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EDITORIAL

Biomarkers and a tailored approach for immune monitoring in kidney transplantation

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Abstract

A literature review on immune monitoring in kidney transplantation produced dozens of research articles and a multitude of promising biomarkers, all in the quest for the much sought after - but perennially elusive - "holy grail" of kidney biomarkers able to unequivocally predict acute transplant rejection *vs* non-rejection. Detection methodologies and study designs were many and varied. Hence the motivation for this editorial, which espouses the notion that in today's kidney transplantation milieu, the judicious use of disease classifiers tailored to specific patient immune risks may be more achievable and productive in the long run and confer a greater advantage for patient treatment than the pursuit of a single "omniscient" biomarker. In addition, we desire to direct attention toward greater scrutiny of biomarker publications and decisions to implement biomarkers in practice, standardization of methods in the development of biomarkers and consideration for adoption of "biomarker-driven" biopsies. We propose "biomarker-driven" biopsies as an adjunctive to and/or alternative to random surveillance (protocol) biopsies or belated indication biopsies. The discovery of a single kidney transplantation biomarker would represent a major breakthrough in kidney transplantation practice, but until that occurs - if ever it does occur, other approaches offer substantial potential for unlocking prognostic, diagnostic and therapeutic options. We conclude our editorial with suggestions and recommendations for productively incorporating current biomarkers into diagnostic algorithms and for testing future biomarkers of acute

rejection in kidney transplantation.

Key words: Acute rejection; Banff classification; Biomarker; Human leukocyte antigen matching; Immune monitoring; Immunological risk; Kidney transplantation; Protocol biopsy

Core tip: In kidney transplantation, a multitude of biomarkers have been proposed to predict transplant rejection *vs* non-rejection, but few - if any - have gained acceptance as reliable tools for predicting rejection. However, an approach more likely to be successful would include improved timing of kidney transplant biopsies and judicious use of multiple diagnostic methodologies based on different immune risks and events throughout transplantation. This approach could also aid in improving diagnostic and prognostic kidney transplantation algorithms and in developing more impactful therapeutic options.

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INTRODUCTION

Kidney transplantation provides kidney failure patients the best opportunity to live longer and fuller lives. Indeed, kidney transplantation is recommended as the first option for suitable patients. However, immunosuppressive drugs currently in use for kidney transplantation are not one-hundred per cent effective in preventing acute or subclinical rejection episodes, or premature transplant failure. In addition, immunosuppressive drugs bring a constellation of side effects linked to significant morbidity and mortality. Thus, until the advent of more targeted and effective, less toxic and tolerogenic immunotherapies, the best strategy appears to be tailoring current immunosuppressive ammunition to the specific immune systems affected by kidney transplant patients. However, the tailored immunosuppressive approach stands in contrast to the current protocolised indiscriminate minimisation of immunosuppression, which has proven to be counterproductive in many instances[1]. Tailoring immune monitoring strategies to a patient's particular risks of rejection, of transplant loss and of transplant-related complications would certainly be more impactful and cost-effective than the non-judicious use of "in vogue" biomarkers.

Conventional monitoring of kidney transplant patients consists of assessing dynamic changes in serum creatinine levels as well as other laboratory parameters such as proteinuria and immunosuppressive drug levels. Additionally, some transplant programmes perform surveillance biopsies, and many measure donor-specific alloantibodies (DSA). DSA are clearly markers of an ongoing anti-allograft response and traditionally viewed as late and ominous markers of rejection that are difficult to counteract. DSA are currently under thorough evaluation in the United Kingdom[2]. Importantly, most immunosuppressive dose changes in kidney transplantation are guided by drug blood levels and their associated toxicities or as a consequence of infections or rejection episodes. But conventional immunomonitoring strategies are unable to reveal the actual state of the immune system and the body's innate defense system. It would be expected then that accurate information on the detector, effector and regulatory arms of the immune system would aid researchers in their quest for more clinically useful biomarkers with improvements in diagnostic accuracy and outcome prediction. It would be anticipated that biomarkers derived from actual immune processes occurring *in vivo* in kidney transplantation would be more likely to guide physicians in choosing the most suitable immunosuppressive strategy.

A substantial impediment to biomarker discovery and application is that the activated targetable immune pathways vary with the immune risk profile of each donor-recipient pair, as well as with the immunosuppressive regimens selected for the recipient. Making the situation even more complex, activated pathways change dynamically throughout the various transplantation stages in response to immunological and infective events occurring throughout the duration of the kidney transplant, and due to modifications in immunosuppressive drugs. Therefore, it is unlikely that one or even a few universal biomarkers can guide transplant physicians in the best use of immunosuppressive regimens throughout all stages of transplantation. However, a combination of clinical parameters and biomarkers revealing distinct immunological, inflammatory and tolerogenic processes occurring at different stages post-transplantation could provide a more useful guide to clinicians. In striving to provide a more accurate picture of the state of the immune system, and hence of the requirements for specific kidney transplant patients, the ideal strategy would complement the immune biomarker analysis with biomarkers revealing parenchymal tissue injury, repair, fibrosis and senescence. Finally, knowledge of the kinetics and interplay of these processes is essential for a proper interrogation and utilization of the biomarker universe.

PERFECT BIOMARKER *VS* TODAY'S REALITY

Biomarker preferred definitions and conceptual framework have been formulated by the Biomarkers Definitions Working Group[3]. Our definition of the perfect tailored immunosuppressive biomarker combines the following properties and characteristics: (1) is easily obtained non-invasively from patients to allow

multiple and sequential analyses; (2) is easily detected and detectable prior to clinically observable events; (3) reflects physiopathogenic mechanisms; (4) demonstrates strong immunodiagnostic and theragnostic value to guide selection and changes in immunosuppressive therapies and possess immunopredictive value; (5) correlates with treatment response; (6) anticipates potential clinical outcomes before and after interventions; (7) indicates over-immunosuppression and risk of infection and cancers; (8) inexpensive with rapid turnaround time; and (9) spares the patient from a kidney transplant biopsy. However, given the complexity of the immune system and alloresponses, the perfect biomarker may be just a pipe-dream.

In kidney transplantation, urine is the most attractive sample source for non-invasive biomarker testing and discovery. Urine is also very accessible, and several urine biomarkers have shown great promise. For instance, chemokines CXCL10 and CXCL9, measured by ELISA, were found to be elevated in urine up to 30 d prior to the episode of acute rejection, and importantly, levels decreased with anti-rejection treatment and displayed prognostic value[4]. Similarly, higher levels of urinary transcripts for cytotoxic cell products like perforin and granzyme B are found in patients with rejection as opposed to non-rejection states[5,6]. Despite the anatomical relationship with the transplant, the kidney does not leak all molecules released by the immune system or injured parenchymal cells into the urine. Many of the leaked molecules are not reliable surrogate markers of rejection, and are even less reliable as markers of tolerance.

On the other hand, whole blood and serum are very accessible, and transcripts for cytotoxic cell products like granzyme B, perforin and granulolysin top the list of promising biomarkers to differentiate rejection from non-rejection[7]. However, many of the molecules participating in transplant rejection or inflammation are not leaked into the blood compartment or they are diluted. Many cells involved in alloimmune processes and detectable in tissue[8] remain or die inside the kidney, or migrate preferentially to draining lymph nodes, which make them inaccessible to the physician's tools. In spite of these limitations, alloreactive memory/effector T cell responses in peripheral blood using an IFN-gamma ELISPOT[9], and the detection of a 17-gene set in peripheral blood using the so-called kidney solid organ response test (kSORT)[10] have shown promise to identify kidney transplant rejection at both the subclinical and clinical stages. Thus, as physicians we must learn to take full advantage of available biomarkers by using them in the correct combinations and at optimal sampling times post-transplantation.

It is important to remember that in many cases serum creatinine levels and glomerular filtration rate are of uninformative for detecting kidney transplant dysfunction as a consequence of rejection. Elevation of serum creatinine levels occurs late in the rejection process and indicates overt kidney transplant injury and nephron loss. At this point, significant alloaggressive mechanisms have commenced, portending the possibility of permanent and irreparable tissue damage and increased risk of refractory rejection. In addition, serum creatinine monitoring precludes the possibility of detecting acute rejection pre-emptively at the state of subclinical rejection. Moreover, small elevations of serum creatinine indicating initiation or progression of the rejection process, may be ignored by patients and physicians with opportunity for early intervention delayed. Serum creatinine is recognized as an imperfect marker for acute kidney dysfunction and a very poor marker for acute rejection; however, its utility might be augmented if taken in combination with other promising non-invasive biomarkers, including certain cytotoxic cell products described above[4-7] or others. Taken in combination, immunodiagnostic and immunopredictive properties might be enhanced.

It would be absurd to suggest that urine and blood biomarkers - given the current state of the art - are able to replace kidney transplant biopsy, which is the gold standard for diagnosis of allograft rejection[11]. However, the realistic and practical utility of these biomarkers would be to aid physicians in decisions that ultimately expedite a confirmatory transplant biopsy and initiation of anti-rejection therapy thereby minimizing damage to the kidney and enhancing chances of therapeutic success.

