ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2012 December 24; 2(6): 84-103

Baishideng Publishing Group
www.wjgnet.com

WJT World Journal of Transplantation

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

Editorial Board

2011-2015

The *World Journal of Transplantation* Editorial Board consists of 100 members, representing a team of worldwide experts in transplantation. They are from 29 countries, including Argentina (1), Australia (1), Belgium (1), Brazil (6), Canada (1), China (9), Czech Republic (1), France (3), Georgia (1), Germany (4), Greece (2), Hungary (1), India (2), Iran (3), Israel (1), Italy (9), Japan (4), Netherlands (3), Norway (1), Poland (1), Saudi Arabia (2), South Korea (2), Spain (2), Switzerland (1), Tunisia (1), Turkey (4), United Kingdom (7), and United States (26).

WJT

World Journal of Transplantation

Contents

Bimonthly Volume 2 Number 6 December 24, 2012

REVIEW

World Journal of Transplantation
Volume 2 Number 6 December 24, 2012

Contents

NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Bimonthly

EDITING
Editorial Board of *World Journal of Transplantation*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: wjt@wjgnet.com
http://www.wjgnet.com

EDITOR-IN-CHIEF
Maurizio Salvadori, MD, Professor, Renal Unit, Careggi University Hospital, Viale Pieraccini 18, Florence 50139, Italy

EDITORIAL OFFICE
Jian-Xia Cheng, Director
Jin-Lei Wang, Vice Director
World Journal of Transplantation
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: wjt@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
December 24, 2012

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2220-3230/g_info_20100722180909.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
wjt@wjgnet.com
doi:10.5500/wjt.v2.i6.84

World J Transplant 2012 December 24; 2(6): 84-94
ISSN 2220-3230 (online)

REVIEW

Polyomavirus-associated nephropathy

Cristina Costa, Rossana Cavallo

Cristina Costa, Rossana Cavallo, Virology Unit, University Hospital San Giovanni Battista di Torino, 10126 Turin, Italy
Author contributions: Costa C wrote the manuscript; Cavallo R reasonable for the supervision and discussion of this manuscript.
Correspondence to: Cristina Costa, MD, PhD, Virology Unit, University Hospital San Giovanni Battista di Torino, Via Santena 9,10126 Turin, Italy. cristina.costa@unito.it
Telephone: +39-11-6705630 Fax: +39-11-6705648
Received: July 14, 2011 Revised: August 14, 2012
Accepted: October 31, 2012
Published online: December 24, 2012

Abstract

Polyomaviruses BK and JC are ubiquitous viruses with high seroprevalence rates in general population. Following primary infection, polyomaviruses BK and JC persist latently in different sites, particularly in the reno-urinary tract. Reactivation from latency may occur in normal subjects with asymptomatic viruria, while it can be associated to nephropathy (PVAN) in kidney transplantat recipients. PVAN may occur in 1%-10% of renal transplant patients with loss of the transplanted organ in 30% up to 80% of the cases. Etiology of PVAN is mainly attributable to BK virus, although approximately 5% of the cases may be due to JC. Pathogenesis of PVAN is still unknown, although viral replication and the lack of immune control play a major role. Immunosuppression represents the *condicio sine qua non* for the development of PVAN and the modulation of anti-rejection treatment represents the first line of intervention, given the lack of specific antiviral agents. At moment, an appropriate immunemodulation can only be accomplished by early identification of viral reactivacation by evaluation of polyomavirus load on serum and/or urine specimens, particularly in the first year post-trasplantation. Viro-immunological monitoring of specific cellular immune response could be useful to identify patients unable to recover cellular immunity posttransplantation, that are at higher risk of viral reactivation with development of PVAN. Herein, the main features of polyomaviruses BK and JC, biological properties, clinical characteristics, etiopathogenesis, monitoring and diagnosing of PVAN will be described and discussed, with an extended citation of related relevant literature data.

Key words: Polyomavirus; Kidney transplantation; Immunosuppressive therapy; Virological monitoring; Cellular immune response

Peer reviewer: Kais Harzallah, Associate Professor, Unit of Organ Transplantation, Military hospital of Tunis, Tunis, 1008, Tunisia

Costa C, Cavallo R. Polyomavirus-associated nephropathy. *World J Transplant* 2012; 2(6): 84-94 Available from: URL: http://www.wjgnet.com/2220-3230/full/v2/i6/84.htm DOI: http://dx.doi.org/10.5500/wjt.v2.i6.84

INTRODUCTION

Polyomavirus-associated nephropathy (PVAN) is one of the most common viral complications in renal transplant recipients and is an increasingly recognized cause of renal transplant dysfunction and graft loss. Since the first description of PVAN in 1995, an increasing prevalence rate from 1% to 10% has been evidenced[1]. The increase in prevalence data could be due to the introduction of new deeply immunosuppressive drugs and/or the relative decline in acute rejection rates. PVAN can lead to kidney graft loss in 10% up to 100% of the cases, determining the return in hemodialysis within 6 to 60 mo, thus significantly and markedly reducing the graft survival.

Viral replication is the single common feature of all patients at risk of nephropathy. Therefore, screening for viral replication is the most useful tool for the identification of patients at risk of developing nephropathy, thus allowing for earlier intervention, in particular a pre-emptive reduction of immunosuppression, with improvement of outcome. Beside virological monitoring, in recent years the role of virus-specific cellular immune response in the control of viral replication has prompted

the development and employment of methods for viro-immunological monitoring.

The complexity regarding diagnosis, monitoring and clinical management of PVAN evidences the need for a multidisciplinary approach, including nephrologists, pathologists, and clinical virologists.

HUMAN POLYOMAVIRUSES AND NEPHROPAHTY

The most characterized polyomaviruses infecting humans and involved in the pathogenesis of PVAN are BK virus (BKV) and JC virus (JCV), named after the initials of the patients in which they were first isolated in 1971[2,3]. In recent years, other polyomaviruses have been described in humans, including WU, KI, Merkel cell virus and others, however their clinical role is still undefined and no association to nephropathy has been evidenced. Moreover, also the non-human primate polyomavirus SV-40 has been detected in human specimens and is associated to nephropathy in these primates in the presence of immunocompromised conditions, such as infection with simian immunodeficiency virus (Table 1). SV-40 was accidentally introduced as a pathogen into the human population as a contaminant of early polio vaccines, both the inactivated one by Salk and the oral one by Sabin, between 1955 and 1964[4]. Apart for polio vaccines, strong serological and molecular evidence suggests that new SV-40 infections may be occurring in the human population, although the route of transmission remains unknown. Pathogenic role of SV-40 in humans is controversial and recent studies evidenced the risk of false positive results because of contamination by common laboratory plasmids containing SV-40 sequences[5-7]. Nevertheless, like other polyomaviruses, SV-40 displays renotropism and is believed to latently persist in the kidney after primary infection.

Polyomaviruses BK and JC are ubiquitous, with seroprevalence rates ranging from 70% to 90% in adult population. Primary infection usually occurs early in the childhood, at a median age of 5 years, and is characterized by low upper respiratory tract morbidity or is asymptomatic. Following primary infection, BKV and JCV remain latent in different sites, including the renourinary tract, as the epidemiologically most relevant latency site, B cells, brain, spleen, and probably other organs. Periodical reactivation may occur in both immunocompetent individuals (in 0% up to 62%) and immunocompromised patients[8] and is evidenced by asymptomatic viruria.

In contrast to BKV, that is found infrequently in the urine of healthy adults, JC viruria occurs universally, increasing with age. In a study on 400 healthy blood donors, Egli and colleagues evidenced that JC viruria was significantly more frequent and at a higher viral load in comparison to BK viruria (19% *vs* 7%, $P < 0.0001$)[9]. According to the Authors, these data indicate significant differences between BKV- and JCV-infected cells with respect to anatomic location and/or accessibility to T cells in mucosal sites.

Table 1 Polyomaviruses detected in humans and involved in the pathogenesis of polyomavirus-associated nephropathy

Virus	Host	Clinical diseases
BKV	Human	PVAN in renal transplantation Hemorrhagic cystitis in bone marrow transplantation
JCV	Human	Progressive multifocal leukoencephalopathy PVAN in renal transplantation
SV-40	Non-human primate	Unknown; PVAN in renal transplantation?

PVAN: Polyomavirus-associated nephropathy; BKV: BK virus; JCV: JC virus.

At the time of first description of BK in 1971, the pathogenic role of BKV remained elusive and it has been considered an orphan virus for many years afterwards. In 1978, Mackenzie and colleagues first described four features of nephropathy in renal transplantation: (1) the detection of urinary decoy cells; (2) the presence of viral inclusions in uroepithelial cells in graft biopsies; (3) the difficulties in the differential diagnosis with acute rejection; and (4) the role of immunosuppression in the development of these findings[10].

In 1995, Purighalla and colleagues described the first case of PVAN and recognized it as a defined disease entity[11]. Subsequently, several reports with increasing prevalence rates from many transplant centres worldwide followed, evidencing a stepwise increase in incidence of PVAN from approximately 1% in 1995 to 5% or even more in 2001. Subsequently, studies from 2002 to 2004 reported PVAN with prevalence rates of 1% to 10% (mean 5.1%). The majority of the cases occur in the first year posttransplantation, although approximately 25% of cases are diagnosed later. Clinical impact is relevant, as loss of the renal graft ranges from 30% up to > 80% of cases[12-14]; in transplant centres where screening for polyomavirus replication in the urine and plasma is performed, the rate of graft loss is lower[15].

Several studies evidenced that BK viremia is only rarely observed in non-kidney solid organ transplant recipients and biopsy-confirmed cases of nephropathy have been described in few case reports[16-22], despite the similar or even higher level of immunosuppression, thus supporting the role of organ determinants in the pathogenesis of PVAN.

In hematopoietic stem cell transplant recipients, polyomavirus BK replication is commonly associated to hemorrhagic cystitis. Its incidence in this population ranges from 5.7% to 7.7%[23,24]. A case of polyomavirus-induced hemorrhagic cystitis in renal transplantation patient with polyomavirus nephropathy has been reported[25].

Polyomavirus JC was first isolated in brain tissue from a patient with progressive multifocal leukoencephalopathy, a demyelinating disease caused by lytic replication of the virus in oligodendrocytes and observed in the setting of profound cellular immunosuppression, such as ac-

quired immunodeficiency-syndrome. JCV has been also associated to nephropathy in kidney transplant recipients.

In fact, the etiological agent of PVAN is BKV in most of the cases, while JCV has been recognized as the etiologic agent in less than 3% of all reported cases, alone[26,27] or in association to BKV[28]. Nevertheless, it is likely that the role of JCV is more relevant than that reported. In a recent study[29], a biopsy-proven PVAN was diagnosed in six kidney graft recipients with exclusive JCV viruria out of 75 patients (8%) with BKV and/or JCV viruria, thus accounting for an overall incidence during the study period of 0.9%.

A potential role for SV-40 in the etiology of PVAN has been also suggested. Butel and colleagues[30] found that SV-40 seropositivity in children increased with age and was significantly associated to kidney transplantation. A co-infection with BKV and SV-40 has been described in two of six renal transplant patients with PVAN[31].

The pathogenesis of PVAN is still only incompletely understood, although it is now recognized that the interaction of multiple factors concurs to the development of PVAN, including patient, organ, and viral determinants.

In all the cases, the *condicio sine qua non* for the development of PVAN is the presence of intense immunosuppression. Nephropathy has been associated particularly, but not exclusively, to triple immunosuppressive therapy, including tacrolimus, mycophenolate mofetil, and steroids. It has been noted that the emergence of PVAN coincided with the diffuse use of tacrolimus and mycophenolate mofetil, although there is no proved causal relationship, due to the different mechanisms of action of these drugs[1,32]. It seems that the overall level of immunosuppression rather than a specific agent is involved in the pathogenesis of PVAN, although we cannot exclude a drug-specific mechanism. The importance of immunosuppression is also underlined by the fact that the main line of intervention is by modulating immunosuppression, in particular reducing, switching or discontinuing a specific immunosuppressive agent.

The preferential manifestation in renal allograft as compared to the native kidney of other solid-organ transplant recipients, suggests the role of other factors.

Among patient-related determinants, the following determinants have been identified as possibly contributing to PVAN: age > 50 years, male gender, white ethnicity, pre-transplantation BKV seronegativity in children, interferon (IFN)-γ producing specific T-cells, presence of comorbidities such as diabetes mellitus and cytomegalovirus coinfection.

Among organ determinants, the following factors have been considered as associated to viral replication and PVAN: HLA mismatching, previous episodes of acute rejection (treated with anti-lymphocyte preparations and intravenous steroid boluses), viral load in the renourinary tract, presence of renal injury (including calcineurin-inhibitor toxicity, in fact the reduction of this class of drug represents the very first line of intervention).

Among viral determinants, BKV genotypes (mutations in domain of *VP1* gene) and rearrangements in NCCR with presumably increased viral fitness.

Determinants involved in determining the risk of PVAN are summarized in Table 2.

Table 2 Determinants in the development of polyomavirus-associated nephropathy

Determinants	
Patient-related	Age > 50 yr
	Male gender
	Comorbidities (diabetes mellitus)
	Negative serostatus before transplantation
Organ-related	Degree of HLA mismatching
	Prior rejection episodes
	Renal injury
	Latent infection load
Viral-related	NCCR rearrangements
	Genotype
	Viral fitness
Immunity-related	Intense triple immunosuppression (calcineurin-inhibitor, antiproliferative agent, steroid)
	Rejection and anti-rejection treatment (anti-lymphocyte preparations, iv steroid boluses)
	Positive serostatus of donor
	Low number of BKV-specific T-cells

NCCR: Noncoding control region; BKV: BK virus.

CLINICAL AND HISTOLOGIC FEATURES

PVAN is typically diagnosed within the first year post-transplantation, although approximately 25% of the cases are seen later (range, 1.3-45.1 mo). Clinical presentation may be inconspicuous and lacks of useful features. Varying degrees of renal dysfunction may be seen, although in early stages even normal serum creatinine levels may be detected. PVAN may consist in interstitial nephritis and/or ureteric stenosis with ureteric obstruction, hydronephrosis, and sometimes associated urinary tract infections. Progressive renal failure is reported in approximately 30%-60% of the cases[33].

