysis limited only to the studies that provided data on time of kidney stone diagnosis after kidney transplantation; the estimated incidence of kidney stones was 0.9% (95%CI: 0.7%-1.2%), and there was evidence of a high level of heterogeneity (I^2 = 60%, P < 0.001; Figure 2). The mean duration to diagnosis of kidney stones after kidney transplantation was 28 ± 22 mo.

Subgroup analyses by geographic information were also performed. The estimated incidences of kidney stones were 0.9% (95%CI: 0.3%-1.7%; I^2 = 94%) and 0.7% (95%CI: 0.5%-0.9%; I^2 = 40%) in the United States and Europe, respectively. Data on the incidence of kidney stones in kidney transplant recipients in other geographical area were limited as shown in Table 1.

Characteristics of kidney transplant recipients with kidney stones

The mean age of patients with kidney stones was 42 ± 7

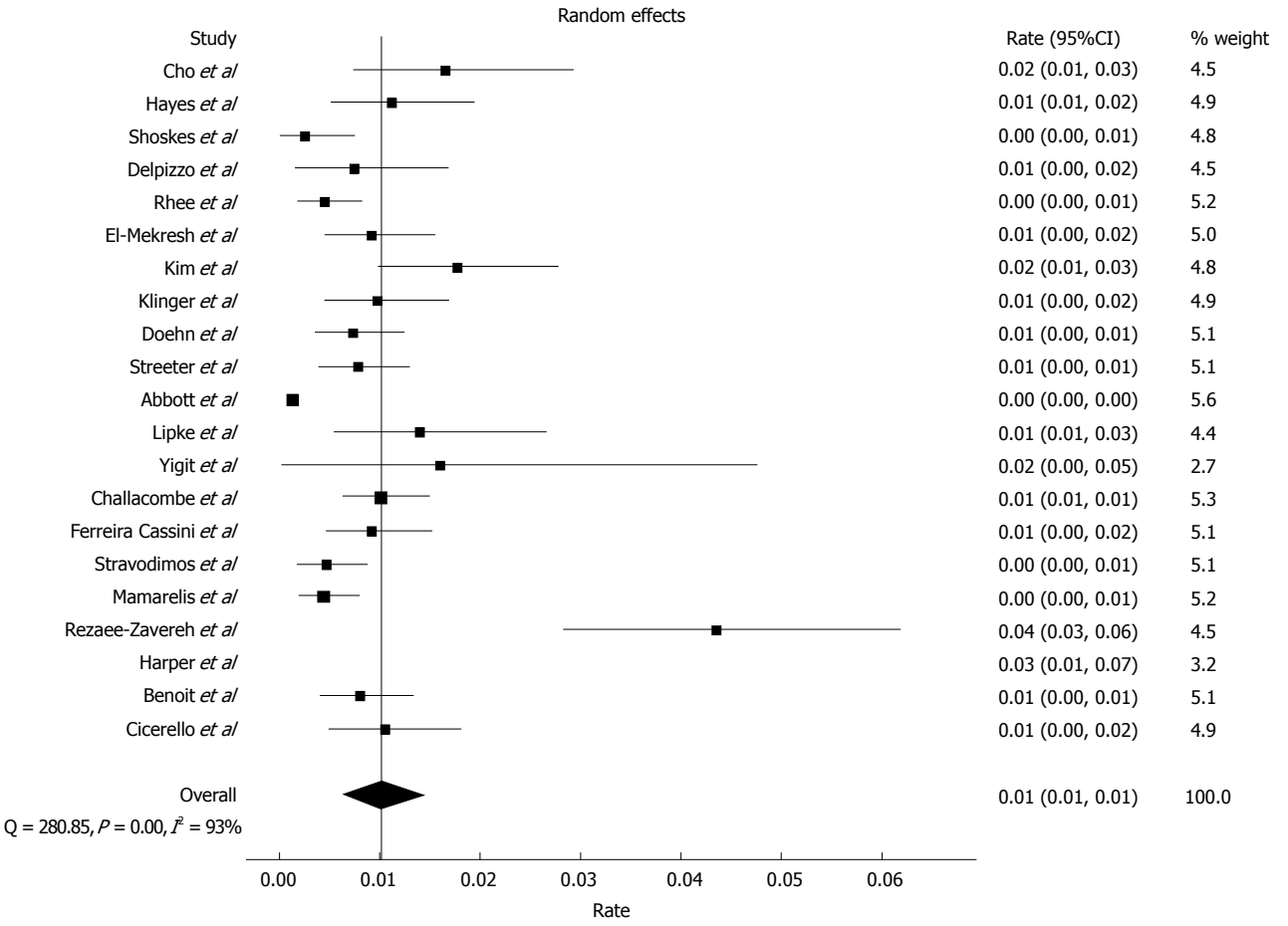

Figure 1 Forest plot of incidence of kidney stones in kidney transplant populations.

years. Within reported studies (Table 1), approximately 50% of kidney transplant recipients with kidney stones were males.