CURSE OF THE SPECIFIC "MAGICAL'' BIOMARKER

The kidney transplant literature is rife with research in pursuit of a "magical" biomarker capable of identifying onset of kidney transplant rejection with perfect accuracy - with an aim to supplanting the kidney transplant biopsy. But inevitably, pre-study optimism is confuted by post-study outcomes demonstrating that tested markers are not specific for kidney rejection - they cannot distinguish indicators of rejection from those of other disease processes such as BK virus infection, non-rejection sources of inflammation or nonspecific tissue injury. Markers are then labelled as "not-very useful" and dismissed - a possibly premature verdict considering a marker might be still useful for signalling at least that some pathologic events are in progress in the transplant kidney and thereby alerting to the need of a confirmatory biopsy.

For researchers engaged in the perennial search for a biomarker to replace the kidney transplant biopsy, a concomitant enterprise could be mining the depth and breadth of information that remains untapped in a transplant biopsy. It is highly unlikely that urine or blood markers can surpass those extracted from the transplant kidney and the draining lymph nodes as more informative of the condition of the kidney transplant - the invasive nature of biopsy and inaccessibility of lymph nodes notwithstanding. In today's world of kidney transplants, current non-invasive biomarkers will not be perfect predictors as they reveal only partially the complex

interplay of immune and non-immune factors and events occurring inside the kidney transplant. However, we can use them more effectively by understanding their precise biological meaning and clinical value.

On the other hand, tissue biomarkers, classifiers and archetypes obtained from the molecular microscope on kidney transplant biopsies, when combined with the constellation of non-invasive biomarkers, could give physicians the most comprehensive and accurate information upon which to base therapeutic decisions. In this respect, citing the INTERCOMEX Study, the analysis of transcripts in kidney transplant biopsies was able to classify patients with acute kidney transplant dysfunction with high accuracy in those having pure T cell-mediated rejection (TCMR), antibody-mediated rejection (ABMR), mixed rejection and no rejection[12].

The most effective solution - although not the simplest - will involve a finer dissection of the immunopathogenesis of rejection. The purpose would be to achieve greater understanding of the biological meaning and derivation of the presently available biomarkers and potential new biomarkers, to rank them physiopathologically and address their clinical contributions individually and in combination with other biomarkers.

SURVEILLANCE KIDNEY TRANSPLANT BIOPSIES RELOADED

Surveillance kidney transplant biopsies play an important role in kidney transplant immune monitoring, especially in patients at high immunological risk for antibody-mediated rejection whose biopsies were performed in the early stages post-transplantation when risk of rejection is higher. The purpose of surveillance biopsies is straightforward: To find remediable problems as early as possible. However, many centres do not perform surveillance biopsies for various reasons, including the following: Biopsies are not part of their academic culture, feasibility issues, historic poor yields and/or poor outcomes - making crucial judicious patient selection - or use of more effective combinations of immunosuppressive drugs. However, surveillance biopsy schedules tend to be somewhat arbitrary and unit specific. They reflect varying physician experience and thresholds among transplant units and are imperfect in consequence of the limited and equivocal signs and symptoms manifested by the alloresponses. Thresholds adjudged warranting a kidney transplant biopsy vary among transplant units and physicians - even thresholds attributable to indication biopsies (also referred to as for-cause or episode biopsies) - when something is obviously going wrong. In addressing arbitrariness in selecting surveillance biopsy time points, current and future biomarkers could be designed not only for diagnosis of rejection - as they might not replace biopsy - but to identify the onset of specific problems or simply to confirm with a transplant biopsy when something wrong (yet to be defined) is occurring at the subclinical stage. These types of biopsies would not be called surveillance biopsies or indication biopsies, but might be referred to as "biomarker-driven or biomarker-triggered biopsies". Biomarker-driven biopsies would enhance the diagnostic yield of the biopsy procedure as accuracy would likely be higher than a conventional and arbitrarily mandated protocol surveillance biopsy, and they would be more opportune than an indication biopsy. An exciting prospect is the potential to enhance the diagnostic yield and outcome prediction potential of any surveillance, indication or "biomarker-triggered" transplant biopsy by coupling gene expression analysis (the molecular microscope) with the conventional histopathologic grading of the Banff classification like in the INTERCOMEX Study[12].

Inherent in the concept of a "biomarker-triggered" transplant biopsy, is the notion of a more impactful search for biomarkers of subclinical rejection rather than markers of acute rejection. Subclinical rejection biomarkers could trigger an opportune diagnostic kidney transplant biopsy enabling initiation of anti-rejection strategies much earlier. Performance of a marker of acute rejection might not be as good if tested for utility in identifying subclinical rejection. Nevertheless, biomarkers of acute rejection could still have a role in confirming suspicious cases of rejection, as prognosticators of transplant outcomes, or for hypothesis generation in the search for novel biomarkers of subclinical rejection.

Although kidney transplant biopsy is considered the gold standard for diagnosing acute rejection, it is far from ideal. The vision provided of what is occurring reveals patchy, non-uniform rejection throughout the kidney tissue. Consequently, acute rejection can be missed by performing biopsies in randomly selected areas of the kidney transplant. In addition, a biopsy cannot quantify the degree to which the renal parenchyma is inflamed. One possible solution - not yet developed - is an imaging technique that could give a quantifiable assessment of inflammation in the kidney parenchyma. Imaging findings in combination with biopsy results would allow quantification of the extent of rejection and guide better "tailoring" of corrective immunosuppression after rejection episodes.

STATISTICAL ADVANTAGE IN IMMUNE MONITORING

From the discussion above and the examples presented, we can also expect that the development of predictors for immune monitoring strategies that incorporate multiple biomarkers, as opposed to just a single biomarker, would have the greatest potential for considerably enhancing prognostic accuracy, especially if incorporated into comprehensive monitoring algorithms that include clinical parameters. One approach to accomplishing this more effectively would be to incorporate tests assessing different biomarkers in prospective studies and clinical trials under more controllable and less heterogeneous

circumstances to investigate potential utility as predictors in kidney transplantation. In clinical trials, biomarkers could be investigated as theragnostic markers to guide the use of interventions or assess response to interventions thereby providing data enabling better kidney transplant outcomes.

LAYING THE FOUNDATIONAL STONES IN IMMUNE MONITORING

A better understanding of the immunopathogenesis of kidney transplant rejection and the mechanisms of immune adaptation that could potentially lead to transplant tolerance is crucial for the development of more accurate and precise biomarkers in kidney transplantation.

Technological advances now allow us to interrogate the immune system in peripheral blood and other fluids and tissues of kidney transplant patients that give a multidimensional and multifaceted perspective. We are currently able to obtain a very detailed picture of the state of many genes involved in the body's response to kidney transplantation, specifically of their transcriptional and translational products. Nevertheless, a multitude of genetic interactions, their hierarchy and precise clinical translation remain to be deciphered. Sophisticated biomolecular technologies and mass spectrometry-based technologies are robust to identify and discover novel biomarkers, which once validated, will open the way for implementation of other less expensive and more accessible technologies to serve in the clinical detection of those biomarkers. Thus, a multidimensional and multisystem interrogation of different biological systems in kidney transplantation would provide a combinatorial (phenotypic and functional picture) of the actual state of the immune system and its inter-relationships with other bodily systems. Well-equipped and experienced labs will be able to eventually reveal the secret world underlying alloresponses, especially if they commit their full resources and capabilities to achieving the goal.

Until the advent of more robust non-invasive biomarkers able to detect subclinical rejection with greater accuracy, *i.e.*, "biomarker-triggered transplant biopsies", protocoled surveillance biopsies and indication biopsies will continue to play a central role in the discovery of molecular signatures and the evaluation and correlation of novel biomarker candidates.

A comprehensive review article on different types of biomarkers tested and those showing promise in kidney transplantation immunodiagnosis was published recently in this journal[13]. However, more critical reviews of the available literature are needed to identify the most promising biomarkers. Admittedly, this is a difficult task given the multitude of biomarker candidates obtained from diverse sources using a range of technologies in typically heterogeneous patient populations. Thus, laboratories aiming to discover and validate biomarkers should consider protocol standardization and judicious selection of testing time points as essential elements of adequate and well-controlled biomarker-led clinical trials. The creation of advisory and work groups, and opportunities for collaboration and grant applications, should also be promoted with the ultimate aim of advancing the science of biomarker use and immunomonitoring in kidney transplantation.

RECOMMENDATIONS AND SUGGESTIONS FOR USING BIOMARKERS AND SURVEILLANCE BIOPSIES IN KIDNEY TRANSPLANTATION

It is quite apparent that we are still far from finding biomarkers that can supplant kidney transplant biopsy. Nevertheless, we can proceed methodically and persistently, perhaps not expecting to find the "magical" biomarker but towards a more in-depth and informative interrogation of the patient immune system. With this view in mind, our recommendations and suggestions for utilizing and testing biomarkers in kidney transplantation are summarized in Table 1. These are presented in the context of eight scenarios representing somewhat typically encountered cases. Given the complexity of clinical kidney transplantation, they are by no means all-inclusive or exhaustive. For each scenario, the necessity for customization in addressing different immunological risks should be recognized. Challenges confronting researchers engaged in biomarker development and utilization in kidney transplantation are encountered as well in other branches of nephrology (*e.g.,* biomarkers of acute kidney injury) and other disciplines of Medicine. We believe that the recommendations and suggestions offered have general applicability in other areas of biomarker research. Standard measures for assessing kidney transplant status are omitted from Table 1 as they are standard practice. Therapeutic recommendations or choice of immunosuppressants are not given as they are not within the scope of our biomarker-centred recommendations and suggestions. The interested reader is referred to the references cited[1,14].