The stereotypical evolution of PVAN has been thoroughly characterized and is as follows[34]. Most of the cases are preceded by an asymptomatic phase of persistent and significant viruria, as demonstrated by the evidence of a urine viral load > 10^5 copies/mL by polymerase chain reaction or by urine cytology (urinary decoy cells for at least 2 mo). Sustained BK viruria is typically followed within few weeks by viremia. A significant and sustained viremia (identified as > 5000 copies/mL plasma for 3 consecutive weeks) identifies patients with uncontrolled viral replication potentially leading to parenchymal injury. Progression of PVAN leads to eventual deterioration of the kidney graft function. Usually, appearance of viruria and viremia precedes the increase in serum creatinine by weeks or months. Options for clinical interventions vary in relation to the stage of PVAN course. In patients with viremia and viruria, a patient's tailored reduction of immunosuppression before a significant renal injury has

occurred leads to the resolution of the infection in up to 90% of the cases with long term preservation of renal function and a low risk of acute rejection (10%-15%). No intervention is required in the sole presence of viruria. In the presence of viruria and viremia, increased serum creatinine level and renal injury at graft biopsy, the intervention by reducing immunosuppression is mandatory. Late diagnosis and intervention, once that graft dysfunction is evident, decreases significantly the likelihood of viral clearance and is associated with higher probability of graft loss (30% *vs* < 10%). In patients with end-stage PVAN, intervention is usually late and ineffectual; the end-stage PVAN is clinically and histologically similar to end-stage renal disease with progressive obliteration of the renal tubules and decrease in viremia and viruria.

PVAN displays a (multi)focal distribution in the kidney. Histologic diagnosis of PVAN is based on the detection of typical basophilic nuclear viral inclusion in epithelial cells (renal tubular and/or Bowman's capsular lining urothelium). The virus is identified by immunohistochemical staining for the so-called SV40 Large T antigen, which cross-reacts with polyomaviruses BKV, JCV, and SV40. Histologic patterns of PVAN are based on the identification and extend of inflammatory infiltrates and fibrosis in association with viral infection[34]. The following patterns have been described: pattern A with viral cytopathic changes within a normal renal parenchyma, scant or no tubular atrophy, interstitial fibrosis and inflammation; pattern B with combination of viral cytopathic changes and focal/multifocal areas of tubular atrophy/interstitial fibrosis/inflammation (< 25% for pattern B1; 25%-50% for pattern B2; > 50% for pattern B3); pattern C (end-stage PVAN) with scarce viral cytopatic changes within a diffusely scarred renal tissue and extensive tubular atrophy/interstitial fibrosis/inflammation.

DIAGNOSIS

The definitive diagnosis of PVAN is made by histopathologic evaluation, however this presents some drawbacks, including limited sensitivity due to focal involvement, thus accounting for sampling errors; varying presentations with cytopatic-inflammatory and/or fibrotic/scarring patterns; difficult differential diagnosis from acute rejection, that may coexist with a opposite impact on intervention strategies. As the main line of intervention is by reducing immunosuppression, early diagnosis is fundamental for a pre-emptive treatment, before the instauration of renal injury. Considering the main pathogenic factors of PVAN, i.e., viral replication and failure of immune surveillance, earl diagnosis may be accomplished by virological and viro-immunological monitoring, consisting in monitoring of viral replication and evaluation of virus-specific immune response, respectively.

VIROLOGICAL MONITORING

Viral replication is the single common feature of all renal transplant recipients at risk of PVAN. Therefore, screening for viral replication allows for the identification of patients at risk of developing PVAN, thus permitting earlier intervention consisting in a pre-emptive reduction of immunosuppression[35], with improvement of the outcome. Screening for viral replication presents a high predictive value (100%), as in the absence of viral replication, PVAN is excluded[36]. Moreover, virological monitoring is the most important tool for evaluating the response to the treatment in patients with confirmed PVAN. Different screening assays are available and include: (1) urine cytology, i.e., the detection of decoy cells that are present in 40% to 60% of transplant recipients, although it represents a good screening test with a 100% negative predictive value, positive predictive value is very low (approximately 20%)[33]; (2) quantification of urinary BKV-DNA, with a load 100-fold higher than plasma viral load that is found in 30% to 40% of transplant recipients, with a positive predictive value of approximately 40%[33]; and (3) quantification of plasma BKV-DNA, with a viral load higher than 10^4 copies/mL recommended for a presumed diagnosis of PVAN[32,37,38]; quantification of urinary VP1 mRNA that is likely to mirror active viral replication.

Different screening methods present some drawbacks, for example urine cytology and urinary VP1 mRNA measurement are susceptible to preanalytic hazards due to the type and duration of processing, viruria may differ depending on the type of specimen (supernatant, cell pellet, resuspended urine), micturition intervals and fluctuations of urine content, inhibition of polymerase chain reaction in urine (e.g., depending on urea concentration) for viruria.

As regards VP1 mRNA, this assay was first proposed as a tool for noninvasive diagnosis of PVAN adopting a cut-off of 6.5×10^5 copies/nanogram of total RNA[39] and has been recently described as a complementary assay to investigate viral replication on the basis of the results of a study having found that the mean copy number in patients without PVAN was significantly lower than that in biopsy-proven nephropathy[40], and the assay has been validated in an independent cohort of renal transplant patients[41]. However, the use of VP1 mRNA measurement has been criticized as it is dependent on the purity of the RNA preparation and contamination by the encoding viral genomic VP1 DNA contaminating the VP1 cDNA preparation has been indicated as being potential responsible for falsely high results[42]. We have recently evaluated the role of urine VP1 mRNA quantification in a large cohort of kidney transplant recipients and found that, by analyzing the operating characteristics, VP1 messenger study was not superior to viremia and was otherwise limited by the technical complexity in comparison to DNA detection[43].

According to the Consensus Recommendations of a panel of international experts, screening for polyomavirus replication should be made by urine cytology or urine DNA load evaluation; recommended screening intervals are as follows: every 3 mo up to 2 years posttranplantation

Table 3 Algorythm for the virological monitoring of polyomavirus BK replication in renal transplantation[49]

Assay	Notes	Timing - intervention
Screening Urine cytology (decoy cells) or urine DNA load	Positive screening test (possible PVAN)	Every 3 mo up to 2 yr post-transplantation or in case of allograft dysfunction or when renal biopsy is performed
Adjunctive quantitative tests (threshold) Urine DNA load > 10^7 copies/mL or plasma DNA load > 10^4 copies/mL	Presumptive PVAN	Pre-emptive reduction of immunosuppression
Allograft biopsy	Definitive diagnosis of PVAN	
Monitoring of response to treatment Urine DNA load decreasing or plasma DNA load decreasing Serum creatinine		Every 2-4 wk
	Negative monitoring test (resolved PVAN)	

PVAN: Polyomavirus-associated nephropathy.

or when allograft dysfunction occurs or when allograft biopsy is performed. In the presence of a positive screening tests (possible PVAN), adjunctive quantitative assays with identification of cut-off levels are recommended, including a urine DNA load > 10^7 copies/mL or plasma DNA load > 10^4 copies/mL. In the presence of a positive adjunctive test above the threshold (presumptive PVAN), allograft biopsy is recommended in order to make a definitive diagnosis of PVAN and prompt intervention. Response to the treatment is monitored every 2-4 wk by evidencing a reduction of urine and plasma DNA load until resolution of PVAN.

The performance characteristics of tests for screening of polyomavirus replication have been evaluated in recent studies. Based on a study by Viscount and colleagues[44] on a cohort of 114 kidney transplant recipients with four cases of confirmed PVAN, BKV-PCR may prove superior to urine cytology, particularly in terms of sensitivity and positive predictive value. A plasma DNA load > 1.6 × 10^4 copies/mL evidenced an improved specificity to 96% (*vs* 91% for plasma load of 10^4 copies/mL, 92% for urine load > 2 × 10^7 copies/mL, and 84% for urine cytology) without reducing sensitivity (100% for all the molecular assays, as all the PVAN cases presented this viral load, *vs* 25% for urine cytology), however positive predictive value was only 50% (*vs* 29% for plasma load of 10^4 copies/mL, 312% for urine load > 2 × 10^7 copies/mL, and 5% for urine cytology). Nevertheless, the important clinical value of a negative polymerase chain reaction test is well established. In a similar study performed at the Renal Transplant Centre of Turin, Italy, on 229 patients with three cases of confirmed PVAN (accounting for an overall prevalence of 1.3%), a viremia level > 1.6 × 10^4 copies/mL was found in four cases, three of which with PVAN. The following operating characteristics for the diagnosis of PVAN were achieved: sensitivity 100%, specificity 99.6%, positive predictive value 75%, and negative predictive value 100%; of course, the low number of PVAN cases represents a limitation of this study[15].

Virological monitoring for PVAN at the Renal Transplant Centre of Turin, Italy, includes screening of viruria and viremia twice monthly in the first 3 mo posttransplantation, thereafter every 3 mo during the first 2 years and then yearly until the 5th year. Due to the possibility of self-limiting (transient) replication, positive screening assays are confirmed within 2-4 wk.

Renal biopsy is performed in case of suspected rejection, PVAN or declining renal function; in fact, also considering the potential for sampling errors due to focal involvement, renal biopsy is necessary to exclude other pathologic processes, such as acute rejection that may coexist[45]. At our centre, considering that in early stages of PVAN viral inclusions may be absent, as well as inflammation may be scarce[46], beside histopathologic evaluation, also quantification of polyomavirus DNA on renal graft biopsies and/or ureteric specimens is performed. Polyomavirus-DNA quantitation could be useful in the presence of little evidence of viral cytopathy[46]. In a study on quantitation of polyomaviruses BK and JC in human kidneys, the highest tissue viral loads (e.g., > 10^3 copies/cell) were found in active PVAN, while it was significantly lower in pre-PVAN biopsies and in specimens from patients with asymptomatic viruria[47]. Similar results were obtained by our group in a study on 109 renal transplant patients with two cases of histologically confirmed PVAN, with tissue BKV load > 10^4 copies/cell in both PVAN cases and < 10^2 copies/cell in patients with asymptomatic viruria or pre-PVAN[48]. Overall, these studies evidenced that viral load are significantly higher in active PVAN and underline the low sensitivity of tissue polymerase chain reaction in early stages of infection, possibly reflecting the low sensitivity due to focal involvement, as already described for histopathology.

Recommended algorithm for virological monitoring of polyomavirus BK replication in renal transplant recipients[49] is summarized in Table 3.

VIRO-IMMUNOLOGICAL MONITORING

Among determinants potentially involved in the pathogenesis of PVAN, there are immunity-related risk factors. These include immunosuppressive therapy, use of steroids, HLA-mismatching, rejection and anti-rejection treatment, and factors related to polyomavirus-specific

immune response[50]. Both humoral and cellular response may be associated to BKV replication and PVAN.

As regards humoral response to BKV and PVAN risk, it has been evidenced that BKV-seropositive recipients are not protected from viral replication and PVAN[12]. For example, in a study on 78 renal transplant patients, PVAN occurred in four of the 59 (76% of the whole study population) seropositive individuals and in one of the 19 (24%) seronegative patients[12]. On the other hand, BKV-seronegative recipients are at higher risk of viral replication and nephropathy[51-53]. For example, BK viruria was significantly more frequent in seronegative recipients in comparison to seropositive patients (58% *vs* 21% in a study population of 24 and 56 pediatric kidney transplant patients, respectively; $P < 0.005$)[51]. Nevertheless, although specific antibodies may accelerate the clearance of primary infection, they seem to play only a minimal role in the containment of PVAN. In fact, although a significant increase in immunoglobulins G titer is seen in patients with decreasing viremia values and after the resolution of PVAN[52-54], individuals with elevated immunoglobulins G levels can still develop PVAN, thus suggesting a role of defective cellular immune response in the onset of nephropathy[55].

Until recently, studies on immune response to BKV have been limited, due to the little pathologic potential in immunocompetent individuals. It has been evidence that BK-seropositive healthy subjects present CD4+ and CD8+ cells specific for BKV Large T antigen and the capsid protein VP1; in particular, CD4+ T cells with cytotoxic potential seem to play a role in maintaining memory responses to BKV and contribute to the immune control of viral replication[56].

In renal transplant patients, it has been evidenced that control of BKV replication and PVAN is correlated with the development or reconstitution of BKV-specific cellular immune response; whereas the lack of a protective immunity may favour the occurrence of active infection and progression to PVAN[54,57]. The fundamental role of T-cell immune response in the control of BKV replication was first observed by the indirect evidence of increased incidence of viral reactivation and development of disease in relation to the degree of immune compromise[8,32,49,58].

Evaluation of BKV-specific cellular immune response could be accomplished by lymphocyte stimulation with inactivated cultured virus or specific epitopes/overlapping peptide pools derived from VP1 and Large T antigens (i.e., virus-specific stimulation step) and detection of cellular immunity by labelled major histocompatibility complex class I tetramers, by intracellular staining and flow cytometry analysis, or by the enzyme-linked immunosorbent spot (ELISPOT) assay for IFN-γ.

By using one of these assays, several studies have evidenced that kidney transplant recipients with with BK viremia or nephropathy failed to mount or expand a virus-specific cellular immune response, in comparison to seropositive healthy individuals or renal transplant patients with no evidence of infection (negativity of viruria and viremia) or with evidence of infection in the presence of good renal function (evidence of viruria, but no increase in serum creatinine)[54]. In particular, in patients with PVAN, no IFN-γ-secreting cell was detectable; whereas, in patients with PVAN, after reduction of immunosuppression, an increase in the number of IFN-γ-secreting cells to levels similar to those of healthy subjects was evidenced, in concomitance with a reduction of viremia and viruria. In seronegative healthy individuals no cellular immunity is detectable[54].

A defined association between viremia dynamics and BKV-specific cellular immunity has been evidenced. In a study on renal transplant recipients, cellular response to both Large T antigen and VP1 resulted significantly lower in patients with increasing or persistent viral load (n = 22 patients) in comparison to those with decreasing (al least 2 $\log_{10}$ copies/mL) viral load or past PVAN (n = 20 patients)[57]. An example of the course of BK viral load and virus-specific cellular immune response in a kidney transplant recipient with polyomavirus reactivation treated with pre-emptive reduction of maintenance immunosuppression has been reported by Comoli and colleagues[55]: the emergence of BKV-specific T-cells coincides with the reduction of viral load; the concomitant reduction of serum creatinine indicates stabilization of graft function. This last finding seems to argue against the hypothesis that mounting of cellular immunity is a major determinant of tissue damage, as previously proposed for hemorrhagic cystitis in bone marrow transplantation[54,57,59]. Nevertheless, an inflammatory reaction mediated by different effectors may contribute to graft damage in case of prolonged viral cytopathic damage[60,61]. Overall, these observations represent the basis by which it seems reasonable to manage therapeutic modulation by complementing quantification of viral load (virological monitoring) with measurement of specific cellular immunity (viro-immunological monitoring).