Types of kidney stones in kidney transplant recipients

Sixty-seven percent of kidney stones were calcium-based stones (30% mixed CaOx/CaP, 27%CaOx and 10%CaP), followed by struvite stones (20%) and uric acid stones (13%) as shown in Table 1.

Risk factors for kidney stones in kidney transplant recipients

Despite limited data on urinary supersaturation and risk factors for kidney stones, studies reported increased risk of kidney stones in kidney transplant recipients with hyperparathyroidism, hypercalciuria, hypocitraturia, hypophosphatemia, and urinary tract infection[28,38]. Harper *et al*[38] found that urinary excretion of magnesium and phosphate was at the lower range for all kidney transplant recipients with kidney stones. Uncommonly, urinary outflow obstruction and foreign bodies were also found as risk factors for kidney stones in kidney transplant patients[28,48].

Allograft failure in kidney transplant recipients with kidney stones

As in general patient populations, kidney stones can also cause acute kidney injury in kidney transplant recipients[49-52]. Since kidney transplant recipients can have obstructed kidney stones without any symptom of pain[26,28], prompt diagnosis and the removal of obstructed stones are the keys to preventing renal allograft failure[18]. Rezaee-Zavareh *et al*[29] reported no significant association between kidney stones after transplantation and graft survival (OR = 1.04; CI: 0.71-1.54). With the prompt removal of stones, Kim *et al*[18] found no significant changes in renal allograft function at diagnosis and after removal of kidney stones.

Evaluation for publication bias

Funnel plot evaluating publication bias for the incidence of kidney stones in kidney transplant recipients demonstrated slight asymmetry of the graph and thus suggested the presence of publication for positive studies regarding the incidence of kidney stones.

DISCUSSION

In this study, we demonstrated that an overall incidence of kidney stones in kidney transplant recipients was 1.0%. The mean age of recipients with kidney stones was 42, and half of stone formers were males. Calcium based (CaOx and CaP) stones were the most common types of kidney stones after kidney transplantation,

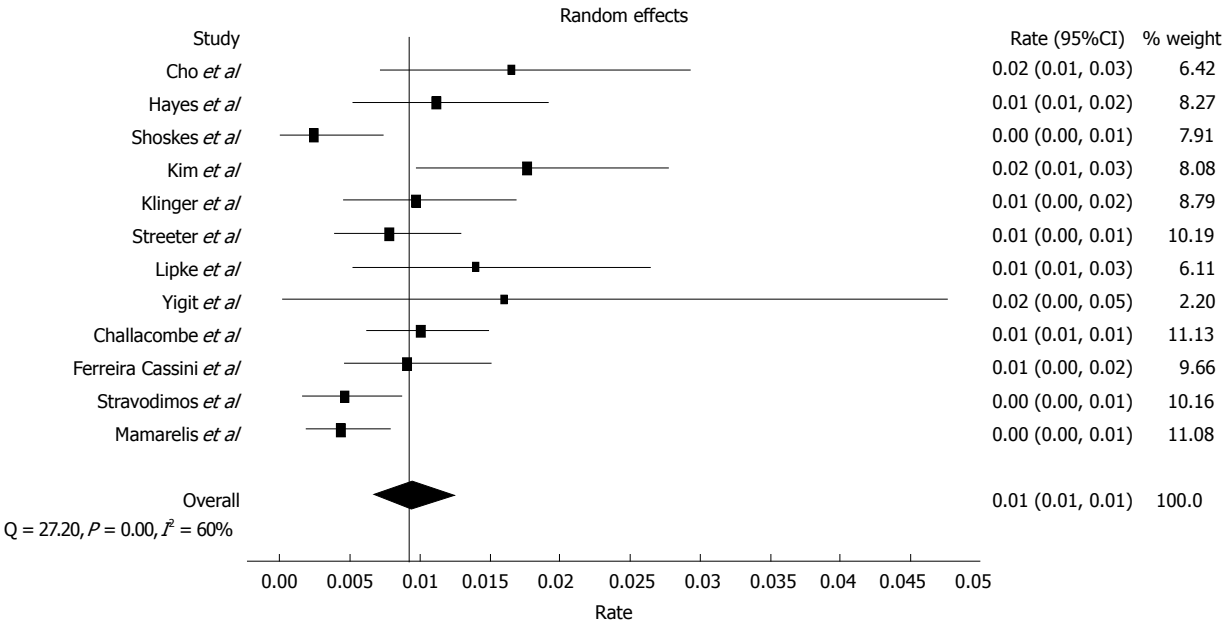

Figure 2 Forest plot of incidence of kidney stones in kidney transplant populations limited only to the studies that provided data on the time of kidney stone diagnosis after kidney transplantation.