IMMUNOLOGICAL RISK AND HOW IT AFFECTS BIOMARKER RESEARCH

Approaches for objective quantification of immunological risk have been attempted but as yet no reliable risk score has been developed. Immunological risk depends largely on the distinct genetic and antigenic differences between recipients and donors (along with other factors), type and amount of immunosuppression used, the degree of activation of the innate defense system and the set of dynamic alloresponses occurring throughout transplantation. The current or proposed attempts to quantify immunological risk would require an editorial or review article of its own - which will likely come with imperfect approximations - but we would like to bring attention one an important point, which is the

Table 1 Recommendations and suggestions on the incorporation of biomarkers and surveillance biopsies in kidney transplantation

Scenario A: Patients with acute kidney transplant dysfunction on whom a kidney transplant biopsy has been performed to exclude rejection	
Recommendations	
A1	Diagnose rejection if present in kidney transplant biopsies according to the Banff classification (using the most current update; now the 2015 update), and report it in a systematic way
A2	Quantify BK viremia[a] and BK virus (BKV) nephropathy by specific staining
A3	Detect anti-HLA antibodies/DSA[d] and define their immunoglobulin class, complement fixing capacities and titres through dilutions
Suggestions	
A4	Bank serum, plasma, urine, peripheral blood mononuclear cells (PBMC) and kidney transplant tissue for future biomarker research[c]
A5	Exclude active infection by cytomegalovirus (CMV) and Epstein-Barr virus (EBV)[a]
A6	Generate a data base with detailed clinical and immunological variables, ideally, using a standardized data base from a consortium or a large multicentre/multinational collaboration
A7	Test any experimental biomarker(s) of your choice and correlate it/them with standard clinical variables and a detailed immune profile. The use of validated disease classifiers and archetypes appears to have more diagnostic accuracy than the use of single biomarkers
A8	Perform a surveillance biopsy if kidney function and other clinical or laboratory parameters do not improve as expected after treatment to exclude persisting rejection or transformation to another type of rejection[b]
Scenario B: Patients with acute kidney transplant dysfunction on whom a kidney transplant biopsy is being considered to exclude rejection	
Recommendations	
B1	Quantify BK viremia[a]
B2	Detect anti-HLA antibodies/DSA[d] and define their immunoglobulin class, complement fixing capacities and titres through dilutions; and perform a kidney transplant biopsy if DSA are detected
B3	Use validated disease classifiers and archetypes (if available) to enhance to pre-test probability for rejection, and perform a kidney transplant biopsy if positive
B4	If a kidney transplant biopsy is performed, consider the recommendations and suggestions for Scenario A
Suggestions	
B4	Bank serum, plasma, urine and PBMC for future biomarker research[c]
B5	Exclude CMV and EBV infection[a]
B6	Generate a data base with detailed clinical and immunological variables, ideally, using a standardized data base from a consortium or a large multicentre/multinational collaboration
B7	Test any experimental biomarker(s) of your choice and correlate it/them with standard clinical variables and a detailed immune profile. The use of validated disease classifiers and archetypes appears to have more diagnostic accuracy than the use of single biomarkers
Scenario C: Patients with: (1) stable kidney function; (2) low immunological risk for ABMR with lack of preformed DSA; and (3) low immunological risk for TCMR or for the synthesis of *de novo* DSA due to no or low degree of HLA mismatch[16-18]	
Recommendations	
C1	Detect anti-HLA antibodies/DSA[d] after a sensitization event (transfusions, pregnancies or other transplants *e.g.*, pancreas after kidney transplantation) and define their immunoglobulin class, complement fixing capacities and titres through dilutions
C2	Perform a kidney transplant biopsy if DSA are detected, diagnose it according to the Banff classification 2015 update and exclude intra-graft BKV infection by specific staining
C3	In case of kidney dysfunction, consider the recommendations and suggestions for Scenarios A or B
Suggestions	
C4	Test any experimental biomarker(s) of your choice at pre-selected time points and correlate it/them with standard clinical variables and a detailed immune profile. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry
C5	Consider surveillance biopsies that exclude subclinical rejection and banking of kidney transplant tissue for biomarker research[c]. Recommendation to select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry
C6	Detect anti-HLA antibodies/DSA[d] at your pre-selected time points, to define their immunoglobulin class, complement fixing capacities and titres through dilutions, and correlate them with standard clinical variables and a detailed immune profile. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry. There are published consensus guidelines[19], but their recommendations are relatively arbitrary as well
C7	Bank serum, plasma, urine and PBMC at your pre-selected sampling time points and when kidney biopsies are performed[c]
C8	Exclude CMV and EBV infection[a]
C9	Perform a biomarker-driven biopsy if your chosen validated biomarker for rejection (or any other anomaly) turns positive, and bank tissue for further biomarker research
Scenario D: Patients with: (1) stable kidney function; and (2) high immunological risk for ABMR due to preformed DSA (desensitized or not)	
Recommendations	
D1	Ensure adequate levels of immunosuppression and prevent non-compliance with treatment[e]
D2	Perform surveillance biopsies to exclude subclinical rejection and banking of kidney transplant tissue for biomarker research[c]. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry, but available guidelines[19] recommend them within the first 3 (or 6) mo post-transplantation
D3	Monitor anti-HLA antibodies/DSA[d] and define their immunoglobulin class, complement fixing capacities and titres through dilutions at your pre-selected time points and correlate them with standard clinical variables and a detailed immune profile. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry; although there are published consensus guidelines[19]
D4	Detect anti-HLA antibodies/DSA[d] after a sensitization event (transfusions, pregnancies or other transplants, *e.g.*, pancreas after kidney transplantation) and define their immunoglobulin class, complement fixing capacities and titres through dilutions
D5	Perform a kidney transplant biopsy if DSA are detected, to diagnose it according to the Banff classification 2015 update and exclude intra-graft BKV infection by specific staining

D6	Perform a biomarker-driven biopsy if your chosen validated biomarker for rejection (or any other anomaly) turns positive, and bank tissue for further biomarker research
D7	In case of kidney dysfunction, we recommend to perform a kidney transplant biopsy and to consider the recommendations and suggestions for Scenario A
Suggestions	
D8	Test any experimental biomarker(s) of your choice at pre-selected time points and correlate it/them with standard clinical variables and a detailed immune profile. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry
D9	Bank serum, plasma, urine and PBMC at your pre-selected sampling time points and when kidney biopsies are performed[c]
D10	Exclude CMV and EBV infection[a]
Scenario E: Patients with: (1) stable kidney function; (2) high immunological risk for TCMR and for the synthesis of *de novo* DSA due to high degree HLA mismatch[16-18]; and (3) without preformed DSA	
Recommendations	
E1	Ensure adequate levels of immunosuppression and prevent non-compliance with treatment[e]
E2	Detect anti-HLA antibodies/DSA[d], especially in those with HLA-B and HLA-DRB1 mismatches, thought to be more immunogenic[16], at your pre-selected time points and correlate them with standard clinical variables and a detailed immune profile. Define immunoglobulin class, complement fixing capacities and titres through dilutions. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry, although there are published consensus guidelines[19]
E3	Detect anti-HLA antibodies/DSA[d] after a sensitization event (transfusions, pregnancies or other transplants, *e.g.*, pancreas after kidney transplantation) and define their immunoglobulin class, complement fixing capacities and titres through dilutions
E4	Perform a kidney transplant biopsy if DSA are detected, diagnose according to the Banff classification 2015 update and exclude intra-graft BKV infection by specific staining
E5	In case of kidney dysfunction, perform a kidney transplant biopsy, especially in those with HLA-B and HLA-DRB1 mismatches, thought to be more immunogenic, and consider the recommendations and suggestions for Scenario A
Suggestions	
E6	Test any experimental biomarker(s) of your choice at pre-selected time points and correlate it/them with standard clinical variables and a detailed immune profile. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry
E7	Suggest surveillance biopsies exclude subclinical rejection and banking of kidney transplant tissue for biomarker research[c]. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry
E8	Bank serum, plasma, urine and PBMC at your pre-selected sampling time points and when kidney biopsies are performed[c]
E9	Exclude CMV and EBV infection[a]
E10	Perform a biomarker-driven biopsy if your chosen validated biomarker for rejection (or any other anomaly) turns positive, and bank tissue for further biomarker research
Scenario F: Patients with: (1) stable kidney function; (2) high immunological risk for ABMR due to preformed DSA; and (3) high immunological risk for TCMR and for the synthesis of *de novo* DSA due to high degree HLA mismatch[16-18]	
Recommendation	
F1	Follow our recommendations and suggestions for Scenarios D and E
Scenario G: Patients with delayed graft function (DGF)	
Recommendations	
G1	Perform a kidney transplant biopsy if DGF extends beyond the first week post-transplantation without an obvious explanation, and subsequently every 7-10 d if DGF persists[14]
G2	Detect anti-HLA antibodies/DSA[d] if DGF extends beyond the first week post-transplantation without an obvious explanation, and subsequently every 7-10 d if DGF persists, and define their immunoglobulin class, complement fixing capacities and titres through dilutions
G3	Perform a kidney transplant biopsy if DSA are detected, to diagnose it according to the Banff classification 2015 update and exclude intra-graft BKV infection by specific staining
Suggestions	
G4	Define lower threshold for performing a kidney transplant biopsy in patients with DGF and pre-formed DSA or with HLA-B and HLA-DRB1 mismatches thought to be more immunogenic[16]
G5	Bank serum, plasma, urine and PBMC at the protocolised sampling time points and when kidney biopsies are performed[c]
G6	Bank kidney transplant tissue for biomarker research whenever a biopsy is performed[c]
G7	Test any experimental biomarker(s) of your choice at protocolised time points and correlate it/them with standard clinical variables and a detailed immune profile[c]
G8	Perform a biomarker-driven biopsy if your chosen validated biomarker for rejection (or any other anomaly) turns positive, and bank tissue for further biomarker research
G9	Exclude active CMV and EBV infection[a]
Scenario H: Every kidney transplant patient included in a clinical trial	
Recommendations	
H1	Bank serum, plasma, urine and PBMC at the protocolised sampling time points and when kidney biopsies are performed[cf]
H2	Bank kidney transplant tissue for biomarker research whenever a biopsy is performed[cf]
H3	Test any experimental biomarker(s) of your choice at the sampling points established by the trial designers and correlate it/them with
H4	Consider performing surveillance biopsies at important assessment points as per trial protocol (which can help to exclude subclinical rejection and to assess histopathological response to interventions) and banking of kidney transplant tissue for biomarker research[c]