Prolonged and deep immunosuppression is considered as the most important determinant in the development of PVAN. In particular, although viral replication and PVAN has been associated to the overall level of immunosuppression rather than a specific drug, triple therapy including tacrolimus, mycophenolate mofetil and prednisone[62-64] seems to be associated with a higher risk than cyclosporine. The mechanism of action of anti-rejection drugs interferes with the T cell activity. Calcineurin inhibitors (i.e., cyclosporine and tacrolimus) interfere with T cell activation (signal-1); whereas mammalian target of rapamycin (mTOR) inhibitors (i.e., sirolimus and everolimus) and anti-proliferative agents (i.e., azathioprine and mycophenolate mofetil) interfere with T cell proliferation downstream of the interleukin-2-receptor activation (signal-3)[35,63,65].

Tacrolimus trogh levels > 8 ng/mL, and higher doses of tacrolimus or mycophenolate mofetil, have been associated to polyomavirus replication and the development of PVAN[66]. Conversely, the reduction of tacrolimus trough levels to 6 ng/mL and of the daily dose of myco-

Table 4 Recommended treatment of polyomavirus-associated nephropathy by reduction or switching of immunosuppression[49]

Switching	Decreasing
Tacrolimus → Cyclosporine A (trough levels 100-150 ng/mL)	Tacrolimus (trough levels < 6 ng/mL)
Mycophenolate mofetil → Azathioprine (dose ≤ 100 mg/d)	Cyclosporine A (trough levels 100-150 ng/mL)
Tacrolimus → sirolimus (trough levels < 6 ng/mL)	Mycophenolate mofetil dose ≤ 1 g/d
Mycophenolate mofetil → sirolimus (trough levels < 6 ng/mL)	Cyclosporine A (trough levels 100-150 ng/mL)
Mycophenolate mofetil → leflunomide	

phenolate mofetil to ≤ 1 g, determined an improvement or stabilization of PVAN in most of the cases[67]. According to current consensus recommendations[49], tacrolimus trough levels should be targeted to 6 ng/mL, cyclosporine A to 100-150 ng/mL, and mycophenolate mofetil should be reduced to a daily dose ≤ 1 g or discontinued.

As no effective antiviral therapy is available, the milestone of treatment is represented by the pre-emptive reduction of immunosuppression on the basis of virological monitoring, although no protocol has been defined.

Based on the knowledge of the mechanisms of action, it can be hypothesized that the frequency of BKV-specific IFN-γ producing T cells is impacted by the immunosuppressive treatment. A recent study, using IFN-γ ELISPOT assays, investigated immunosuppressive drug levels and BKV Large T antigen-specific T cell responses in kidney transplant recipients *in vivo* and in healthy donors after titrating immunosuppression *in vitro*[68]. Based on their results, in kidney transplant patients *in vivo* ($n = 16$), responses resulted inversely correlated with tacrolimus trough levels ($P < 0.002$), but not with mycophenolate mofetil, prednisone or the overall immunosuppressive dosing. *In vitro* tacrolimus concentrations > 6 ng/mL resulyed in inhibition of BKV-specific T cell responses more than 50%, while inhibition was less than 30% with tacrolimus concentration < 3 ng/mL. Cyclosporine A resulted in > 50% inhibition of BKV-specific cellular responses at concentrations of 1920 ng/mL and less than 30% at concentrations below 960 ng/mL (corresponding to clinical C_0 trough levels of 200 and 100 ng/nL, respectively). No inhibition of BKV-specific T cell responses was observed for mycophenolate mofetil levels up to 8 μg/mL, leflunomide 50 g/mL, or sirolimus concentrations of 64 ng/mL. Overall, the results obtained by Egli and colleagues[68] suggested that calcinuerin-inhibitor concentrations are crucial for the development and/or recovery of BKV-specific T cell responses. Therefore, reduction of calcineurin inhibitors should be considered as the first line of intervention in the presence of a presumptive diagnosis of PVAN, whereas switching to mTOR inhibitors may represent an alternative or the second line of intervention. These data should be confirmed in clinical trials.

TREATMENT

There is no approved and defined treatment for PVAN. The main line of intervention is by reducing immmunosuppression. Antiviral agents have been used, but no defined treatment is recommended and results are conteoversial. More recently, the use of the immunomodulant agent leflunomide, together with the reduction of immunosuppression has been proposed.

As the majority of cases of PVAN have been associated to triple immunosuppressive therapy including combinations of calcineurin inhibitors (tacrolimus, cyclosporine A), antiproliferative agents (mycophenolate mofetil, azathioprine) and corticosteroids, most of recommended strategies includes decreasing, switching or stopping the ongoing treatment.

Immunosuppressive reduction

The recommended treatment of PVAN by modification of maintenance immunosuppression is summarized in Table 4[49]. Discontinuation of mycophenolate mofetil or azathioprine and reduction of immunosuppression by 25%-50% were commonly used strategies[69]. Graft failure after immunosuppression reduction alone can be observed in approximately 8% of the patients[69]. Following reduction of immunosuppression, biopsy-proven acute rejection has been observed in approximately 25% of patients[49]. These episodes of rejection may be treated by steroid without recurrence of PVAN[12,63].

Acute rejection and PVAN may coexist. In cases of concurrent biopsy-confirmed acute rejection and PVAN, a two-step approach of anti-rejection treatment followed by the reduction of immunosuppressive treatment has been adopted by several studies, that obtained the stabilization or improvement of allograft function[12,31,70,71].

The response to the immunosuppression reduction should be monitored by viruria and viremia evaluation every 2-4 wk[49]. Some studies evidenced clearance of viruria and viremia in most of the patients, with clearance rates ranging from 7% to 80% for viruria and from 40% to 96% for viremia. However, based on creatininemia evaluation, renal function did not always improve with the reduction of immunosuppression[72-74].

Immunosuppression reduction with antivirals

Antiviral agents, in particular cidofovir, have been investigated in addition to the reduction of immunosuppression for the management of PVAN. However, results evidenced a scarce, if any, significant effect in clearing viruria or viremia[75-78]. Other studies reported clearance of viremia in 50% to 100% of the cases[75,79-82]. The effect of cidofovir on renal function was variable, with some studies evidencing stabilization of creatinine[79-81] and others

showed no effect on renal function[75,77]. The pronounced nephrotoxicity limits the use of cidofovir particularly in renal transplantation and an adequate hydratation is required. Vidarabine is used in the treatment of BKV-associated cystitis in bone marrow transplant recipients; its efficacy in nephritis is unknown[33].

Immunosuppression reduction with leflunomide

More recently, the use of the immunomodulant agent leflunomide has been proposed for the treatment of patients with PVAN, in addition to the reduction of immunosuppression. Leflunomide is an immunosuppressive agent, however its metabolite A77 1726 exhibits antiviral activity *in vitro*. Among the few studies that have evaluated the role of leflunomide in treating PVAN, three reported results of viral clearance with treatment[83-85] with significant decreases in viremia and viruria with leflunomide alone or leflunomide plus cidofovir. Moreover, treatment with leflunomide stabilized or improved renal function in most of the cases.

Other interventions and retransplantation

Among drugs having evidenced a polyomavirus-inhibitory activity *in vitro*, beside cidofovir and leflunomide, there are certain quinolone antibiotics. Based on the results of a recent study, a 1-mo fluroquinolone course after transplantation is associated with significantly lower rates of BK viremia at 1 year in comparison to patients with no fluoroquinolone, therefore suggesting the usefulness of these antibiotics at preventing viremia in kidney graft recipients[86]. However, these results should be further confirmed by other studies, given the lack of clinical trials.

Amantadine has been used in the treatment of PVAN with poor effect[33]. γ globulin has been used in order to augment the immune response, however the real efficacy is unknown[33].

Consideration of retransplantation in the context of PVAN has become an increasingly relevant issue, with some unsolved questions regarding timing of retransplantation, i.e., preemptive *vs* after progression to renal failure. There is only limited experience about this in patients who have lost a previous graft due to PVAN. Retransplantation has been reported in a total of 21 cases[87-90], four of which were performed pre-emptively. All preemptive cases were performed with concomitant graft nephrectomy because of the risk of possible reinfection. Nevertheless, Cooper and colleagues[90] reported for the first time a successful preemptive retransplantation for PVAN in a patient without simultaneous graft nephrectomy. This cases evidenced that retransplantation could be pursued without nephrectomy for patients with PVAN provided the absence of viral replication and an active surveillance protocol. The need for close monitoring of viral replication both in the immediate posttransplantation setting (to minimize the risk of development of PVAN) and in the context of graft failure (to indicate proper management and retransplant option) remains critical.

CONCLUSION

In the past decade, PVAN has emerged as one of the most relevant viral diseases occurring in renal transplantation. Its increasing incidence has underlined the role played by immunosuppression in its pathogenesis. The major determining factors are now recognized as the occurrence of uncontrolled viral replication and the failure of immune surveillance. Therefore, since a preemptive reduction of immunosuppression represents the mile stone for the treatment of PVAN, virological and viro-immunological monitoring are necessary and should be performed according to current recommendations. At moment, the reduction of immunosuppression represents the first line of intervention, however clinical controlled trials are required to identify the best therapeutic strategies. A multidisciplinary approach is fundamental to optimize the clinical management of renal transplant recipients and, despite of the relevance of consensus recommendations, these cannot substitute for clinical judgement and individualized care taking into account the different points of view.

ACKNOWLEDGMENTS

The authors want to acknowledge Dr. Maria Messina and Professor Giuseppe P Segoloni of the Renal Transplant Unit of the University Hospital San Giovanni Battista of Turin, for their precious support and counselling.

REFERENCES

1 **Hirsch HH**, Drachenberg CB, Steiger G, Ramos E. Polyomavirus-associated nephropathy in renal transplantation: critical issues of screening and management. In: Ahsan N, editor. Polyomaviruses and human diseases. Georgetown: Landes Bioscience, 2006: 117-147
2 **Gardner SD**, Field AM, Coleman DV, Hulme B. New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet* 1971; **1**: 1253-1257
3 **Padgett BL**, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet* 1971; **1**: 1257-1260
4 **Butel JS**, Lednicky JA. Cell and molecular biology of simian virus 40: implications for human infections and disease. *J Natl Cancer Inst* 1999; **91**: 119-134
5 **Butel JS**, Jafar S, Wong C, Arrington AS, Opekun AR, Finegold MJ, Adam E. Evidence of SV40 infections in hospitalized children. *Hum Pathol* 1999; **30**: 1496-1502
6 **López-Ríos F**, Illei PB, Rusch V, Ladanyi M. Evidence against a role for SV40 infection in human mesotheliomas and high risk of false-positive PCR results owing to presence of SV40 sequences in common laboratory plasmids. *Lancet* 2004; **364**: 1157-1166
7 **Poulin DL**, DeCaprio JA. Is there a role for SV40 in human cancer? *J Clin Oncol* 2006; **24**: 4356-4365
8 **Hirsch HH**, Steiger J. Polyomavirus BK. *Lancet Infect Dis* 2003; **3**: 611-623
9 **Egli A**, Infanti L, Dumoulin A, Buser A, Samaridis J, Stebler C, Gosert R, Hirsch HH. Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. *J Infect Dis* 2009; **199**: 837-846
10 **Mackenzie EF**, Poulding JM, Harrison PR, Amer B. Human