followed by struvite stones and then uric acid stones.

The incidence of kidney stones after kidney transplantation from our meta-analysis is much lower than reported in the general adult populations[5-8]. Although the mechanisms behind the lower incidence of kidney stones after kidney transplantation, when compared with the general population, are only speculative, there are several plausible explanations. First, with the observation that new kidney stones are usually formed in transplanted allograft kidney but not in native non-functioning kidneys, kidney transplant recipients have significantly improved but still lower GFRs than those in healthy general populations, which may be "protective" for stone disease[9,10]. Second, transplanted kidneys are from healthy donors ideally without tubulointerstitial defects, one not uncommon cause of kidney stones. Third, it is possible that kidney stones after kidney transplantation are underdiagnosed since recipients may spontaneously pass them from the transplanted kidney/ureter without pain or awareness.

Calcium based (CaOx and CaP) stones are the most common types of kidney stones in the general population as well as after kidney transplantation as demonstrated in our meta-analysis. Interestingly, struvite stones (ammonium magnesium phosphate) or "infection stones" is more common in kidney transplant recipients (20%) than in the general population (10%-15%)[53]. Since struvite stones are associated with infection with urea-splitting bacteria and the principles of treating struvite stones are different than other stones types, including removal of all stone fragments and use of antibiotics[53], this information is important for future studies targeting prevention and management of kidney stones after kidney transplantation.

There are several limitations to our study. First, there were statistical heterogeneities in the analysis of the incidence of kidney stones. The potential sources of this heterogeneity included differences in diagnostic methodology of kidney stones and follow-up duration. However, a sensitivity analysis that limited studies to those that only provided data on time of kidney stone diagnosis still showed a similar incidence rate of kidney stones, consistent with the finding of our primary analysis. Second, most included studies were conducted in developed Western countries with the majority of the subjects being Caucasian. Thus, our findings may not represent renal transplant populations from other parts of the world. Lastly, the data on urinary supersaturation and risk factors for kidney stones were limted. Although struvite stones represent an association with urinary tract infection, it is still unclear the risk factors for other stone types after kidney transplantation, and future studies are needed.

Our meta-analysis demonstrates that the estimated incidence of kidney stones in patients after kidney transplantation is 1.0%. Although calcium based stones are the most common kidney stones after transplantation, struvite stones are the second common type. These findings may impact clinical prevention and management of kidney stones in kidney transplant recipients.

COMMENTS

Background

Renal stones are one of the most prevalent metabolic disorders and urological problems. However, with reduced kidney functions, patients with advanced chronic kidney disease (CKD) or end-stage kidney disease (ESRD) may encounter less stone disease. After successful kidney transplantation, ESRD patients have significant improvement in kidney functions and may develop

kidney stones in their allograft kidney.

Research frontiers

The incidence and characteristics of kidney stones in kidney transplant recipients are not well studied. It is thus necessary to assess the incidence and types of kidney stones after kidney transplantation.

Innovations and breakthroughs

In this study, the authors demonstrated that an overall incidence of kidney stones in kidney transplant recipients was 1.0%. The mean age of recipients with kidney stones was 42, and half of stone formers were males. Calcium based (CaOx and CaP) stones were the most common types of kidney stones after kidney transplantation, followed by struvite stones and then uric acid stones.

Applications

The data in this study demonstrates an estimated incidence of kidney stones in patients after kidney transplantation of 1.0%. Calcium based stones and struvite stones are common types of kidney stones after transplantation. These findings may impact the clinical management of kidney stones prevention in kidney transplant recipients.

Terminology

CaOx: Calcium oxalate; CaP: Calcium phosphate; CKD: Chronic kidney disease; GFR: Glomerular filtration rate; NR: Not reported.

Peer-review

This is a reasonable first meta-analysis of incidence of kidney stones in kidney transplant recipients. The results have potential clinical applications.

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