[a]These infections can present with kidney dysfunction, trigger or appear around a rejection episode, but importantly viraemia, especially at high levels, will elicit cytotoxic-type and other immune responses that can interfere with the interpretation of biomarkers. [b]This is another opportunity for biomarker testing, especially if its kinetics post-treatment are known or being tested. [c]When banking samples, we suggest to process them and store them with the vision that they could be analysed using different technologies (*e.g.*, RNA- or proteomics-friendly sample processing), even if those technologies are not available in your lab, as the research world is developing towards more constructive collaborations and cross-validation approaches. In such way,

laboratories will end up with legacy sample banks from highly characterized patients with several follow up times points, in which future technologies (pending improvements or not developed yet) could be easily applied, saving huge time to researchers (no further recruitment and sample acquisition), minimizing the risk of including patients to similar protocols (just because the technology has changed) and maximizing previous patients effort and kindness; at least for pilot, exploratory and cross-validation studies. Seek advice on how to maximise your sample banking from an experienced laboratory. Strict protocols should be devised and followed up and biobanking details of the samples should be recorded (time and date of collection, type of tube, type of anti-coagulant, additives for preservation, if centrifuged the speed of centrifugation in "g", sample processor – if a person – or a machine, *etc.*). It is important to consider the easiness of the retrieval process of the data as it is inputted (any free text or absence of drop-down lists from choice answers will result in manual-dependent retrieval, which will be time consuming and expensive. [d]We recommend high resolution tissue typing of HLA-A; -B; -C; -DP; -DQ; and -DRB1,3,4,5 alleles for both donor and recipient. This will ensure more accurate detection of anti-HLA DSA, and the use of algorithms to assess degree HLA mismatching like the HLAMatchmaker[17,18]. [e]This recommendation is important for every kidney transplant patient, but seems crucial for patients with augmented immunological risk. [f]For clinical trials, we prefer to recommend rather than just suggest the inclusion of biomarker testing as the incorporation of biomarkers in diagnostic well-designed clinical trials is the best channel to validate biomarkers in a standardized controlled setting and maximize all the benefits from the trial.

differentiation of risk conferred by pre-formed anti-human leukocyte antigen (HLA) alloantibodies from risk conferred by HLA mismatches.

Many centres stratify patients according to the degree of immunological risk based primarily on presence or absence (or titres) of preformed anti-HLA alloantibodies (greater risk if DSA) and cross-match characteristics, and whether or not they have been desensitised. Some centres pay appropriate attention to the degree of HLA mismatches but others do not. Thus, a patient with a negative crossmatch and no anti-HLA antibodies could be deemed in some programmes to have a low immunological risk even with a high degree of HLA mismatch. This type of stratification, based on the presence of preformed alloantibodies, represents risk primarily for immediate or early ABMR due to preformed DSA, particularly in the absence of desensitization or subsequent ABMR episodes (either acute or chronic) and depicts previous sensitization events in the recipient (*e.g.*, pregnancies, transfusions, previous transplants). However, the immunological risk derived from the degree of HLA mismatch between recipients and donors must be considered more explicitly. The antigenic differences provided by the donor genes not present in the recipient are the main drivers of strong *de novo* alloresponses. These can trigger either the development of TCMR or the formation of *de novo* DSA with consequent progression to ABMR[15] or both, especially when current immunosuppression is not 100% effective to prevent rejection. In fact, pre-formed alloantibodies are derived from the same principle, *i.e.,* from mismatches in HLA molecules (or other polymorphic antigens) between the fetus and the mother, and the blood or tissue donor and the "pre-transplant" recipient. The degree of HLA mismatch has been traditionally quantified by counting, enumerating or stratifying the number and type of HLA mismatches[16], but more robust algorithms like the HLAMatchmaker[17,18] that more specifically assess HLA epitope mismatches can be applied to assess the risk for TCMR (acute or chronic variants) and synthesis of *de novo* DSA. To make things even more complex, kidney transplant patients usually have a combination of immunological factors that put them at risk for both types of rejection. So our recommendations and suggestions have to be tailored to the specific clinical and immunological characteristics of specific patient populations, and they would need to be implemented in the context of other available useful guidelines[14,19].

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REVIEW

De novo glomerular diseases after renal transplantation: How is it different from recurrent glomerular diseases?

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Abstract

The glomerular diseases after renal transplantation can occur *de novo*, *i.e.*, with no relation to the native kidney disease, or more frequently occur as a recurrence of the original disease in the native kidney. There may not be any difference in clinical features and histological pattern between *de novo* glomerular disease and recurrence of original glomerular disease. However, structural alterations in transplanted kidney add to dilemma in diagnosis. These changes in architecture of histopathology can happen due to: (1) exposure to the immunosuppression specifically the calcineurin inhibitors (CNI); (2) in vascular and tubulointerstitial alterations as a result of antibody mediated or cell-mediated immunological onslaught; (3) post-transplant viral infections; (4) ischemia-reperfusion injury; and (5) hyperfiltration injury. The pathogenesis of the *de novo* glomerular diseases differs with each type. Stimulation of B-cell clones with subsequent production of the monoclonal IgG, particularly IgG3 subtype that has higher affinity to the negatively charged glomerular tissue, is suggested to be included in PGNMID pathogenesis. *De novo* membranous nephropathy can

be seen after exposure to the cryptogenic podocyte antigens. The role of the toxic effects of CNI including tissue fibrosis and the hemodynamic alterations may be involved in the *de novo* FSGS pathophysiology. The well-known deleterious effects of HCV infection and its relation to MPGN disease are frequently reported. The new concepts have emerged that demonstrate the role of dysregulation of alternative complement pathway in evolution of MPGN that led to classifying into two subgroups, immune complex mediated MPGN and complement-mediated MPGN. The latter comprises of the dense deposit disease and the C3 GN disease. *De novo* C3 disease is rather rare. Prognosis of *de novo* diseases varies with each type and their management continues to be empirical to a large extent.

Key words: *De novo* glomerulonephritis; Renal transplantation; New concepts of therapy

Core tip: The role of post-transplant glomerulonephritis in affecting both patient and allograft survival is well documented. For decades recurrent glomerular diseases after renal transplantation have been thoroughly investigated. On the other hand a group of a newly classified *de novo* glomerular diseases attained an increasing interest. However, the paucity of data concerned with *de novo* glomerular diseases after renal transplantation have been shown to be a great obstacle necessitating more active cooperation between transplant centers. A thorough work up is clearly warranted to declare not only their pathogenesis, but also to draw the proper therapeutic plan.

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INTRODUCTION

De novo glomerular disease is a glomerular disease that damages the renal allograft and it is totally different from the native renal disease. The most common types of *de novo* glomerulonephritis (GN) are: Membranous nephropathy (MN), focal segmental glomerulosclerosis (FSGS), membranoproliferative glomerulonephritis (MPGN) and TMA secondary to drug intake[1,2]. Since immunofluorescence technique (IF) and electron microscopy (EM) are not used that often when assessing histopathology of a biopsy specimen in early post-transplant period, and the possibility of a range of renal diseases of unknown etiology, make it difficult to evaluate the real prevalence of *de novo* GN diseases[3]. *De novo* GN disease is reportedly uncommon[4-9]. In this review we shall discuss the most common *de novo* GN after renal transplantation in addition to the recently presented *de novo* proliferative GN with monoclonal IgG deposits (PGNMID). The *de novo* GN disease presents late, usually one year after renal transplantation, on the other hand recurrent GN might present earlier, sometimes within the first few weeks of renal transplantation. Unfortunately, both types of patterns of GN, whether *de novo* or recurrent, do have a lower graft survival as compared to patients without glomerular involvement[3].