polyoma virus (HPV)--a significant pathogen in renal transplantation. *Proc Eur Dial Transplant Assoc* 1978; **15**: 352-360
11 **Purighalla R**, Shapiro R, McCauley J, Randhawa P. BK virus infection in a kidney allograft diagnosed by needle biopsy. *Am J Kidney Dis* 1995; **26**: 671-673
12 **Hirsch HH**, Knowles W, Dickenmann M, Passweg J, Klimkait T, Mihatsch MJ, Steiger J. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. *N Engl J Med* 2002; **347**: 488-496
13 **Ramos E**, Drachenberg CB, Portocarrero M, Wali R, Klassen DK, Fink JC, Farney A, Hirsch H, Papadimitriou JC, Cangro CB, Weir MR, Bartlett ST. BK virus nephropathy diagnosis and treatment: experience at the University of Maryland Renal Transplant Program. *Clin Transpl* 2002; 143-153
14 **Randhawa P**, Brennan DC. BK virus infection in transplant recipients: an overview and update. *Am J Transplant* 2006; **6**: 2000-2005
15 **Costa C**, Bergallo M, Astegiano S, Terlizzi ME, Sidoti F, Segoloni GP, Cavallo R. Monitoring of BK virus replication in the first year following renal transplantation. *Nephrol Dial Transplant* 2008; **23**: 3333-3336
16 **Haririan A**, Ramos ER, Drachenberg CB, Weir MR, Klassen DK. Polyomavirus nephropathy in native kidneys of a solitary pancreas transplant recipient. *Transplantation* 2002; **73**: 1350-1353
17 **Haririan A**, Hamze O, Drachenberg CB, Ramos E, Weir MR, Klassen DK. Polyomavirus reactivation in native kidneys of pancreas alone allograft recipients. *Transplantation* 2003; **75**: 1186-1190
18 **Razonable RR**, Brown RA, Humar A, Covington E, Alecock E, Paya CV. A longitudinal molecular surveillance study of human polyomavirus viremia in heart, kidney, liver, and pancreas transplant patients. *J Infect Dis* 2005; **192**: 1349-1354
19 **Muñoz P**, Fogeda M, Bouza E, Verde E, Palomo J, Bañares R. Prevalence of BK virus replication among recipients of solid organ transplants. *Clin Infect Dis* 2005; **41**: 1720-1725
20 **Barton TD**, Blumberg EA, Doyle A, Ahya VN, Ferrenberg JM, Brozena SC, Limaye AP. A prospective cross-sectional study of BK virus infection in non-renal solid organ transplant recipients with chronic renal dysfunction. *Transpl Infect Dis* 2006; **8**: 102-107
21 **Salama M**, Boudville N, Speers D, Jeffrey GP, Ferrari P. Decline in native kidney function in liver transplant recipients is not associated with BK virus infection. *Liver Transpl* 2008; **14**: 1787-1792
22 **Egli A**, Helmersen DS, Taub K, Hirsch HH, Johnson A. Renal failure five years after lung transplantation due to polyomavirus BK-associated nephropathy. *Am J Transplant* 2010; **10**: 2324-2330
23 **García Ligero J**, Mora Peris B, García García F, Navas Pastor J, Tomás Ros M, Sempere Gutiérrez A, Rico Galiano JL, Fontana Compiano LO. [Hemorrhagic cystitis caused by BK and JC polyomavirus in patients treated with bone marrow transplantation: clinical features and urologic management]. *Actas Urol Esp* 2002; **26**: 104-110
24 **Vögeli TA**, Peinemann F, Burdach S, Ackermann R. Urological treatment and clinical course of BK polyomavirus-associated hemorrhagic cystitis in children after bone marrow transplantation. *Eur Urol* 1999; **36**: 252-257
25 **Singh D**, Kiberd B, Gupta R, Alkhudair W, Lawen J. Polyoma virus-induced hemorrhagic cystitis in renal transplantation patient with polyoma virus nephropathy. *Urology* 2006; **67**: 423.e11-423.e12
26 **Kazory A**, Ducloux D, Chalopin JM, Angonin R, Fontanière B, Moret H. The first case of JC virus allograft nephropathy. *Transplantation* 2003; **76**: 1653-1655
27 **Wen MC**, Wang CL, Wang M, Cheng CH, Wu MJ, Chen CH, Shu KH, Chang D. Association of JC virus with tubulointerstitial nephritis in a renal allograft recipient. *J Med Virol* 2004; **72**: 675-678
28 **Cavallo R**, Costa C, Bergallo M, Messina M, Mazzucco G, Segoloni GP. A case of ureteral lesions in a renal transplant recipient with a co-infection of BK virus and JC virus. *Nephrol Dial Transplant* 2007; **22**: 1275
29 **Drachenberg CB**, Hirsch HH, Papadimitriou JC, Gosert R, Wali RK, Munivenkatappa R, Nogueira J, Cangro CB, Haririan A, Mendley S, Ramos E. Polyomavirus BK versus JC replication and nephropathy in renal transplant recipients: a prospective evaluation. *Transplantation* 2007; **84**: 323-330
30 **Butel JS**, Arrington AS, Wong C, Lednicky JA, Finegold MJ. Molecular evidence of simian virus 40 infections in children. *J Infect Dis* 1999; **180**: 884-887
31 **Li RM**, Mannon RB, Kleiner D, Tsokos M, Bynum M, Kirk AD, Kopp JB. BK virus and SV40 co-infection in polyomavirus nephropathy. *Transplantation* 2002; **74**: 1497-1504
32 **Hirsch HH**. BK virus: opportunity makes a pathogen. *Clin Infect Dis* 2005; **41**: 354-360
33 **Hariharan S**. BK virus nephritis after renal transplantation. *Kidney Int* 2006; **69**: 655-662
34 **Ramos E**, Drachenberg CB, Wali R, Hirsch HH. The decade of polyomavirus BK-associated nephropathy: state of affairs. *Transplantation* 2009; **87**: 621-630
35 **Ginevri F**, Azzi A, Hirsch HH, Basso S, Fontana I, Cioni M, Bodaghi S, Salotti V, Rinieri A, Botti G, Perfumo F, Locatelli F, Comoli P. Prospective monitoring of polyomavirus BK replication and impact of pre-emptive intervention in pediatric kidney recipients. *Am J Transplant* 2007; **7**: 2727-2735
36 **Mischitelli M**, Fioriti D, Anzivino E, Bellizzi A, Ferretti G, Gussman N, Mitterhofer AP, Tinti F, Barile M, Dal Maso M, Chiarini F, Pietropaolo V. BKV QPCR detection and infection monitoring in renal transplant recipients. *New Microbiol* 2007; **30**: 271-274
37 **Marchetti S**, Graffeo R, Siddu A, Santangelo R, Ciotti M, Picardi A, Favalli C, Fadda G, Cattani P. BK virus DNA detection by real-time polymerase chain reaction in clinical specimens. *New Microbiol* 2007; **30**: 119-126
38 **Smith TF**, Espy MJ, Mandrekar J, Jones MF, Cockerill FR, Patel R. Quantitative real-time polymerase chain reaction for evaluating DNAemia due to cytomegalovirus, Epstein-Barr virus, and BK virus in solid-organ transplant recipients. *Clin Infect Dis* 2007; **45**: 1056-1061
39 **Ding R**, Medeiros M, Dadhania D, Muthukumar T, Kracker D, Kong JM, Epstein SR, Sharma VK, Seshan SV, Li B, Suthanthiran M. Noninvasive diagnosis of BK virus nephritis by measurement of messenger RNA for BK virus VP1 in urine. *Transplantation* 2002; **74**: 987-994
40 **Dadhania D**, Snopkowski C, Ding R, Muthukumar T, Chang C, Aull M, Lee J, Sharma VK, Kapur S, Suthanthiran M. Epidemiology of BK virus in renal allograft recipients: independent risk factors for BK virus replication. *Transplantation* 2008; **86**: 521-528
41 **Dadhania D**, Snopkowski C, Ding R, Muthukumar T, Lee J, Bang H, Sharma VK, Seshan S, August P, Kapur S, Suthanthiran M. Validation of noninvasive diagnosis of BK virus nephropathy and identification of prognostic biomarkers. *Transplantation* 2010; **90**: 189-197
42 **Hirsch HH**. VP1 messenger RNA levels in urine for diagnosing BK virus nephropathy? *Transplantation* 2003; **75**: 2160
43 **Astegiano S**, Bergallo M, Terlizzi ME, Sidoti F, Gambarino S, Messina M, Costa C, Segoloni GP, Cavallo R. Combined measurement of serum DNA and urine VP1 messenger RNA in monitoring BK virus replication in kidney graft recipients. *Transplant Proc* 2011; **43**: 1052-1054
44 **Viscount HB**, Eid AJ, Espy MJ, Griffin MD, Thomsen KM, Harmsen WS, Razonable RR, Smith TF. Polyomavirus polymerase chain reaction as a surrogate marker of polyomavirus-associated nephropathy. *Transplantation* 2007; **84**: 340-345
45 **Drachenberg CB**, Papadimitriou JC. Polyomavirus-associated nephropathy: update in diagnosis. *Transpl Infect Dis* 2006;

8: 68-75
46 **Schmid H**, Nitschko H, Gerth J, Kliem V, Henger A, Cohen CD, Schlöndorff D, Gröne HJ, Kretzler M. Polyomavirus DNA and RNA detection in renal allograft biopsies: results from a European multicenter study. *Transplantation* 2005; **80**: 600-604
47 **Randhawa P**, Shapiro R, Vats A. Quantitation of DNA of polyomaviruses BK and JC in human kidneys. *J Infect Dis* 2005; **192**: 504-509
48 **Costa C**, Bergallo M, Sidoti F, Astegiano S, Terlizzi ME, Mazzucco G, Segoloni GP, Cavallo R. Polyomaviruses BK- And JC-DNA quantitation in kidney allograft biopsies. *J Clin Virol* 2009; **44**: 20-23
49 **Hirsch HH**, Brennan DC, Drachenberg CB, Ginevri F, Gordon J, Limaye AP, Mihatsch MJ, Nickeleit V, Ramos E, Randhawa P, Shapiro R, Steiger J, Suthanthiran M, Trofe J. Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. *Transplantation* 2005; **79**: 1277-1286
50 **Comoli P**, Binggeli S, Ginevri F, Hirsch HH. Polyomavirus-associated nephropathy: update on BK virus-specific immunity. *Transpl Infect Dis* 2006; **8**: 86-94
51 **Ginevri F**, De Santis R, Comoli P, Pastorino N, Rossi C, Botti G, Fontana I, Nocera A, Cardillo M, Ciardi MR, Locatelli F, Maccario R, Perfumo F, Azzi A. Polyomavirus BK infection in pediatric kidney-allograft recipients: a single-center analysis of incidence, risk factors, and novel therapeutic approaches. *Transplantation* 2003; **75**: 1266-1270
52 **Chen Y**, Trofe J, Gordon J, Du Pasquier RA, Roy-Chaudhury P, Kuroda MJ, Woodle ES, Khalili K, Koralnik IJ. Interplay of cellular and humoral immune responses against BK virus in kidney transplant recipients with polyomavirus nephropathy. *J Virol* 2006; **80**: 3495-3505
53 **Schaub S**, Mayr M, Egli A, Binggeli S, Descoeudres B, Steiger J, Mihatsch MJ, Hirsch HH. Transient allograft dysfunction from immune reconstitution in a patient with polyoma BK-virus-associated nephropathy. *Nephrol Dial Transplant* 2007; **22**: 2386-2390
54 **Comoli P**, Azzi A, Maccario R, Basso S, Botti G, Basile G, Fontana I, Labirio M, Cometa A, Poli F, Perfumo F, Locatelli F, Ginevri F. Polyomavirus BK-specific immunity after kidney transplantation. *Transplantation* 2004; **78**: 1229-1232
55 **Comoli P**, Hirsch HH, Ginevri F. Cellular immune responses to BK virus. *Curr Opin Organ Transplant* 2008; **13**: 569-574
56 **Zhou W**, Sharma M, Martinez J, Srivastava T, Diamond DJ, Knowles W, Lacey SF. Functional characterization of BK virus-specific CD4+ T cells with cytotoxic potential in seropositive adults. *Viral Immunol* 2007; **20**: 379-388
57 **Binggeli S**, Egli A, Schaub S, Binet I, Mayr M, Steiger J, Hirsch HH. Polyomavirus BK-specific cellular immune response to VP1 and large T-antigen in kidney transplant recipients. *Am J Transplant* 2007; **7**: 1131-1139
58 **Vasudev B**, Hariharan S, Hussain SA, Zhu YR, Bresnahan BA, Cohen EP. BK virus nephritis: risk factors, timing, and outcome in renal transplant recipients. *Kidney Int* 2005; **68**: 1834-1839
59 **Prosser SE**, Orentas RJ, Jurgens L, Cohen EP, Hariharan S. Recovery of BK virus large T-antigen-specific cellular immune response correlates with resolution of bk virus nephritis. *Transplantation* 2008; **85**: 185-192
60 **Comoli P**, Basso S, Azzi A, Moretta A, De Santis R, Del Galdo F, De Palma R, Valente U, Nocera A, Perfumo F, Locatelli F, Maccario R, Ginevri F. Dendritic cells pulsed with polyomavirus BK antigen induce ex vivo polyoma BK virus-specific cytotoxic T-cell lines in seropositive healthy individuals and renal transplant recipients. *J Am Soc Nephrol* 2003; **14**: 3197-3204
61 **Hammer MH**, Meyer S, Brestrich G, Moosmann A, Kern F, Tesfa L, Babel N, Mittenzweig A, Rooney CM, Hammerschmidt W, Volk HD, Reinke P. HLA type-independent generation of antigen-specific T cells for adoptive immunotherapy. *Eur J Immunol* 2005; **35**: 2250-2258
62 **Binet I**, Nickeleit V, Hirsch HH, Prince O, Dalquen P, Gudat F, Mihatsch MJ, Thiel G. Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. *Transplantation* 1999; **67**: 918-922
63 **Brennan DC**, Agha I, Bohl DL, Schnitzler MA, Hardinger KL, Lockwood M, Torrence S, Schuessler R, Roby T, Gaudreault-Keener M, Storch GA. Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. *Am J Transplant* 2005; **5**: 582-594
64 **Dharnidharka VR**, Cherikh WS, Abbott KC. An OPTN analysis of national registry data on treatment of BK virus allograft nephropathy in the United States. *Transplantation* 2009; **87**: 1019-1026
65 **Halloran PF**. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004; **351**: 2715-2729
66 **Mengel M**, Marwedel M, Radermacher J, Eden G, Schwarz A, Haller H, Kreipe H. Incidence of polyomavirus-nephropathy in renal allografts: influence of modern immunosuppressive drugs. *Nephrol Dial Transplant* 2003; **18**: 1190-1196
67 **Trofe J**, Cavallo T, First MR, Weiskittel P, Peddi VR, Roy-Chaudhury P, Alloway RR, Safdar S, Buell JF, Hanaway MJ, Woodle ES. Polyomavirus in kidney and kidney-pancreas transplantation: a defined protocol for immunosuppression reduction and histologic monitoring. *Transplant Proc* 2002; **34**: 1788-1789
68 **Egli A**, Köhli S, Dickenmann M, Hirsch HH. Inhibition of polyomavirus BK-specific T-Cell responses by immunosuppressive drugs. *Transplantation* 2009; **88**: 1161-1168
69 **Johnston O**, Jaswal D, Gill JS, Doucette S, Fergusson DA, Knoll GA. Treatment of polyomavirus infection in kidney transplant recipients: a systematic review. *Transplantation* 2010; **89**: 1057-1070
70 **Barri YM**, Ahmad I, Ketel BL, Barone GW, Walker PD, Bonsib SM, Abul-Ezz SR. Polyoma viral infection in renal transplantation: the role of immunosuppressive therapy. *Clin Transplant* 2001; **15**: 240-246
71 **Mayr M**, Nickeleit V, Hirsch HH, Dickenmann M, Mihatsch MJ, Steiger J. Polyomavirus BK nephropathy in a kidney transplant recipient: critical issues of diagnosis and management. *Am J Kidney Dis* 2001; **38**: E13
72 **Limaye AP**, Jerome KR, Kuhr CS, Ferrenberg J, Huang ML, Davis CL, Corey L, Marsh CL. Quantitation of BK virus load in serum for the diagnosis of BK virus-associated nephropathy in renal transplant recipients. *J Infect Dis* 2001; **183**: 1669-1672
73 **Ramos E**, Drachenberg CB, Papadimitriou JC, Hamze O, Fink JC, Klassen DK, Drachenberg RC, Wiland A, Wali R, Cangro CB, Schweitzer E, Bartlett ST, Weir MR. Clinical course of polyoma virus nephropathy in 67 renal transplant patients. *J Am Soc Nephrol* 2002; **13**: 2145-2151
74 **Alméras C**, Foulongne V, Garrigue V, Szwarc I, Vetromile F, Segondy M, Mourad G. Does reduction in immunosuppression in viremic patients prevent BK virus nephropathy in de novo renal transplant recipients? A prospective study. *Transplantation* 2008; **85**: 1099-1104
75 **Burgos D**, López V, Cabello M, Sola E, Gutiérrez C, García I, Siles J, Florez P, González Molina M. Polyomavirus BK nephropathy: the effect of an early diagnosis on renal function or graft loss. *Transplant Proc* 2006; **38**: 2409-2411
76 **Kuypers DR**, Vandooren AK, Lerut E, Evenepoel P, Claes K, Snoeck R, Naesens L, Vanrenterghem Y. Adjuvant low-dose cidofovir therapy for BK polyomavirus interstitial nephritis in renal transplant recipients. *Am J Transplant* 2005; **5**: 1997-2004
77 **Wadei HM**, Rule AD, Lewin M, Mahale AS, Khamash HA, Schwab TR, Gloor JM, Textor SC, Fidler ME, Lager DJ, Larson TS, Stegall MD, Cosio FG, Griffin MD. Kidney transplant function and histological clearance of virus following diag-