DE NOVO GLOMERULAR DISEASES AFTER RENAL TRANSPLANTATION

De novo MN

Definition: *De novo* MN, is rather uncommon etiology among causes of allograft failure, can be defined as a MN lesion that is developed in the renal allograft of a patient originally suffered from another renal disease in native kidney[10].

***De novo* or recurrent MN:** The type of IgG subclass deposition is different in recurrent MN when compared to *de novo* MN, where IF is of immense use. Kearney *et al*[11] (2011) reported that IgG4 was dominant in glomerular deposits of recurrent MN, IgG1 was the dominant subtype in *de novo* MN. Honda *et al*[12] (2011) and others reported a clear predominance of IgG4 in idiopathic MN in comparison with the *de novo* type[13]. Another vital difference is the lack of phospholipase A2 receptor (PLA2R) staining in *de novo* MN, in contrast to the *recurrent* MN that is characterized by positive glomerular PLA2R staining[14,15].

Incidence: Of 1000 allograft biopsy, 19 cases of *de novo* MN were reported in a large French series[16], while the incidence was 1.8% in another French study[17], which means that 2% of renal transplant recipients can develop *de novo* MN[14]. In United Kingdom, *de novo* MN is considered to be the second most common cause of nephrotic syndrome after kidney transplantation[18]. The disease was reported to be 9% in a pediatric series[19]. *De novo* MN can be associated with: Alport's syndrome, ureteral obstruction, newly diagnosed HCV and recurrent IgA[10].

Pathogenesis: The new autoimmune disease IgG-related lesions have been recently shown to affect the renal allograft in several ways including *de novo* MN[20]. A novel regulatory protein (named: Pdlim2) has been recognized, with an observed decline of this protein in the podocytes of MN patients. A possible role of this protein in *de novo* MN pathogenesis has been suggested[21]. Various types of injury, *e.g.*, viral, ischemic, immunological and mechanical can induce podocyte damage, exposing the hidden or cryptogenic antigens, which could be different from that of the idiopathic

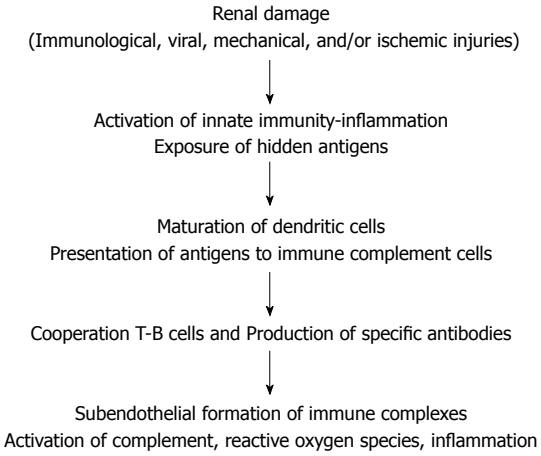

Figure 1 Any type of kidney injury can cause tissue damage. The danger signals released by the damaged tissue alert the recognition receptors, which activate the inflammatory cells and mediators of the innate immunity system. In this inflammatory environment, hidden podocyte antigens may be exposed, whereas dendritic cells become mature, migrate to lymphatic system, and present the antigen to immune competent cells. T cells cooperate with B cells favoring the production of antibodies directed against the exposed antigens planted in the subepithelium, with *in situ* formation of immune complexes, activation of complement, formation of free oxygen radicals, and inflammation. Adapted from: Ponticelli *et al*[10], 2012. *De novo* membranous nephropathy (MN) in kidney allografts. A peculiar form of all immune disease? With permission.

MN. This is quite evident, for example, in allogenic hematopoietic stem cell transplantation[22]. Consequently, these damaged cells will generate danger signals that intercepted by toll-like receptors and other receptors, which in turn initiate a cascade of signals activating transcription factors encoding the inflammatory gene[23]. Finally, the inflammatory cells of the innate system (PNLC, monocytes, macrophages and natural killers' cells) eventually release cytokines, inflammatory mediators and other mediators. Dendritic cells present the antigen to immunocompetent CD4 T cells that trigger B-cell induced antibody production. The end result is subepithelial immune complex deposition, complement activation and glomerular effector cell induced injury[24] (Figure 1).

No one single antigen can be "blamed" to be responsible of evolution of *de novo* MN, but rather a wide array of various antigens. An alloimmune response, viral infection and may be mechanical injury can create an environment that lead to release of various cryptogenic (hidden) autologous podocyte-antigen with subsequent production of auto- and alloantibodies (namely IgG1 subtype) that ultimately results in "*in situ*" immune complex formation, subepithelial deposits and eventually histological form of MN[10]. A thorough search for underlying malignancy and a hidden viral infection should be performed in view of the clear general association between MN and both cancer and infection[14]. Honda *et al*[12] (2011) reported frequent association of the AMR with *de novo* MN. El Kossi *et al*[25] (2008) suggested a possible evolutional role of DSA in development of *de novo* MN.

Role of HCV: HCV is a small RNA virus (30-38 nm) with lipid envelope and related to the *Flaviviridae* family. A robust relation to many glomerular (FSGS, immunotactoid, IgA, post-infectious and fibrillary GN)[26-31] and non-glomerular (tubulointerstitial and TMA) diseases has been reported[30,32]. Prevalence of HCV exceeds 8%-10% in many dialysis centers. HCV is known to be related to a range of renal diseases, such as MPGN Type Ⅰ associated type Ⅱ mixed cryoglobulinemia being the most common, other less common pathologies include MNGN and non-cryoglobulinemic MPGN[3]. Thereby, chronic HCV infection is a serious risk factor for development of *de novo* MN[27]. Another series reported an incidence of 3.6% in patients with positive HCV infection as compared to those with lack of HCV infection (0.36%)[13]. Genesis of *de novo* GN can be influenced by several factors on long-run including impact of immunosuppressive agents, HCV-induced modulation of lymphocyte response and the production of antibodies, so that an imbalance between antigens and antibodies will be created and the subjective allograft susceptibility of the allograft itself[33]. For patients with chronic HCV and post-transplant AMR, a particular focus of attention should be directed to *de novo* MN, with the IgG subtype staining being much helpful to differentiate recurrent from *de novo* MN[13].

The LM features of allograft biopsy of *de novo* lesions are similar to that in idiopathic MN, but with more foam cells in arterial intima and possibly with signs of AMR[34]. IF shows diffuse granular deposits of IgG in the subepithelial side of the glomerular basement membrane, with the IgG1 subclass being dominant in *de novo* MN, while the IgG4 is usually seen in *recurrent* type[11].

Clinical presentation: Clinical features vary much from no symptoms up to nephrotic range proteinuria[7,35,36], with some 25% of them would present with allograft dysfunction[19]. *De novo* MN usually presents a few years after renal transplantation[11,12,37,38].

Prognosis: There are no established risk factors for poor prognosis. In pediatric patients, 60% of Antignac *et al*[19] (1988) patients, for example, lost their grafts in 6 years after diagnosis of *de novo* MN, despite 20% have no proteinuria. In another series, 4 of 7 patients who received a second transplant, developed *de novo* MN for the second time[11]. Prognosis of *de novo* MN in adults is different. *De novo* MN was reported to have no impact on allograft function in a large French series as well as in Schwarz *et al*[39] (1991) study that reported similar 5 year survival in 21 patients with *de novo* MN to other RTR. On the other hand, Monga *et al*[34] (1993) reported a progression of the pathological stage and deposit extension to more glomeruli in serial biopsies. Accelerated allograft loss was also reported by Dische *et al*[40] (1981). Of note, most cases with deleterious outcome showed signs of chronic rejection in allograft biopsy[10]. The observed poor prognosis of *de novo* MN may be attributed by some authors to the associated AMR[41]. The latter is responsible of 20%-30% of allograft losses

in the literature[37]. The impact of *de novo* MN on allograft survival continues to be debatable. While a higher rate of allograft loss associated with signs of chronic rejection was reported in some series[37], no impact on allograft survival has been shown by others[14,37].

Anti-vascular endothelial growth factor therapy related de novo PLA2R-negative membranous nephropathy

The role of vascular endothelial growth factor (VEGF) in angiogenesis is well documented[42,43]. Local (intravitreal) and systemic (IV) anti-VEGF therapy have been recently introduced in many diseases. Systemic (IV) therapy has been used in the management of advanced cancer therapy. Unfortunately, this type of therapy has been associated with several untoward effects, *e.g.*, hypertension, hemorrhage, proteinuria and thromboembolic events[44]. On the other hands, local (intravitreal) route is usually well tolerated[45], due to its low administrated dose and the localized nature of injection. However, clearance of these agents has individual variations that may be reflected as systemic insults[46].

Recently, Wisit Cheungpasitporn *et al*[46] (2015) reported two cases of allograft dysfunction that are related to the administration of intravitreal anti-VEGF therapy. First case developed MN with spherular deposits after one year of initiation of therapy[47]. Moreover, PLA2R antibodies were reported to be negative in biopsy and no anti-PLA2R antibodies were detected in serum[48,49], which favours the *de novo* nature of MN, as only one third of idiopathic MN can express the lack of anti-PLA2R antibodies[48,49]. The increased level of proteinuria was not due to MN, as no evidence of immune complex GN in subsequent biopsy (4 mo), which is in agreement with other reports[49,50]. The second case has long standing decline but stable renal function, it showed progressing proteinuria observed few months after initiation of therapy with a clear evidence of both acute and chronic AMR in allograft biopsy[51].

Relation to proteinuria: Appearance of proteinuria in anti-VEGF treated patients is reported to be related to the start of anti-VEGF therapy, a finding that is supported by allograft biopsy findings (microspherule substructure variant of MN) properly due to a new antibody formation or unmasking of already present anti-HLA antibodies. The appearance of proteinuria is clearly related to the systemic use of anti-VEGF in cancer patients[44]. An observation that can be explained by the well documented effect of VEGF on preserving the glomerular filtration barrier integrity[42,52]. Moreover, an altered VEGF activity has been proposed to be a potential aetiology of mTOR inhibitors-induced proteinuria[53]. On the other hand local (intravitreal) administration of anti-VEGF may lack this effect[45]. A given explanation may be due to its different formulation and the local route of administration. However, clearance of these agents is ultimately systemic[45]. Furthermore, a recent report recorded a precipitous decline of allograft function (GFR < 25 mL/min per 1.73 m^2) in a group of anti-VEGF treated patients[54].

Mechanism of renal injury: The following mechanisms have been postulated as a given explanations for allograft injury: (1) Disruption of the normal survival signals mediated by VEGF leading to creation of alloreactive antibodies or exaggerated renal allograft injury induced by the already present antibodies; (2) Loss of the mitigating effect exerted by VEGF on CyA toxicity[55]; (3) Unmasking action on the already present anti-HLA antibodies; (4) Renal allograft susceptibility to anti-VEGF-induced injury leading to an increased tissue marker expression, including HLA and non-HLA antibodies; and (5) Evolution of antibody-mediated rejection through anti-HLA antibodies production[46]. The exact role of anti-VEGF agents' interference in allograft biology is complex, necessitating more extensive investigations[56-58].

Recommendations: Two recommendations have been proposed in the context of anti-VEGF therapy: (1) RTR should be strictly monitored through at least monthly determination of urinary proteins; and (2) The threshold index for allograft biopsy should be lowered, with application of both IF and EM studies[46].

De novo MPGN

The recent classification of MPGN relies primarily on the immunofluorescence (IF) findings. While cases with only capillary and mesangial complement deposition with lack of the Ig deposits are categorized as C3 glomerulopathy (C3 GN or DDD) or complement-mediated GN (CGN)[14,59], other cases with Ig mesangial and capillary deposits can be classified as immune complex-mediated GN (ICGN) (Table 1) (monoclonal, oligoclonal and polyclonal). Recurrence of MPGN post renal transplantation is frequent (mostly ICGN)[59]. On the other hand, *de novo* C3 glomerulopathy has not been reported[14]. In regards to *de novo* IC-mediated MPGN, it can be seen, but not frequently after renal transplantation, usually in association with HCV infection in about 50% of patients[60].

Incidence: In a large French study, only 13 of 399 (3.25%) patients develop *de novo* MPGN[60]. According to Ponticelli *et al*[14] (2014), *de novo* C3 glomerulopathy (CGN) subtype has not been reported, however, some case reports appear thereafter (see below section "VI").

Allograft biopsy: Typical pattern of hypercellularity with broad capillary loops due to reduplication of the glomerular basement membrane. In IF study, mesangial and subendothelial deposition of Ig as well as complement glomerular subendothelial electronic dense deposits (EDD), while fibrillary pattern is usually seen with cryoglobulinemia, most probably as a result of the associated HCV infection[61]. The impact of the associated systemic diseases is usually responsible of

Table 1 Prevalence of the *de novo vs* recurrent membranoproliferative glomerulonephritis according to the new membranoproliferative glomerulonephritis pathological classification depending on the mechanism of glomerular injury instead of deposits distribution[14,59]

No.	MPGN subtype	Pathological criteria	Recurrent MPGN	*De novo* MPGN
1	ICGN (immune complex-mediated GN)	Contains immune complexes + complement compounds	More common (most of the recurrent cases are ICGN)	Reported (3.25%)
2	CGN (complement-mediated GN)	Contains complement compounds only	Less prevalent (change from one type to another)	Not reported (Ponticelli *et al*[14], 2014)

MPGN: Membranoproliferative glomerulonephritis; ICGN: Immune complex mediated glomerulonephritis; CGN: Complement-mediated glomerulonephritis.

Table 2 Case reports in the literatures on *de novo* proliferative glomerulonephritis with monoclonal IgG deposits in renal allografts

Case	Age at diagnosis	Gender	Onset time (mo)	Type of IgG deposits	C1q deposition	Native kidney disease	Pattern of glomerular injury	Monoclonal gammopathy	Ref.
1	24	M	43	IgG3κ	N/A	T1DM	MPGN	None	Albawardi *et al*[64] (2011)
2	68	F	156	IgG1κ	N/A	PKD	MPGN	None	Albawardi *et al*[64] (2011)
3	38	F	72	IgG3κ	1+	T1DM	MesGN or EC	N/A	Hussain *et al*[72] (2017)
4	61	F	98	IgG3κ	C1q	MPGN	EC	None	Al-Rabadi *et al*[73] (2015)
5	40	F	132	IgG3κ	N/A	MPGN	MPGN	None	Al-Rabadi *et al*[73] (2015)
6	46	M	49	IgG1κ	1+	FSGS	MesGN	N/A	Li *et al*[77] (2017)
7	69	M	6	IgG3κ	1+	Obesity (FSGS?)	MPGN	N/A	Merhi *et al*[75] (2017)

EPGN: Endocapillary proliferative glomerulonephritis; FSGS: Focal segmental glomerulosclerosis; MesGN: Mesangioproliferaitve glomerulonephritis; MPGN: Membranoproliferative glomerulonephritis; N/A: Not available; PGNMID: Proliferative glomerulonephritis with monoclonal IgG deposits; PKD: Polycystic kidney disease; T1DM: Type 1 diabetes mellitus; EC: Endocapillary proliferative; M: Male; F: Female.

the *de novo* pattern of allograft biopsy[14].

Pathogenesis: The pathogenesis is not completely understood. However, the glomerular deposits of the hepatitis C virus as well as the anti-HCV antibodies may be responsible of the histological patterns in HCV positive patients[60]. Presence of cryoglobulin is seen in some patients[62]. Evolution of the clinical and histological pattern associated with *de novo* MPGN may be also triggered by the stress of rejection, calcineurin inhibitors (CNI) toxicity as well as viral infection[14].

Clinical features: Nearly, about 50% of cases presents with nephrotic syndrome, but the majority usually show non-nephrotic range proteinuria (*i.e.*, < one gram). Presence of signs of thrombotic microangiopathy in allograft biopsy is usually associated with the clinical and laboratory manifestations of hemolytic uremic syndrome. Some patients with normal kidney function and non-nephrotic range proteinuria usually experience a slow and silent course, while in others the evolution of *de novo* MPGN can trigger rapid graft loss[33].

De novo proliferative GN with monoclonal IgG deposits

De novo proliferative GN with monoclonal IgG deposits (PGNMID) is an extremely rare disease[63-68]. PGNMID is a unique type of GN that was first presented in the literature for the first time in 2004[69], 5 years later the largest series (37 case) was presented in 2009[70]. PGNMID is a proteinuria/hematuria syndrome with a reported incidence of only 0.17%, usually with a normal workup for paraproteinemia[70]. While the recurrent PGNMID presents early (within the initial two years after renal transplantation), *de novo* PGNMID appears several years later[63,64]. A handful of cases of *de novo* PGNMID have been reported in the literature (Table 2), since Nasr *et al*[70] (2009) presented his largest series of the native PGNMID. After a 30 mo follow up of these patients, 38% had complete or partial recovery, 22% developed ESRF, and (38%) of these patients experienced persistent allograft dysfunction. Only 10% of patients expressed low complement level. No M protein bands were detected, which indicates that PGNMID disease should not be considered a precursor for multiple myeloma development[9]. However, Batal *et al*[68] (2014) reported that 18% of their patient with native PGNMID disease showed an evidence of low grade lymphoma. Moreover, Barbour *et al*[71] (2011) and others also reported two patients with native PGNMID kidney disease with evidence of chronic lymphocytic lymphoma.

Case reports of *de novo* PGNMID in the literature: A detailed summary of the case reports of the *de novo* PGNMID in the literature, as regard age, gender, time elapsed since kidney transplantation, type of the deposited IgG, presence of C1q, native kidney disease, pattern of glomerular injury as well as presence of monoclonal gammopathy have been shown in Table 2[64,72-75].

Clinical presentation: Like recurrent PGNMID, *de novo* PGNMID usually manifests with allograft dysfunction associated with a variable degrees of proteinuria with or without hematuria in a white female patient, the disease generally can be seen in adults above 50 years of age[72,73].