nosis of polyomavirus-associated nephropathy (PVAN). *Am J Transplant* 2006; **6**: 1025-1032
78 **Benavides CA**, Pollard VB, Mauiyyedi S, Podder H, Knight R, Kahan BD. BK virus-associated nephropathy in sirolimus-treated renal transplant patients: incidence, course, and clinical outcomes. *Transplantation* 2007; **84**: 83-88
79 **Araya CE**, Lew JF, Fennell RS, Neiberger RE, Dharnidharka VR. Intermediate-dose cidofovir without probenecid in the treatment of BK virus allograft nephropathy. *Pediatr Transplant* 2006; **10**: 32-37
80 **Lim WH**, Mathew TH, Cooper JE, Bowden S, Russ GR. Use of cidofovir in polyomavirus BK viral nephropathy in two renal allograft recipients. *Nephrology* (Carlton) 2003; **8**: 318-323
81 **Tong CY**, Hilton R, MacMahon EM, Brown L, Pantelidis P, Chrystie IL, Kidd IM, Tungekar MF, Pattison JM. Monitoring the progress of BK virus associated nephropathy in renal transplant recipients. *Nephrol Dial Transplant* 2004; **19**: 2598-2605
82 **Scantlebury V**, Shapiro R, Randhawa P, Weck K, Vats A. Cidofovir: A method of treatment for BK virus-associated transplant nephropathy. *Graft* 2002; **5**: S82-S87
83 **Faguer S**, Hirsch HH, Kamar N, Guilbeau-Frugier C, Ribes D, Guitard J, Esposito L, Cointault O, Modesto A, Lavit M, Mengelle C, Rostaing L. Leflunomide treatment for polyomavirus BK-associated nephropathy after kidney transplantation. *Transpl Int* 2007; **20**: 962-969
84 **Josephson MA**, Gillen D, Javaid B, Kadambi P, Meehan S, Foster P, Harland R, Thistlethwaite RJ, Garfinkel M, Atwood W, Jordan J, Sadhu M, Millis MJ, Williams J. Treatment of renal allograft polyoma BK virus infection with leflunomide. *Transplantation* 2006; **81**: 704-710
85 **Leca N**, Muczynski KA, Jefferson JA, de Boer IH, Kowalewska J, Kendrick EA, Pichler R, Davis CL. Higher levels of leflunomide are associated with hemolysis and are not superior to lower levels for BK virus clearance in renal transplant patients. *Clin J Am Soc Nephrol* 2008; **3**: 829-835
86 **Gabardi S**, Waikar SS, Martin S, Roberts K, Chen J, Borgi L, Sheashaa H, Dyer C, Malek SK, Tullius SG, Vadivel N, Grafals M, Abdi R, Najafian N, Milford E, Chandraker A. Evaluation of fluoroquinolones for the prevention of BK viremia after renal transplantation. *Clin J Am Soc Nephrol* 2010; **5**: 1298-1304
87 **Hirsch HH**, Ramos E. Retransplantation after polyomavirus-associated nephropathy: just do it? *Am J Transplant* 2006; **6**: 7-9
88 **Womer KL**, Meier-Kriesche HU, Patton PR, Dibadj K, Bucci CM, Foley D, Fujita S, Croker BP, Howard RJ, Srinivas TR, Kaplan B. Preemptive retransplantation for BK virus nephropathy: successful outcome despite active viremia. *Am J Transplant* 2006; **6**: 209-213
89 **Mindlova M**, Boucek P, Saudek F, Jedinakova T, Voska L, Honsova E, Lipar K, Adamec M, Hirsch HH. Kidney retransplantation following graft loss to polyoma virus-associated nephropathy: an effective treatment option in simultaneous pancreas and kidney transplant recipients. *Transpl Int* 2008; **21**: 353-356
90 **Cooper JE**, Huskey J, Chan L, Wiseman AC. Preemptive retransplant for BK virus nephropathy without concurrent transplant nephrectomy. *Transplantation* 2010; **90**: 331-332

S- Editor Wang JL **L- Editor** A **E- Editor** Zheng XM

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
wjt@wjgnet.com
doi:10.5500/wjt.v2.i6.95

World J Transplant 2012 December 24; 2(6): 95-103
ISSN 2220-3230 (online)

REVIEW

Where are we at with short bowel syndrome and small bowel transplant?

Baris Dogu Yildiz

Baris Dogu Yildiz, Transplantation Surgery, Ankara Numune Teaching and Research Hospital, Ankara 06100, Turkey
Author contributions: Yildiz BD solely contributed to this paper.
Correspondence to: Baris Dogu Yildiz, MD, Head of Transplantation Surgery, Ankara Numune Teaching and Research Hospital, Ankara 06100, Turkey. baris104@yahoo.com
Telephone: +90-532-4454655 Fax: +90-312-4241520
Received: September 2, 2011 Revised: June 26, 2012
Accepted: October 31, 2012
Published online: December 24, 2012

Abstract

Intestinal failure can be defined as the critical reduction of functional gut mass below the minimal amount necessary for adequate digestion and absorption to satisfy body nutrient and fluid requirements in adults or children. Short bowel syndrome (SBS) is characterized by a state of malabsorption following extensive resection of the small bowel. SBS may occur after resection of more than 50% and is certain after resection of more than 70% of the small intestine, or if less than 100 cm of small bowel is left. Several treatment modalities other than total parenteral nutrition, including hormones (recombinant human growth hormone, glucagon-like peptide-2) and tailoring surgeries (Bianchi procedure, serial transverse enteroplasty), had been proposed, however these were either experimental or inefficient. Small bowel transplant is a rather new approach for SBS. The once feared field of solid organ transplantation is nowadays becoming more and more popular, even in developing countries. This is partially secondary to the developments in immunosuppressive strategy. In this regard, alemtuzumab deserves special attention. There are more complex surgeries, such as multivisceral transplantation, for multi-organ involvement including small bowel. This latter technique is relatively new when compared to small bowel transplant, and is performed in certain centers worldwide. In this review, an attempt is made to give an insight into small bowel syndrome, small bowel transplantation, and related issues.

Key words: Short bowel syndrome; Small bowel transplantation; Nutrition; Immunosuppression

Peer reviewer: Eleazar Chaib, PhD, Associate Professor of Surgery, Department of Gastroenterology, Liver Transplantation Surgery Unit, 455 Av. Dr. Arnaldo, 3rd floor, Room 3206, 01246-903, Sao Paulo, Brazil

Yildiz BD. Where are we at with short bowel syndrome and small bowel transplant? *World J Transplant* 2012; 2(6): 95-103 Available from: URL: http://www.wjgnet.com/2220-3230/full/v2/i6/95.htm DOI: http://dx.doi.org/10.5500/wjt.v2.i6.95

INTRODUCTION

Intestinal failure (IF) can be defined as the critical reduction of functional gut mass below the minimal amount necessary for adequate digestion and absorption to satisfy body nutrient and fluid requirements in adults or children. IF itself is a general term used in combination with short bowel syndrome (SBS)[1]. SBS is characterized by a state of malabsorption following extensive resection of the small bowel[2,3]. There is no exact current data regarding the incidence and prevalence of SBS. Data derived from patients receiving home parenteral nutrition (PN) indicate an incidence of severe SBS of 1-2 cases per 100 000 people per year[4].

Several conditions requiring intestinal resection lead to SBS in adults. In a reported series of 210 cases, these conditions included: 52 postoperative (25%), 51 irradiation/cancer (24%), 46 mesenteric vascular disease (22%), 34 Crohn's disease (16%), and 27 other benign causes (13%)[5].

Causes of IF in children include: SBS, congenital diseases of enterocyte development, and severe motility disorders (total or subtotal aganglionosis or chronic intestinal pseudo-obstruction syndrome) as shown in Table 1[6].

SBS may occur after resection of more than 50% and is certain after resection of more than 70% of the small intestine, or if less than 100 cm of small bowel is left. It is particularly severe after resection of the ileocecal region or if the colon has been additionally removed. Function is not dependent on length alone, since 150 cm of diseased bowel might function worse than 75 cm of healthy intestine. For this reason, some definitions of SBS and IF have been based on measurements of the functional capacity of the remaining bowel. A 48-h nutritional balance study in patients dependent on home total PN (TPN) compared with patients who were not, demonstrated that IF could be predicted by an absorption rate below 1.4 kg/d of wet weight and 84% of the calculated basal metabolic rate (1171 kilocalories/d of energy). It is important to note that nutritional balance studies are very difficult to perform accurately in practice, as they require the analysis of food portions and accurate stool collections[7-10].

After the insult on the gastrointestinal system, intestines show an adaptation process. This intestinal adaptation process in SBS has three phases. The acute phase starts directly after resection and generally lasts less than 4 wk. This serves for the patient's stabilization. The second phase is the adaptation phase, which lasts 1-2 years and represents maximal stimulation of intestinal adaptation achieved by gradually increasing intestinal nutrient exposure. The last phase is the maintenance phase, which requires permanent individualized dietetic treatment[11].

SURGICAL THERAPY FOR SBS

A small number of patients will acquire intestinal autonomy (i.e., PN weaning) very slowly because of major motility disorders or a small bowel without an ileocecal valve. In such patients, different surgical approaches have been proposed for increasing nutrient and fluid absorption by either slowing intestinal transit or increasing surface area.

Although surgical procedures aimed at slowing intestinal transit have been attempted and extensively reviewed, the clinical results are conflicting. Such procedures include intestinal valves, reversed intestinal segments, colon interposition, and electrical retrograde small bowel stimulation[12].

For selected patients with dilated bowel segments, longitudinal intestinal lengthening and tailoring (Bianchi procedure) was first proposed in 1980. The Bianchi procedure has the advantage of tapering the dilated segment and using the divided intestine to increase total small bowel length. Anatomic criteria have been suggested for patient selection for this procedure: (1) intestinal diameter > 3 cm; (2) length of residual small bowel > 40 cm; and (3) length of dilated bowel > 20 cm. This procedure allows improvement in more than 50% of patients in terms of intestinal transit, stool frequency, intestinal absorption rate, weight gain, and PN weaning[13].

Table 1 Causes of intestinal failure in children

Atresia	Necrotizing enterocolitis
Midgut volvulus	Arterial thrombosis
Abdominal wall defects	Venous Thrombosis
Gastrochisis	Intussusception
Ompalocele	Inflamatory bowel disease
Hirschsprung's disease	Post traumatic resection

The Bianchi procedure does not create any additional surface area for absorption, but has been demonstrated to increase the function of the remnant small bowel. Specific improvements have been shown in fat absorption, carbohydrate absorption, and the slowing of transit time through the intestine in children at 4 centers. Outcome is influenced by age and clinical status, especially liver status, of the patient at the time of surgery. It is yet not recommended to perform the Bianchi procedure in patients with severe liver disease or cirrhosis. However, this procedure may be successfully achieved after isolated liver transplantation for SBS[14].

A new procedure called serial transverse enteroplasty (STEP) was introduced in 2003 for infants and children with SBS. Experience with this procedure still remains too limited to make any confident recommendation[15,16].

A study comparing the outcomes of Bianchi type longitudinal lengthening to STEP lengthening stated that surgical lengthening with both Bianchi and STEP procedures results in an improvement in enteral nutrition, reverses complications of TPN, and avoids intestinal transplantation (ITx) in the majority, with few surgical complications. ITx can salvage most patients who later develop life-threatening complications or fail to wean TPN.

Surgical lengthening may therefore be useful in selected patients without complications of portal hypertension as a bridge to ITx, primarily in the youngest and jaundiced infants who are below 8 or 10 kg in body weight and unlikely to find an appropriate organ donor. Patients with advanced liver disease are poor candidates for lengthening and should instead be referred for ITx[17].

HORMONAL THERAPY FOR SBS

Recombinant human growth hormone (rhGH) was used in adult patients with SBS in both open and randomized clinical trials[18]. Scolapio *et al*[19] did not show benefits from the use of rhGH in short bowel adult patients, whereas Seguy *et al*[20] recently showed a significant improvement of the absorption rates, with a decrease in PN requirements of adult patients with SBS. In another study, growth hormone administration (0.5 IU/kg per day or 0.024 mg/kg per day) alone for 8 wk had no effect on the absorptive capacity of energy, protein, or fluid in 10 patients[21]. The use of rhGH treatment in adults remains controversial, whereas the incidence rate of secondary ef-

fects is high. To date, few studies have been reported in children with SBS[22-24]. An open-label clinical trial was performed in infants who received 0.3 IU/kg per day rhGH for a 10 d period of treatment[22]. A significant weight gain during treatment was reported, whereas no information was given about PN weaning. An open-label trial involving 8 PN-dependent children with neonatal SBS receiving > 50% of their protein energy requirements from PN was also performed[23]. They received 0.6 IU/kg per day rhGH for 3 mo. All were weaned from PN during the treatment period. However, only 2 children remained free of PN 1 year later. More recently, PN-dependent children with neonatal SBS received 0.14 mg/kg per day and glutamine for 3 mo[24]. Preliminary results suggested a beneficial effect of rhGH by decreasing the need for PN, but with mild effects on body composition and gut mucosa. More prolonged, and perhaps earlier, use of rhGH in SBS infants or children might be helpful for future management.

Glucagon-like peptide-2 (GLP-2), a 33 amino acid peptide-encoded carboxy-terminal to the sequence of GLP-1 in the proglucagon gene, is produced by L cells in the ileum in response to luminal nutrients[25]. The effect of GLP-2 on gastrointestinal function was assessed in patients without a terminal ileum or colon who had functional SBS with severe malabsorption and no postprandial secretion of GLP-2[26]. Balance studies were performed before and after treatment with GLP-2; 400 μg subcutaneously twice a day for 35 d. Treatment with GLP-2 improved the intestinal absorption of energy and increased body weight. Thus, GLP-2 improves intestinal absorption and nutritional status in short-bowel patients with impaired postprandial GLP-2 secretion in whom the terminal ileum and the colon have been resected, based on the hypothesis that distal small bowel and caecal resection would decrease GLP-2 levels and reduce adaptation[27]. GLP-2 might be the most logical medical approach for early management of short bowel patients, especially those with ileal resection. Genetically engineered GLP-2 analogs should be commercially available in the near future for clinical use.

ENTERAL NUTRITION IN SBS

Enteral nutrition is the most significant single factor in promoting intestinal adaptation, and may play a part in reducing the frequency of IF-associated liver disease. Detailed evidence on the management of SBS has recently been published[28]. Breast milk may be the best choice in the first few months, because of the presence of trophic factors such as epidermal growth factor. Amino acid based formulas may be beneficial in weaning children from PN, perhaps due to a smaller antigenic load[29].

Continuous nasogastric (NG) feeding initially, followed by overnight NG feeding and bolus feeding during the day, is recommended in order to utilize existing small bowel function and encourage oral feeding. Maintaining a urinary sodium/potassium ratio of at least 2:1 with an absolute urinary sodium concentration of over 10-20 mmol/L is important in children with ongoing fluid and electrolyte losses[30].

Currently, insufficient evidence exists in the literature to support the routine use of pectin, glutamine, growth hormone, insulin like growth factor 1, or *Saccharomyces boulardii* as trophic factors in the process of adaptation[31].