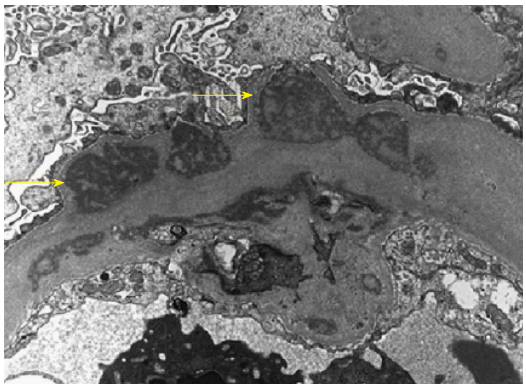

Figure 2 Glomerular capillaries are greatly distorted and thickened by the presence of numerous, sometimes large and/or confluent subendothelial electron-dense deposits (arrows). The electron-dense deposits have a variegated ("two-toned") appearance and are finely granular, but they do not show organized substructures. Adapted from: Al-Rabadi *et al*[73] (2015) (open access).

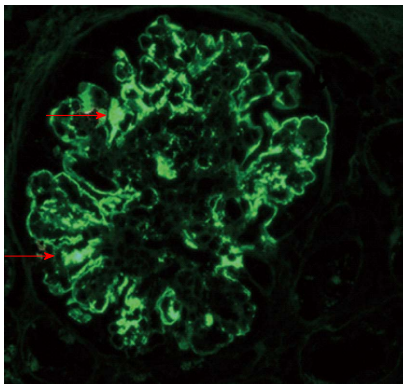

Figure 3 Diffuse irregular granular and pseudo linear deposition of IgG (3+/4+) (arrows). No staining is found in Bowman's capsule or the tubular BM. Adapted from: Al-Rabadi *et al*[73] (2015) (open access).

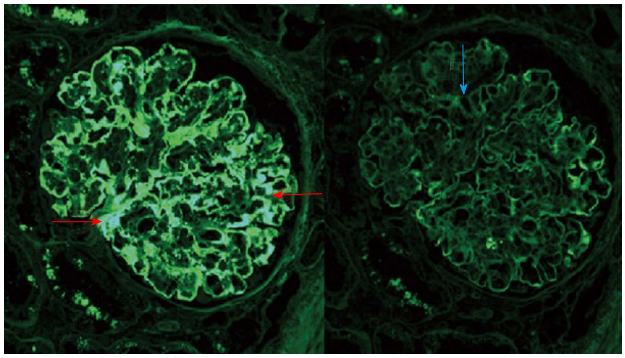

Figure 4 Kappa light chains stain strongly positive (3+/4+) (Rt side, red arrows) along the peripheral capillary walls and mesangial areas. Lambda light chains are negative in the deposits (Lt side, blue arrow). Adapted from: Al-Rabadi *et al*[73] (2015) (open access).

Pathogenesis: Pathogenesis of PGNMID is not clear. However, the reported recurrence of this disease suggests the presence of a circulating factor in RTR[72]. Other reports suggest deposition of a circulating non-deleted monoclonal IgG in the glomeruli, followed by complement fixation with outburst of inflammatory mediators[69]. A variety of intrinsic and extrinsic antigens would cause glomerular injury through stimulation of B-cell clones with subsequent production of the monoclonal IgG, particularly IgG3 subtype (8% of the total IgG). The latter is rapidly absorbed by the glomeruli so that it cannot be detected by immunofixation. Three criteria have been postulated to increase the avidity of IgG3 to glomerular deposition: (1) Positively charged nature; (2) The heaviest molecular weight; and (3) The greatest complement fixing capacity. So, these criteria would augment the affinity of IgG3 to the negatively charged glomerular elements, making it highly nephritogenic[63].

Histopathology: LM usually shows mesangioproliferative or endocapillary GN. EM shows prominent granular mesangial and subendothelial electron dense deposits (EDD) (Figure 2)[73]. Finally, IF study could ultimately establish the PGNMID diagnosis. A positive staining of one of the monoclonal IgG subtypes, with IgG3 being the most common and either *kappa* (most common) or the less common *lambda* subtype, strictly and exclusively in the glomerular constituents. C1q and complement 3 may be positive denoting complement activation (Figures 3 and 4)[73].

Differential diagnosis: PGNMID should be differentiated from other entities, *e.g.*, Type I cryoglobulinemic GN, transplant glomerulopathy, primary MPGN, post infectious GN, immunotactoid and fibrillary GN. In comparison to Type I cryoglobulinemic GN, PGNMID lacks the serologic evidence of cryoglobulinemia, and also the annular-tubular as well as the fibrillary substructure by EM are absent. The microtubules of 30-40 nm in EM that characterizing immunotactoid are missed, so did the negative Congo red randomly organized fibrils of 16-24 nm diameter of the fibrillary GN. Lack of IF and EM studies can lead to a suspicion of transplant glomerulopathy, but the absence of monoclonality and the faint staining of the IgG can differentiate it from PGNMID[64]. PGNMID may simulate LHCDD in many aspects, but the pathogenesis is not alike[73]. While the heavy and the light chains deposition involve the glomerular as well as the tubular basement membrane in LHCDD, deposition of the intact monoclonal Ig is usually confined to the glomerular constituents in PGNMID. Also, the EDD are of granular nature in PGNMID as opposed to the powdery nature of LCDD deposits[73].

In the last few years, PGNMID disease attained a particular entity. Given the lack of monoclonal bands either in urine or serum, with normal appearance of bone marrow biopsy, this entity should be differentiated from other diseases with similar presentation, a challenging insight during RTR preparation[73]. Once the features of MPGN have been observed in the LM of allograft kidney biopsy, PGNMID disease should be considered

among differential diagnoses. Long term monitoring of PGNMID is recommended to look for occult hematological malignancy[71].

De novo non-collapsing FSGS

De novo FSGS was reported to be the commonest form of *de novo* GN in some Canadian series[76]. While the recurrent FSGS can develop early post renal transplantation, usually in the form of nephrotic syndrome, *de novo* FSGS usually presents more than 12 mo after renal transplantation.

Clinically: *De novo* FSGS presents with a variable amounts of proteinuria up to nephrotic syndrome. Hypertension and progressive decline in allograft function can be seen[14].

Pathogenesis: The size discrepancy between the recipient's body mass and the nephron mass of his allograft as a single kidney will induce compensatory hyperfiltration of the residual nephrons. DM, hypertension, BK polyomavirus[77], or parvovirus B19[78] can be also included in the pathogenesis of FSGS in addition to any pathological event that results in nephron loss. CNI toxicity can result in development of *de novo* FSGS months after transplant procedures in the form of proteinuria, hypertension and progressive decline in allograft function. The robust vasoconstrictor effect in addition to the typical microvascular lesions induced by CNI could ultimately induce arteriolar ischemic changes with subsequent characteristic histopathological lesions. A pivotal role of transforming growth factor-β (TGF-β), the multipotent protein responsible of cell growth regulation, differentiation and matrix formation can be observed. CNI can augment the expression of the podocyte TGF-β[79], leading to podocyte apoptosis and detachment from the glomerular basement membrane with synechia and glomerulosclerosis[14].

Allograft biopsy: Focal and/or global glomerular sclerosis in addition to arteriolar occlusion, tubular atrophy, interstitial nephritis and striped interstitial fibrosis could be seen. On the other hand, RTR who converted their immunosuppression protocol from CNI to a high dose of sirolimus (SRL) have developed *de novo* FSGS with proteinuria and similar lesion to that seen in the classic FSGS. The immunohistochemical studies show decreased or lack of expression of the podocyte-specific epitopes synaptopodin p57 with acquired expression of cytokeratin and PAX2, which reflects an immature fetal phenotype[80]. This pattern reflects a state of podocyte dysregulation, which was confirmed in human by exposure of human podocytes to sirolimus. Decreased VEGF and protein kinase B phosphorylation have been also observed. Dose-dependent decline in Wilms' tumor 1, a transcription factor responsible of podocyte integrity was also observed[81]. *De novo* non-collapsing FSGS usually presents months or year after renal transplantation, with expected poor prognosis. The 5 years graft survival is only 40% after disease diagnosis in cases associated with CAN findings[82,83].

De novo collapsing FSGS

Collapsing FSGS (CG) is a distinct clinical and pathological variant from FSGS[14]. The reported incidence of *de novo* collapsing FSGS is about 0.6%[84]. *De novo* CG usually present 4-5 years after renal transplantation with heavy proteinuria and rapid decline of allograft function. Allograft biopsy usually shows segmental and/or global collapse of the glomerular capillary tuft. Prominent podocytes occupying the Bowman's capsule with marked tubulointerstitial damage as well as obliterative vascular disease are usually seen[85]. Pathogenesis is not clear, but these histological changes could be seen with acute rejection, diabetic nephropathy and immune complex GN disease. Altered hemodynamic stability may be included in the *de novo* CG behavior. Certain infections, *e.g.*, CMV and parvovirus B19 may be also associated with *de novo* CG disease[86,87]. Post-transplant antibody to angiotensin Ⅱ Type Ⅰ receptors, have been implicated in development of AMR[88].

A deleterious impact to the glomerular visceral and parietal epithelial cells integrity leading to cellular dedifferentiation with loss of glomerular filtration barrier function has been suggested[89-91]. Mitochondrial function disturbance has also been postulated as a deleterious mechanism in CG pathogenesis[92]. Ten cases of CG have been reported in serial biopsies in Mayo Clinic performed between 1994 and 2003[93]. CG is found to be prevalent in deceased donor kidney, presented usually with heavy proteinuria, higher serum creatinine level and poor response to plasmapheresis and ultimately allograft loss[94].