PN IN SBS

The North American Home Parenteral and Enteral Nutrition patient registry indicates a 4-year survival on a home PN of 80% for SBS patients and 70% for motility disorders[32]. The quality of life (QOL) of home PN patients of all ages is reported to not be significantly different from the scores in a reference population of healthy children and adolescents[33,34]. The main complications commonly associated with long term use of PN are: (1) Central venous catheter (CVC) related infections; (2) Thrombosis of the vessels leading to impaired venous access; and (3) IF-associated liver disease.

Episodes of line infection can cause a greater than 30% rise in bilirubin level, and cholestasis may develop in 90% of infants after first line infection[35,36]. The reduction in the overall incidence of CVC infection is crucial to sustained good health. Failure to prevent CVC infection greatly contributes to progression of liver disease. Involvement of a multidisciplinary nutritional care team and early discharge on home PN has been shown to reduce the incidence of CVC infection[37,38].

The repeated episodes of line infections with multiple surgical procedures to remove and replace new catheters may predispose to thrombosis of the major vessels, leading to impaired venous access (defined as the loss of two vascular sites in the neck to thrombosis)[39,40]. Despite meticulous care and aggressive strategies to prevent line infections, some children may develop end stage loss of venous access and need referral for ITx[41].

Pulmonary thromboembolism is another potentially fatal complication of long term venous access, occurring in 39% of children[42]. In asymptomatic children, yearly echocardiography and ventilation-perfusion scanning are recommended, unless there is clinical suspicion or the child is exhibiting symptoms suggestive of pulmonary embolism.

TPN failure was defined by Medicare as any one of the following: (1) impending or overt liver failure (jaundice, elevated liver enzymes, cirrhosis, portal hypertension); (2) thrombosis of central veins (at least two); (3) frequent central-line sepsis (more than two per year, fungemia, shock, acute respiratory distress syndrome); and (4) frequent severe dehydration. Prospective analyses of home TPN patients have shown that an ultra-short bowel of less than 20-30 cm is associated with a high risk of liver failure and poor survival in children and adults. Similarly, infants with total intestinal agangliosis or microvillus inclusion disease have low life expectancy. Transplantation in this situation has been termed "preemptive", and is being increasingly applied in the major

centers. "Preemptive" indications are (1) the high risk of death attributable to the underlying disease resulting from desmoid tumors associated with familial adenomatous polyposis; (2) congenital mucosal disorders such as microvillous inclusion disease; and (3) ultra-SBS with residual small intestine < 10 cm in infants and < 20 cm in adults[43].

SMALL BOWEL TRANSPLANTATION

The successful emergence of small bowel transplantation as a curative alternative has provided many patients with bowel failure to have an improved QOL, better nutrition, and a reduction in PN-associated complications. Since the initial small bowel transplants first performed in the 1980s[44,45], there have been technical improvements, novel immunosuppressive agents, better understanding of immune and gastrointestinal physiology, and increased clinical program experience. All of these factors have contributed to a remarkable improvement in bowel transplant, 1-year graft, and patient survival (estimated 80% and 80%, respectively), compared with only several years ago[46-48].

The spectrum of underlying diseases causing SBS in patients who have been transplanted is extensive and variable between pediatric and adult populations (Table 2). Generally, nonmalignant conditions are the norm for recipients, although occasional tumors such as desmoids[49] have been successfully treated with ITx.

Contraindications to small bowel transplantation include non-resectable or disseminated malignancy, unreconstructable vascular anatomy, diseases that are likely to recur after transplantation, profound disabilities that will not be corrected by transplantation, a loss of vascular access sufficient to allow transplantation, or an inability or unwillingness to comply with the post-transplant management plan (Table 3)[50].

Transplantation of the intestine can be performed as an isolated graft or in combination with other abdominal organs, since patients with IF often experience other complex abdominal pathologies that require organ replacement. As a result, there have been several variants of intestinal transplants, all derivatives of the "cluster" concept originally proposed by Starzl *et al*[51].

Isolated ITx is transplantation of the small intestine with or without the large intestine, and is more commonly performed in adults, whereas combined liver-intestinal transplant (LITx), performed *en bloc* or separately, is more commonly performed in children. The latter scenario occurs when there is concomitant liver failure (typically PN induced). With ITx, the entire jejunum and ileum is transplanted in the majority of cases and, when taken from a living donor and in cases in which reduction of the size of the graft is required, a 200-cm segment[52] is usually transplanted. In this regard, it is important to match size because of the need for closure of the abdomen. There is maintenance of as much native bowel as possible, particularly with recent data suggesting that increased residual or allograft bowel provides some protection from PN-associated injury. This is particularly relevant because there may be some supplementation of transplanted patients with PN for a period of time. When ITx is performed *en bloc*, the duodenum with a segment (or the entire pancreas) (Omaha technique) may be included to avoid the need for biliary reconstruction. Upper gastrointestinal continuity is maintained through the native stomach and pancreaticoduodenal complex, which are retained. In LITx, intestinal transplant is combined with the liver. These organs are transplanted *en bloc* or separately. When the liver and intestine are transplanted separately, the two organs can be transplanted at the same session or sequentially from the same or a different donor. The great majority of the donors for these two forms of ITx are from cadaveric donors, although living donors are also an option[53].

Multivisceral transplantation (MVTx) is the removal and replacement of both native foregut and midgut[54], in which the native abdominal viscera are resected and the composite graft, which includes the stomach, pancreaticoduodenal complex, and small intestine, are transplanted *en bloc* and form the new gastrointestinal tract. The liver, kidneys, and large intestine of the donor may or may not be included depending on the clinical scenario. Removal of the native organs is facilitated by early dearterialization, achieved by mass clamping of the celiac and superior mesenteric arteries. This can be achieved through a cephalad approach after division of the esophagus or proximal stomach, or a caudal approach between the inferior surface of the pancreas and left renal vein. Since 2000, the use of MVTx is increasing, and despite the fact

Table 2 Indications for bowel transplantation in children and adults

Children	Adults
Gastroschisis	Ischemia
Volvulus	Crohn's disease
Necrotizing enterocolitis	Trauma
Pseudoobstruction	Volvulus
Intestinal atresia	Motility disorders
Aganglionosis/Hirschsprung	Desmoids
Retransplant	Retransplant
Microvillous inclusion	Miscellaneous
Malabsorption	Gardner's syndrome
Tumors	

Table 3 Contraindications to small bowel transplant

Absolute contraindications
Neurological disabilities
Life threatening disease unrelated to the digestive system
Non-resectable malignancy
Relative contraindications
Severe immunological deficiencies
Multi-system autoimmune diseases
Inadequate vascular anatomy to warrant long term patency
Prematurity with lung disease

that the donors for MVTx are exclusively cadaveric, the 1-year graft and patient survival is at least as good as the other forms of ITx. As of mid-2005, an isolated intestinal graft has been performed in 44% of cases, an intestine transplant in combination with the liver in 38%, and a multivisceral transplant in 18%[55].

The decision to use one form of ITx *vs* another is typically determined by the individual patient's particular needs (type of underlying disorder, surgical history of the patient, type, and size of the donor). The emergence of promising data suggesting improved survival data and long-term sequelae, as well as possible immunologic advantage for MVTx, is allowing the clinical team more options as it determines which form of transplantation should be recommended.

OUTCOMES OF LIVING DONOR ITx

The technical aspects of living donor intestinal transplantation (LDIT) were standardized by Gruessner *et al*[56] in 1997. The donor operation consists of harvesting 200 cm (150 cm for pediatric recipients) of distal ileum, preserving at least 20 cm of terminal ileum and ileocecal valve. The vascular pedicle of the graft is formed by the distal branches of the superior mesenteric artery and vein, and is anastomosed to the infrarenal aorta and cava of the recipient. LDIT has several potential advantages, such as elimination of waiting time, the elective nature of the procedure, better human leukocyte antigen (HLA) matching, and a short cold ischemia time. LDIT tends to performed with well HLA-matched grafts. The significance of HLA matching in ITx is still to be determined. In fact, experienced programs have obtained good outcomes and low rates of rejection with poorly-matched deceased ITx[57,58]. A significant risk of antibody-mediated graft injury in settings of positive cross-match has been demonstrated[59].

In normal physiologic conditions, a significant amount of the energy produced in the enterocytes is used to maintain the integrity of the mucosa. Obviously, during period of ischemia, decreased energy production will affect the mucosal resistance, leading to an increased chance for bacterial translocation and septic complications in the post-transplant period[60,61]. The direct correlation between the duration of ischemia and the degree of mucosal injury is well known[62]. As shown in animal models, the process of mucosal damage starts even before organ harvesting, during the brain-dead state[63]. Irreversible damage has been seen after 5 h of cold ischemia and the rate of bacterial translocation increases significantly after 9 h[60]. A significant reduction of ischemia time has been achieved in the settings of LDIT.

NON-HEART BEATING DONOR INTESTINAL TRANSPLANT

Intestinal mucosa is sensitive to ischemic injury. When the intestinal graft is harvested from non-heart beating donors (NHBDs), the infectious-related mortality was higher and the absorptive function lower. Histological examination confirmed a higher grade of ischemic injury in the NHBD grafts that correlated with the clinical data. An experimental study suggested that non-heart-beating donation may not be indicated for small bowel transplantation[64].

IMMUNOSUPPRESSION IN ITx

Many therapies and combinations of immunosuppression (IS) have been used for ITx, but what remain undefined are the optimal IS regimens to achieve the required goals while preserving graft function and not predisposing the recipient to increased infections or malignancy.

Tacrolimus is a drug that allowed the development of a consistently successful intestinal transplant series and, to date, is the maintenance IS drug of choice[65]. One of the most significant changes to occur with ITx is the near ubiquitous use of induction IS therapy, with an estimated 90% of cases now using this as part of the overall regimen. The most common induction IS agent is anti-IL2-receptor antibody therapy followed by anti-lymphocyte globulin and Campath-1[66,67]. Their use has been associated with a reduction in the incidence and severity of rejection episodes, and an improvement of survival results, which have allowed maintenance with lower levels of tacrolimus. This latter issue has become important because there is now increasing evidence of calcineurin-inhibitor toxicities in patients receiving non-renal transplants[68]. Conversion to non-calcineurin-inhibitor drugs (such as rapamycin), use of steroid-sparing protocols, and a determination as to which IS therapy best maintains graft acceptance still need explanation.

COMPLICATIONS OF ITx

Besides general complications seen in small bowel surgeries (like anastomotic leaks), several common complications are worth mentioning in small bowel transplantation.

Acute cellular rejection

The diagnosis of intestinal acute cellular rejection (ACR) requires close correlation of clinical, endoscopic, and pathologic findings. The clinical symptoms of intestinal ACR include fever, nausea, vomiting, increased stomal output, abdominal pain, and distension. In severe cases, acute rejection may manifest as septic shock, with metabolic acidosis, hypotension, and adult respiratory distress syndrome, which likely results from loss of mucosal integrity and bacterial translocation across the intestinal wall.

The endoscopic appearances of intestinal ACR range from edema and hyperemia in mild cases, to granularity, loss of the fine mucosal vascular pattern, diminished peristalsis, and mucosal ulceration in more severe cases. The final diagnosis depends on histologic analysis of

Table 4 Histological characteristics of acute rejection of intestinal graft

Mild	> 6 apoptotic bodies/10 crypts, no mucosal ulceration, mild epithelial injury
Moderate	Diffuse crypt epithelial injury, focal confluent apoptosis, intimal arteritis
Severe	Mucosal ulceration, transmural arteritis

endoscopy-guided mucosal biopsy specimens. A grading system was used to retrospectively evaluate 3268 small bowel allograft biopsies from 52 adult patients who underwent ITx between 1990 and 1999 at the Thomas E Starzl Transplant Institute, University of Pittsburgh Medical Center (Table 4).

The results demonstrated that a grade indicating a more severe rejection episode was associated with a greater probability of an unfavorable outcome. Significantly increased levels of eosinophils with coexistent activated lymphocytes and crypt apoptosis suggest acute rejection. Peyer's patches are commonly sampled in mucosal biopsies, especially from the ileum. Although localized Peyer's patches without significant lymphoid activation do not indicate acute rejection, Peyer's patches with lymphoid activation (characterized by lymphoid cells with open chromatin, diffuse infiltration into the surrounding mucosa, or mixtures with eosinophils and neutrophils) are frequently associated with acute rejection.

The significance of lymphocytic cryptitis (increased numbers of lymphocytes in the crypt epithelium) is unclear. Although cryptitis is present in some cases of acute rejection, it is also observed in biopsy tissues without ACR. Because the distribution of acute rejection may be patchy, multiple biopsies (three to five) are often required. Biopsies from either the ileum or the jejunum are sufficient for histologic evaluation in most cases, although sampling from both the ileum and the jejunum may be required in some cases with ambiguous diagnoses. Most of the histologically diagnosed mild-acute rejection episodes are treated with increased IS. Various pathologic conditions must be differentiated from acute rejection, the most common of which include: nonspecific enteritis, cytomegalovirus infection, Epstein-Barr virus (EBV) infection, and post-transplant lymphoproliferative disorder (PTLD)[69].

Graft vs host disease

Graft *vs* host disease (GVHD) has the highest occurrence after small intestine transplantation (5.6%)[70], followed by liver transplantation (1%-2%)[71,72], with the mortality rate of solid organ transplant-associated acute GVHD ranging from 30% to more than 75%[73-75]. The amount of lymphoid tissue in the small bowel is much higher compared with other solid organ transplants, and this may explain the fact that the rate of GVHD in the recipients of small bowel transplants is increased (5.6%)[70]. Therapy consists mainly of increasing IS, support of hematopoiesis with cytokines, and discontinuation of antibiotics or any drugs that might be myelosuppressive. However, it is difficult to determine whether this is effective, as mortality normally exceeds more than 75%. Approximately 86 cases have been reported in the literature since 1987, and among them only 18 patients survived[74]. In 13 of the survivors, IS had been increased, while in 5 other cases, IS had been withdrawn. It could be argued that reducing IS and allowing the patient's immune system to have the opportunity to reject the engrafting donor lymphocytes, as well as helping the patient to respond to infections, could be an effective method of treatment[75-77]. Any treatment is more likely to work if it is begun before the onset of severe pancytopenia.

PTLD

The vast majority of PTLDs are EBV driven and arise either as a consequence of the reactivation of latent infection or, more commonly, infection of the host by latent virus from donor B cells[78]. The particularly high incidence of PTLD reported after ITx is a consequence of the high levels of IS traditionally used to prevent GVHD and rejection, along with the fact that a large load of donor lymphocytes is transplanted with the graft[79]. The gastrointestinal tract is frequently affected and it is important to distinguish between PTLD and other causes of graft infiltration, including rejection. *In situ* hybridization of tumor tissue for EBV RNA is a quick and sensitive way of confirming the diagnosis. It is crucial to distinguish PTLD from rejection because many patients will respond to a reduction in IS alone. If this fails, second-line treatment includes antivirals, chemotherapy, or interferon-α.

Experimental treatment using adoptive immunotherapy with donor leukocytes or specific anti-EBV specific cytotoxic T lymphocytes may be effective in aggressive cases.

CONCLUSION

Despite advances in medicine and surgery, SBS still remains a burden on the healthcare system and the economy. Along with novel medical therapies, various surgical techniques had been developed to overcome the consequences of a short bowel. Some of these approaches are still experimental, and the rest have limited success.

This limited success lead to the invention of ITx, which further branched into living donor ITx and MVTx. With the help of novel immunosuppressive regimens, the outcomes of ITx improved. The once feared field of solid organ transplantation is nowadays becoming more and more popular, even in developing countries, in the form of living donor transplantation.

In developing countries the cost of maintenance therapies for SBS and transferring patients for further treatment to developed countries far exceeds the cost of ITx performed on site. Thus ITx must be encouraged and take its place in abdominal organ transplantation departments worldwide.

REFERENCES

1 **Buchman AL**, Scolapio J, Fryer J. AGA technical review on short bowel syndrome and intestinal transplantation. *Gastroenterology* 2003; **124**: 1111-1134
2 **Buchman AL**. The medical and surgical management of short bowel syndrome. *MedGenMed* 2004; **6**: 12
3 **Jeejeebhoy KN**. Short bowel syndrome: a nutritional and medical approach. *CMAJ* 2002; **166**: 1297-1302
4 **Bakker H**, Bozzetti F, Staun M, Leon-Sanz M, Hebuterne X, Pertkiewicz M, Shaffer J, Thul P. Home parenteral nutrition in adults: a european multicentre survey in 1997. ESPEN-Home Artificial Nutrition Working Group. *Clin Nutr* 1999; **18**: 135-140
5 **Thompson JS**, DiBaise JK, Iyer KR, Yeats M, Sudan DL. Postoperative short bowel syndrome. *J Am Coll Surg* 2005; **201**: 85-89
6 **Goulet O**, Ruemmele F. Causes and management of intestinal failure in children. *Gastroenterology* 2006; **130**: S16-S28
7 **Jeppesen PB**, Mortensen PB. Intestinal failure defined by measurements of intestinal energy and wet weight absorption. *Gut* 2000; **46**: 701-706
8 **Nordgaard I**, Mortensen PB. Digestive processes in the human colon. *Nutrition* 1995; **11**: 37-45
9 **Royall D**, Wolever TM, Jeejeebhoy KN. Evidence for colonic conservation of malabsorbed carbohydrate in short bowel syndrome. *Am J Gastroenterol* 1992; **87**: 751-756
10 **Crenn P**, Vahedi K, Lavergne-Slove A, Cynober L, Matuchansky C, Messing B. Plasma citrulline: A marker of enterocyte mass in villous atrophy-associated small bowel disease. *Gastroenterology* 2003; **124**: 1210-1219
11 **Keller J**, Panter H, Layer P. Management of the short bowel syndrome after extensive small bowel resection. *Best Pract Res Clin Gastroenterol* 2004; **18**: 977-992
12 **Aigrain Y**, Cornet D, Cezard JP, Boureau M. Longitudinal division of small intestine: a surgical possibility for children with the very short bowel syndrome. *Z Kinderchir* 1985; **40**: 233-236
13 **Boeckman CR**, Traylor R. Bowel lengthening for short gut syndrome. *J Pediatr Surg* 1981; **16**: 996-997
14 **Weber TR**, Powell MA. Early improvement in intestinal function after isoperistaltic bowel lengthening. *J Pediatr Surg* 1996; **31**: 61-63; discussion 63-64
15 **Figueroa-Colon R**, Harris PR, Birdsong E, Franklin FA, Georgeson KE. Impact of intestinal lengthening on the nutritional outcome for children with short bowel syndrome. *J Pediatr Surg* 1996; **31**: 912-916
16 **Kim HB**, Fauza D, Garza J, Oh JT, Nurko S, Jaksic T. Serial transverse enteroplasty (STEP): a novel bowel lengthening procedure. *J Pediatr Surg* 2003; **38**: 425-429
17 **Sudan D**, Thompson J, Botha J, Grant W, Antonson D, Raynor S, Langnas A. Comparison of intestinal lengthening procedures for patients with short bowel syndrome. *Ann Surg* 2007; **246**: 593-601; discussion 601-604
18 **Byrne TA**, Persinger RL, Young LS, Ziegler TR, Wilmore DW. A new treatment for patients with short-bowel syndrome. Growth hormone, glutamine, and a modified diet. *Ann Surg* 1995; **222**: 243-254; discussion 254-255
19 **Scolapio JS**, Camilleri M, Fleming CR, Oenning LV, Burton DD, Sebo TJ, Batts KP, Kelly DG. Effect of growth hormone, glutamine, and diet on adaptation in short-bowel syndrome: a randomized, controlled study. *Gastroenterology* 1997; **113**: 1074-1081
20 **Seguy D**, Vahedi K, Kapel N, Souberbielle JC, Messing B. Low-dose growth hormone in adult home parenteral nutrition-dependent short bowel syndrome patients: a positive study. *Gastroenterology* 2003; **124**: 293-302
21 **Szkudlarek J**, Jeppesen PB, Mortensen PB. Effect of high dose growth hormone with glutamine and no change in diet on intestinal absorption in short bowel patients: a randomised, double blind, crossover, placebo controlled study. *Gut* 2000; **47**: 199-205
22 **Socha J**, Ksiazyk J, Fogel WA, Kierkus J, Lyszkowska M, Sasiak K. Is growth hormone a feasible adjuvant in the treatment of children after small bowel resection? *Clin Nutr* 1996; **15**: 185-188
23 **Goulet O**, Dabbas-Tyan M, Talbotec C, Kapel N, Rosilio M, Souberbielle JC, Corriol O, Ricour C, Colomb V. Effect of recombinant human growth hormone on intestinal absorption and body composition in children with short bowel syndrome. *JPEN J Parenter Enteral Nutr* 2010; **34**: 513-520
24 **Lifschitz C**, Duggan C, Lazngston C, Vanderhoof J, Shulman RJ. Growth Hormone (GH) therapy in children with short bowel syndrome (SBS): a randomized, placebo controlled study. Orlando: Digestive Disease Week, 2003: A100
25 **Drucker DJ**. Biological actions and therapeutic potential of the glucagon-like peptides. *Gastroenterology* 2002; **122**: 531-544
26 **Jeppesen PB**, Hartmann B, Hansen BS, Thulesen J, Holst JJ, Mortensen PB. Impaired meal stimulated glucagon-like peptide 2 response in ileal resected short bowel patients with intestinal failure. *Gut* 1999; **45**: 559-563
27 **Jeppesen PB**, Hartmann B, Thulesen J, Graff J, Lohmann J, Hansen BS, Tofteng F, Poulsen SS, Madsen JL, Holst JJ, Mortensen PB. Glucagon-like peptide 2 improves nutrient absorption and nutritional status in short-bowel patients with no colon. *Gastroenterology* 2001; **120**: 806-815
28 **Booth IW**, Lander AD. Short bowel syndrome. *Baillieres Clin Gastroenterol* 1998; **12**: 739-773
29 **Andorsky DJ**, Lund DP, Lillehei CW, Jaksic T, Dicanzio J, Richardson DS, Collier SB, Lo C, Duggan C. Nutritional and other postoperative management of neonates with short bowel syndrome correlates with clinical outcomes. *J Pediatr* 2001; **139**: 27-33
30 **Vanderhoof JA**. New and emerging therapies for short bowel syndrome in children. *J Pediatr Gastroenterol Nutr* 2004; **39** Suppl 3: S769-S771
31 **Jeppesen PB**, Szkudlarek J, Høy CE, Mortensen PB. Effect of high-dose growth hormone and glutamine on body composition, urine creatinine excretion, fatty acid absorption, and essential fatty acids status in short bowel patients: a randomized, double-blind, crossover, placebo-controlled study. *Scand J Gastroenterol* 2001; **36**: 48-54
32 **Kaufman SS**, Atkinson JB, Bianchi A, Goulet OJ, Grant D, Langnas AN, McDiarmid SV, Mittal N, Reyes J, Tzakis AG. Indications for pediatric intestinal transplantation: a position paper of the American Society of Transplantation. *Pediatr Transplant* 2001; **5**: 80-87
33 **Gottrand F**, Staszewski P, Colomb V, Loras-Duclaux I, Guimber D, Marinier E, Breton A, Magnificat S. Satisfaction in different life domains in children receiving home parenteral nutrition and their families. *J Pediatr* 2005; **146**: 793-797
34 **Agostoni C**, Axelson I, Colomb V, Goulet O, Koletzko B, Michaelsen KF, Puntis JW, Rigo J, Shamir R, Szajewska H, Turck D. The need for nutrition support teams in pediatric units: a commentary by the ESPGHAN committee on nutrition. *J Pediatr Gastroenterol Nutr* 2005; **41**: 8-11
35 **Sondheimer JM**, Asturias E, Cadnapaphornchai M. Infection and cholestasis in neonates with intestinal resection and long-term parenteral nutrition. *J Pediatr Gastroenterol Nutr* 1998; **27**: 131-137
36 **Beath SV**, Davies P, Papadopoulou A, Khan AR, Buick RG, Corkery JJ, Gornall P, Booth IW. Parenteral nutrition-related cholestasis in postsurgical neonates: multivariate analysis of risk factors. *J Pediatr Surg* 1996; **31**: 604-606
37 **Knafelz D**, Gambarara M, Diamanti A, Papadatou B, Ferretti F, Tarissi De Iacobis I, Castro M. Complications of home parenteral nutrition in a large pediatric series. *Transplant Proc* 2003; **35**: 3050-3051

38 **Beath SV**, Booth IW, Murphy MS, Buckels JA, Mayer AD, McKiernan PJ, Kelly DA. Nutritional care and candidates for small bowel transplantation. *Arch Dis Child* 1995; **73**: 348-350
39 **Juno RJ**, Knott AW, Racadio J, Warner BW. Reoperative venous access. *Semin Pediatr Surg* 2003; **12**: 132-139
40 **McBride KD**, Fisher R, Warnock N, Winfield DA, Reed MW, Gaines PA. A comparative analysis of radiological and surgical placement of central venous catheters. *Cardiovasc Intervent Radiol* 1997; **20**: 17-22
41 **Newall F**, Barnes C, Savoia H, Campbell J, Monagle P. Warfarin therapy in children who require long-term total parenteral nutrition. *Pediatrics* 2003; **112**: e386
42 **Dollery CM**, Sullivan ID, Bauraind O, Bull C, Milla PJ. Thrombosis and embolism in long-term central venous access for parenteral nutrition. *Lancet* 1994; **344**: 1043-1045
43 **O'Keefe SJ**. Candidacy for intestinal transplantation. *Am J Gastroenterol* 2006; **101**: 1644-1646
44 **Pironi L**, Goulet O, Buchman A, Messing B, Gabe S, Candusso M, Bond G, Gupte G, Pertkiewicz M, Steiger E, Forbes A, Van Gossum A, Pinna AD; the Home Artificial Nutrition and Chronic Intestinal Failure Working Group of ESPEN. Outcome on home parenteral nutrition for benign intestinal failure: A review of the literature and benchmarking with the European prospective survey of ESPEN. *Clin Nutr* 2012; **31**: 831-845
45 **Di Benedetto F**, Lauro A, Masetti M, Cautero N, De Ruvo N, Quintini C, Sassi S, Di Francesco F, Diago Usò T, Romano A, Dazzi A, Molteni G, Begliomini B, Siniscalchi A, De Pietri L, Bagni A, Merighi A, Codeluppi M, Girardis M, Ramacciato G, Pinna AD. [Outcome of isolated small bowel transplantation in adults: experience from a single Italian center]. *Minerva Chir* 2005; **60**: 1-9
46 **Fryer JP**. Intestinal transplantation: an update. *Curr Opin Gastroenterol* 2005; **21**: 162-168
47 **Pritchard TJ**, Kirkman RL. Small bowel transplantation. *World J Surg* 1985; **9**: 860-867
48 Intestinal Transplant Registry Data. Available from: URL: http://www.intestinaltransplant.org/
49 **Tryphonopoulos P**, Weppler D, Levi DM, Nishida S, Madariaga JR, Kato T, Mittal N, Moon J, Selvaggi G, Esquenazi V, Cantwell P, Ruiz P, Miller J, Tzakis AG. Transplantation for the treatment of intra-abdominal fibromatosis. *Transplant Proc* 2005; **37**: 1379-1380
50 **Gilroy RK**, Botha JF, Sudan DL. Intestinal and multivisceral transplantation. Accessed February 11, 2005. Available from: URL: http://www.emedicine.com/med/topic3502.htm
51 **Ruiz P**, Kato T, Tzakis A. Current status of transplantation of the small intestine. *Transplantation* 2007; **83**: 1-6
52 **Starzl TE**, Todo S, Tzakis A, Alessiani M, Casavilla A, Abu-Elmagd K, Fung JJ. The many faces of multivisceral transplantation. *Surg Gynecol Obstet* 1991; **172**: 335-344
53 **Kato T**, Gaynor JJ, Selvaggi G, Mittal N, Thompson J, McLaughlin GE, Nishida S, Moon J, Levi D, Madariaga J, Ruiz P, Tzakis A. Intestinal transplantation in children: a summary of clinical outcomes and prognostic factors in 108 patients from a single center. *J Gastrointest Surg* 2005; **9**: 75-89; discussion 89
54 **Testa G**, Holterman M, John E, Kecskes S, Abcarian H, Benedetti E. Combined living donor liver/small bowel transplantation. *Transplantation* 2005; **79**: 1401-1404
55 **Tzakis AG**, Kato T, Levi DM, Defaria W, Selvaggi G, Weppler D, Nishida S, Moon J, Madariaga JR, David AI, Gaynor JJ, Thompson J, Hernandez E, Martinez E, Cantwell GP, Augenstein JS, Gyamfi A, Pretto EA, Dowdy L, Tryphonopoulos P, Ruiz P. 100 multivisceral transplants at a single center. *Ann Surg* 2005; **242**: 480-490; discussion 491-493
56 **Gruessner RW**, Sharp HL. Living-related intestinal transplantation: first report of a standardized surgical technique. *Transplantation* 1997; **64**: 1605-1607
57 **Tzvetanov IG**, Oberholzer J, Benedetti E. Current status of living donor small bowel transplantation. *Curr Opin Organ Transplant* 2010; **15**: 346-348
58 **Reyes J**, Mazariegos GV, Abu-Elmagd K, Macedo C, Bond GJ, Murase N, Peters J, Sindhi R, Starzl TE. Intestinal transplantation under tacrolimus monotherapy after perioperative lymphoid depletion with rabbit anti-thymocyte globulin (thymoglobulin). *Am J Transplant* 2005; **5**: 1430-1436
59 **Langnas AN**. Advances in small-intestine transplantation. *Transplantation* 2004; **77**: S75-S78
60 **Wu T**, Abu-Elmagd K, Bond G, Demetris AJ. A clinicopathologic study of isolated intestinal allografts with preformed IgG lymphocytotoxic antibodies. *Hum Pathol* 2004; **35**: 1332-1339
61 **Cicalese L**, Sileri P, Green M, Abu-Elmagd K, Kocoshis S, Reyes J. Bacterial translocation in clinical intestinal transplantation. *Transplantation* 2001; **71**: 1414-1417
62 **Cicalese L**, Sileri P, Asolati M, Rastellini C, Abcarian H, Benedetti E. Low infectious complications in segmental living related small bowel transplantation in adults. *Clin Transplant* 2000; **14**: 567-571
63 **Fryer J**, Angelos P. Is there a role for living donor intestine transplants? *Prog Transplant* 2004; **14**: 321-329
64 **Cobianchi L**, Zonta S, Vigano J, Dominioni T, Ciccocioppo R, Morbini P, Bottazzi A, Mazzilli M, De Martino M, Vicini E, Filisetti C, Botrugno I, Dionigi P, Alessiani M. Experimental small bowel transplantation from non-heart-beating donors: a large-animal study. *Transplant Proc* 2009; **41**: 55-56
65 **Grant D**, Wall W, Mimeault R, Zhong R, Ghent C, Garcia B, Stiller C, Duff J. Successful small-bowel/liver transplantation. *Lancet* 1990; **335**: 181-184
66 **Pinna AD**, Weppler D, Nery J, Ruiz P, Kato T, Khan F, Levi D, Nishida S, De Faria W, Berho M, Tzakis AG. Intestinal transplantation at the University of Miami-five years of experience. *Transplant Proc* 2000; **32**: 1226-1227
67 **Tzakis AG**, Kato T, Nishida S, Levi DM, Tryphonopoulos P, Madariaga JR, De Faria W, Nery JR, Regev A, Vianna R, Miller J, Esquenazi V, Weppler D, Ruiz P. Alemtuzumab (Campath-1H) combined with tacrolimus in intestinal and multivisceral transplantation. *Transplantation* 2003; **75**: 1512-1517
68 **Ojo AO**, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, Arndorfer J, Christensen L, Merion RM. Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003; **349**: 931-940
69 **Wu T**, Abu-Elmagd K, Bond G, Nalesnik MA, Randhawa P, Demetris AJ. A schema for histologic grading of small intestine allograft acute rejection. *Transplantation* 2003; **75**: 1241-1248
70 **Taylor AL**, Gibbs P, Bradley JA. Acute graft versus host disease following liver transplantation: the enemy within. *Am J Transplant* 2004; **4**: 466-474
71 **Smith DM**, Agura E, Netto G, Collins R, Levy M, Goldstein R, Christensen L, Baker J, Altrabulsi B, Osowski L, McCormack J, Fichtel L, Dawson DB, Domiati-Saad R, Stone M, Klintmalm G. Liver transplant-associated graft-versus-host disease. *Transplantation* 2003; **75**: 118-126
72 **Assi MA**, Pulido JS, Peters SG, McCannel CA, Razonable RR. Graft-vs.-host disease in lung and other solid organ transplant recipients. *Clin Transplant* 2007; **21**: 1-6
73 **Kohler S**, Pascher A, Junge G, Sauer IM, Nagy M, Schönemann C, Koch M, Neumann U, Pratschke J, Neuhaus P. Graft versus host disease after liver transplantation - a single center experience and review of literature. *Transpl Int* 2008; **21**: 441-451
74 **Taylor AL**, Gibbs P, Sudhindran S, Key T, Goodman RS, Morgan CH, Watson CJ, Delriviere L, Alexander GJ, Jamieson NV, Bradley JA, Taylor CJ. Monitoring systemic donor lymphocyte macrochimerism to aid the diagnosis of graft-

versus-host disease after liver transplantation. *Transplantation* 2004; **77**: 441-446

75 **Chinnakotla S**, Smith DM, Domiati-Saad R, Agura ED, Watkins DL, Netto G, Uemura T, Sanchez EQ, Levy MF, Klintmalm GB. Acute graft-versus-host disease after liver transplantation: role of withdrawal of immunosuppression in therapeutic management. *Liver Transpl* 2007; **13**: 157-161

76 **Lu Y**, Wu LQ, Zhang BY, Cao JY. Graft-versus-host disease after liver transplantation: successful treatment of a case. *Transplant Proc* 2008; **40**: 3784-3786

77 **Zhang Y**, Ruiz P. Solid organ transplant-associated acute graft-versus-host disease. *Arch Pathol Lab Med* 2010; **134**: 1220-1224

78 **Chadburn A**, Cesarman E, Knowles DM. Molecular pathology of posttransplantation lymphoproliferative disorders. *Semin Diagn Pathol* 1997; **14**: 15-26

79 **Lee RG**, Nakamura K, Tsamandas AC, Abu-Elmagd K, Furukawa H, Hutson WR, Reyes J, Tabasco-Minguillan JS, Todo S, Demetris AJ. Pathology of human intestinal transplantation. *Gastroenterology* 1996; **110**: 1820-1834

S- Editor Cheng JX **L- Editor** Rutherford A **E- Editor** Zheng XM

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
wjt@wjgnet.com
www.wjgnet.com

World J Transplant 2012 December 24; 2(6): I
ISSN 2220-3230 (online)

Acknowledgments to reviewers of *World Journal of Transplantation*

We acknowledge our sincere thanks to our reviewers. Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of our World Series Journals. Both the editors of the journals and authors of the manuscripts submitted to the journals are grateful to the following reviewers for reviewing the articles (either published or rejected) over the past period of time.

Andrea De Gottardi, MD, PhD, Assistant Professor, Clinic of Visceral Surgery and Medicine, Hepatology, Freiburgstrasse, CH-3010 Berne, Inselspital, Switzerland

Yu-Fan Cheng, MD, Department of Radiology, Chang Gung Memorial Hospital Kaohsiung Medical Center, 123, TA Pei Road, Niao Sung Hsiang, Kaohsiung Hsien 833, Taiwan, China

Sarah Anne Hosgood, Miss, BSc, Department of Infection, Immunity and Inflammation, Transplant Group, Leicester General Hospital, University of Leicester, LE5 4PW, United Kingdom

Ahad Eshraghian, MD, Department of Internal medicine, Namazi hospital, Shiraz University of medical science, Shiraz, 71345-1377, Iran

Frank JMF Dor, MD, PhD, Division of Transplant Surgery, Department of Surgery, Erasmus MC Rotterdam, room H-811, PO BOX 2040, 3000 CA Rotterdam, The Netherlands

Olivier Detry, MD, PhD, Associate Professor, Department of Abdominal Surgery and Transplantation, University of Liège, CHU Liège, Sart Tilman B35, B4000 Liège, Wallonia, Belgium

Andres Beiras-Fernandez, MD, PhD, Department of Cardiac Surgery, University Hospital Munich, Marchioninistraße 15, 81377 Munich, Germany

Ilka FSF Boin, MD, PhD, Associate Professor, Director of Unit of Liver Transplantation, HC, Unicamp, Surgery Department, Faculty of Medical Sciences, State University of Campinas, Av. Carlos Chagas, 420, Postal Code 13983-000, Campinas, SP, Brazil

Costas Fourtounas, MD, PhD, Associate Professor, Department of Nephrology, Patras University Hospital, Rio-Patras 26500, Greece

Wenda Gao, PhD, Assistant Professor, Department of Medicine, Transplant Institute, Beth Israel Deaconess Med Ctr, SL-427, Harvard Medical School, Boston, MA 02215, United States

Mehdi Hamadani, MD, Assistant Professor of Medicine, Hematology, Oncology, West Virginia University, PO Box 9162, 1 Medical Center Drive, Morgantown, WV 26506, United States

Kuzhuvelil B Harikumar, Post Doctoral Associate, Department of Biochemistry, Virginia Commonwealth University, 1101 East Marshall St, Richmond VA 23298, United States

Walid Mohamed El Moghazy, MD, PhD, Department of Hepatobiliary, Pancreas and Transplant Surgery, Kyoto University Hospital, 54 Kawara-cho, Shogoin, Sakyo-ku, Kyoto city, Kyoto, 606-8507, Japan

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
wjt@wjgnet.com
www.wjgnet.com

World J Transplant 2012 December 24; 2(6): I
ISSN 2220-3230 (online)

Events Calendar 2012

January 29 - 31, 2012
2nd Joint AIDPIT and EPITA Winter Symposium & 31st AIDPIT Workshop
Innsbruck, Austria

February 1 - 5, 2012
2012 BMT Tandem Meetings
American Society for Blood and Marrow Transplantation
Manchester Grand Hyatt,
San Diego, CA, United States

February 22 - 24, 2012
British Transplantation Society 15th Annual Congress
Glasgow, Scotland

February 23 - 25, 2012
2012 Canadian Society of Transplantation Annual Scientific Conference
Fairmont Château Frontenac,
Québec, Canada

March 8 - 10, 2012
3rd International Conference on Transplantomics and Biomarkers in Organ Transplantation
La Jolla/San Diego,
CA, United States

April 18 - 21, 2012
The International Society for Heart and Lung Transplantation (ISHLT), 32nd Annual Meeting and Scientific Sessions
Prague, Czech Republic

April 25 - 27, 2012
United Network for Organ Sharing's 20th Annual Transplant Management Forum
Wyndham Rio Mar Beach Resort,
Puerto Rico

June 2 - 6, 2012
2012 American Transplant Congress
John B. Hynes Convention Center,
Boston, MA, United States

July 15 - 19, 2011
24th International Congress of the Transplantation Society
Berlin, Germany

September 13 - 15, 2012
ELITA - LICAGE LIVER MEETING and 4th ELITA Split-Liver Course
Ghent, Belgium

September 29 - 30, 2012
Advances in nephrology, dialysis, Kidney Transplantation
Odessa, Ukraine

October 5 - 7, 2012
V Congress of Transplantologists
Kharkiv, Ukraine

October 5 - 7, 2012
2012 European Organ Donation Congress, 24th ETCO-EDC
Dubrovnik, Croatia

October 12 - 14, 2012
ESOT and AST Joint Meeting - Transformational therapies and diagnostics in transplantation
Nice, France

November 2 - 4, 2012
5th ELPAT Invitational Working Groups Meeting
Sicily, Italy

World Journal of Transplantation

Online Submissions: http://www.wjgnet.com/esps/
wjt@wjgnet.com
www.wjgnet.com

World J Transplant 2012 December 24; 2(6): I-V
ISSN 2220-3230 (online)

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Transplantation (*World J Transplant*, *WJT*, online ISSN 2220-3230, DOI: 10.5500) is a bimonthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 100 experts in transplantation from 29 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJT* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJT* is an OA journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJT* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

WJT aims to report rapidly new theories, methods and techniques for prevention, diagnosis, treatment, rehabilitation and nursing in the field of transplantation. *WJT* covers topics concerning organ and tissue donation and preservation; tissue injury, repair, inflammation, and aging; immune recognition, regulation, effector mechanisms, and opportunities for induction of tolerance, thoracic transplantation (heart, lung), abdominal transplantation (kidney, liver, pancreas, islets), transplantation of tissues, cell therapy and islet transplantation, clinical transplantation, experimental transplantation, immunobiology and genomics, xenotransplantation, and transplantation-related traditional medicine, and integrated Chinese and Western medicine. The journal also publishes original articles and reviews that report the results of transplantation-related applied and basic research in fields such as immunology, physiopathology, cell biology, pharmacology, medical genetics, and pharmacology of Chinese herbs.

Columns

The columns in the issues of *WJT* will include: (1) Editorial: To introduce and comment on the substantial advance and its importance in the fast-developing areas; (2) Frontier: To review the most representative achievements and comment on the current research status in the important fields, and propose directions for the future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (6) Review: To systemically review the most representative progress and unsolved problems in the major scientific disciplines, comment on the current research status, and make suggestions on the future work; (7) Original Articles: To originally report the innovative and valuable findings in transplantation; (8) Brief Articles: To briefly report the novel and innovative findings in transplantation; (9) Case Report: To report a rare or typical case; (10) Letters to the Editor: To discuss and make reply to the contributions published in *WJT*, or to introduce and comment on a controversial issue of general interest; (11) Book Reviews: To introduce and comment on quality monographs of transplantation; and (12) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on the research in transplantation.

Name of journal

World Journal of Transplantation

ISSN

ISSN 2220-3230 (online)

Editor-in-Chief

Editorial Office
World Journal of Transplantation
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjt@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-85381891
Fax: +86-10-85381893

Indexed and Abstracted in
Digital Object Identifier.

Published by
Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc.* The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJT* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions
Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2220-3230/g_info_20100722180909.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjt@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJT*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/2220-3230/g_info_20100725072755.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2220-3230/g_info_20100725073806.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

Editorial: http://www.wjgnet.com/2220-3230/g_info_20100725071851.htm

Frontier: http://www.wjgnet.com/2220-3230/g_info_20100725071932.htm

Topic highlight: http://www.wjgnet.com/2220-3230/g_info_20100725072121.htm

Observation: http://www.wjgnet.com/2220-3230/g_info_20100725072232.htm

Guidelines for basic research: http://www.wjgnet.com/2220-3230/g_info_20100725072344.htm

Guidelines for clinical practice: http://www.wjgnet.com/2220-3230/g_info_20100725072543.htm

Review: http://www.wjgnet.com/2220-3230/g_info_20100725072656.htm

Original articles: http://www.wjgnet.com/2220-3230/g_info_20100725072755.htm

Brief articles: http://www.wjgnet.com/2220-3230/g_info_20100725072920.htm

Case report: http://www.wjgnet.com/2220-3230/g_info_20100725073015.htm

Letters to the editor: http://www.wjgnet.com/2220-3230/g_info_20100725073136.htm

Book reviews: http://www.wjgnet.com/2220-3230/g_info_20100725073214.htm

Guidelines: http://www.wjgnet.com/2220-3230/g_info_20100725073300.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade B certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com..

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2220-3230/g_info_20100725073726.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2220-3230/g_info_20100725073445.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJT will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

WJT is an international, peer-reviewed, OA, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 600 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.