Prognosis: Outcomes of *de novo* CG is ultimately poor. All cases reported by Swaminathan *et al*[93] (2006), for example, lost their allograft within three years.

De novo C3 glomerulopathy

C3GN is a recently presented rare GN disease, characterized by predominant C3 glomerular deposits with similar morphology to that seen in DDD. However, in C3 GN there is lack of the ribbon-like intramembranous EDD. Recurrence of C3GN is reported, however, *de novo* C3GN disease is very rare[95].

In 2012, Sethi *et al*[96] (2012) presented the first two cases of recurrent C3GN, with subsequently reported 14 cases more in the next two years[97]. On the other hand, in 2008, Boyer *et al*[98] (2008) present two cases of *de novo* C3GN, however, these cases were presented as an aHUS or complement H deficiency. Furthermore, Nahm *et al*[95] (2016), reported a case of *de novo* C3 GN in a patient with no past history of alternative complement pathway abnormality, family history of renal disease or any symptoms related to glomerular disease. Tests related to complement factor H, complement factor H-related protein 5 genes and C3 nephritic factors were all negative[95]. They postulated an acquired complement

Table 3 Main characteristics of the more frequent *de novo* glomerulonephritis after transplantation (minimal change disease, nephrotic syndrome, membranous nephropathy, membranoproliferative glomerulonephritis, hepatitis C virus, IgAN)

Disease	Presentation	Time of onset	Difference with native GN	Treatment	Prognosis
MN	Proteinuria sometimes in nephrotic range	Late after transplant	Associated with trans-plant complications; IgG1 deposits instead of IgG4	No specific treatment	Slowly progressive
MPGN	Proteinuria, hematuria, NS, nephritic sediment	Months or years after transplant	Often associated with HCV, or with other diseases	Steroids + cytotoxic drugs if crescentic GN (?)	Slowly progressive; poor with many crescents.
FSGS	Proteinuria, rarely in nephrotic range	Months or years after transplant	NS is rare; signs of rejection or CNI toxicity at biopsy	Removal of associated events	Usually poor, particularly in collapsing GN
MCD	NS	Early after transplant	Mild mesangial sclerosis, hypercellularity	Steroids	Good

Adapted from: Ponticelli *et al*[14] (2014). *De novo* Glomerular Diseases after Renal Transplantation. *Clin J Am Soc Nephrol* 2014; 9: 1479-1487, with permission. MCD: Minimal change disease; NS: Nephrotic syndrome; MN: Membranous nephropathy; MPGN: Membranoproliferative GN; HCV: Hepatitis C virus.

abnormality after renal transplantation.

Histopathology: The C3GN early pathological changes usually show minimal mesangial expansion which may progress later to mesangial proliferation. EDD initially located in the mesangium, extend later to the subepithelial and subendothelial areas[97]. The EDD that present early in tubular basement membrane and in Bowman's capsule may change to band-like simulating that present in dense deposition disease (DDD) that is characterizing and specified to its diagnosis[99]. However, C3 GN showed segmental tubular basement membrane deposits[100]. The DDD disease may experience phenotypical transformation to C3GN in the native kidney[101]. However, DDD usually shows more profound MP features as well as more intense complement abnormalities as compared to C3 GN[102]. The presence of an overlap may justify using the term "C3 glomerulopathy" instead of exerting to separate the two pathological identities, DDD and C3 GN[95]. *De novo* C3GN is a rare subtype of post renal transplantation GN diseases. The fundamental role observed through both IF and E/M studies in diagnosis and serial follow up is quite mandatory[95]. Of note that despite the observed decline in C3 deposition, renal function as well as histopathological changes continue to progress.

De novo minimal change disease

De novo minimal change disease (MCD) is a rarely reported disease in RTR. Fulfilled criteria of MCD diagnosis is not always present in some cases, which suggests a misdiagnosis of FSGS disease. While Markowitz *et al*[103] (1998) succeeded to report eight cases with full criteria of MCD, Truong and his associates (2002) added five more cases[104]. Furthermore, *de novo* MCD have been reported in incompatible ABO transplants[105]. With evolution of *de novo* MCD, a nephrotic range proteinuria developed rapidly after renal transplantation, however, some cases reported eight years after transplantation[106].

Histopathology: LM show typically normal appearance of the glomeruli. Some cases show hypercellularity and IgM/C3 deposition[103,105].

Pathogenesis: The pathogenesis of *de novo* MCD still uncertain. An activation of the innate and/or the adaptive immunity with T cell dysfunction and cytokines release, *e.g*., cardiotrophin-like cytokine-1[107] or the soluble urokinase-plasminogen receptor[108], leading to alteration of the glomerular capillary wall permeability has been suggested. The initial culprit agent is unknown, but certain viral-induced activity has been postulated. Another suggested factor, the costimulatory molecule B7-1 (CD80) in podocytes, has an additional impact on glomerular permselectivity. This agent [B7-1 (CD80)] has been proved to have a role in inducing an experimental nephrotic syndrome[109]. The role of this factor in inducing foot process fusion and proteinuria in the renal allograft is to be determined. The reported development of *de novo* MCD in a patient was on SRL therapy with clearance of the disease with drug withdrawal, has suggested a possible role of certain drugs in *de novo* MCD pathogenesis[110].

Prognosis: *De novo* MCD has a favorable prognosis in most cases[14]. Owing to its potential reversibility, *de novo* MCD has no deleterious impact on allograft survival on the long run. However, this disease is possibly still underestimated as a pivotal cause of nephrotic syndrome in the renal allograft (Table 3).

De novo IgAN

IgAN has been one of the most common GN worldwide. Graft loss has been frequently reported with *recurrent* IgAN[8]. On the other hand, this fate is rarely reported with *de novo* IgAN[111].

Incidence: *De novo* IgAN has been reported to be less common than recurrent IgAN[112]. Considering the high frequency of asymptomatic IgAN, some authors argue that *de novo* IgAN might be considered as "transmitted disease", which means that recipient received an allograft that already had a "latent" form of IgAN[14], this argument is supported by the finding that a considerable percentage of mesangial IgA deposition (16.1%) has been reported in 0-hour protocol biopsy performed by a

Table 4 The risk of recurrence of *de novo* glomerulonephritis after retransplantation is unknown

Disease	Indications to retransplant
MN	In view of the slow progression, there is no contraindication to retransplant
MPGN	The risk of recurrence is high in carriers of HCV, active autoimmune disease, or monoclonal gammopathy. These risk factors should be removed or inactivated before retransplant
FSGS	If FSGS was caused by calcineurin inhibitor or mTOR inhibitor toxicity, there is no contraindication to retransplant, but the dosage of the offending drug should be minimized. If FSGS was associated with AMR, the risk of recurrence is increased. Circulating antibodies should be removed before retransplant
Collapsing nephropathy	Risk of recurrence is probably high. Antiviral and/or removal of circulating AB before retransplant are recommended according to the possible role played by virus infection or AMR in the 1st transplant
MCD	In view of the favorable prognosis, there is no contraindication to retransplant
IgAN	No contraindication to retransplant

Adapted from: Ponticelli *et al*[14] (2014), De Novo Glomerular Diseases after Renal Transplantation. *Clin J Am Soc Nephrol* 2014; 9: 1479-1487. Published online 2014, with permission. MCD: Minimal change disease; NS: Nephrotic syndrome; MN: Membranous nephropathy; MPGN: Membranoproliferative GN; HCV: Hepatitis C virus; FSGS: Focal segmental glomerulosclerosis.

Japanese study[113].

Histopathology: Intracapillary proliferation with a possibility of crescent formation can be observed in many biopsies. IgA and C3 granular deposits in the glomerular capillary wall and mesangium are frequently seen in IF studies.

Clinical features: despite the presence of frequent IgA deposition, *de novo* IgA is frequently asymptomatic especially in Asian population that may be discovered only in protocol biopsy.

Course and prognosis: In case of presence of crescent formation in allograft biopsy, prognosis of *de novo* IgA is ultimately poor, otherwise course and prognosis is quiescent with mild mesangial hypercellularity[8]. For example, Robles *et al*[4] (1991) reported a case of *de novo* IgAN with progressive proteinuria, microscopic hematuria and rapid deterioration of allograft function after renal transplantation in a patient with ESRD due to MPGN. On the other hand, *de novo* Henoch-Schönlein purpura has been described post renal transplantation with a rapid graft loss[114,115].

THERAPY OF *DE NOVO* GN DISEASES

Treatment of de novo MN

Options of *de novo* MN therapy are variable, including rituximab, bortezomib, PE, and intravenous Ig[116-118]. Unfortunately, absence of randomized control prospective studies and the high cost would be an obvious obstacles[13]. Therapy of *de novo* MN is still unclear. There is no enough data to support the use of rituximab in *de novo* MN therapy and there no clear base supporting the introduction of cytotoxic therapy or the intensified immunosuppressive agents would be efficacious[37,119].

Indications for retransplantation: MN is a slowly progressive disease, there is no contraindication to retransplant (Table 4).

Treatment of de novo MPGN

Therapy of *de novo* MPGN is still elusive. Trial of intensification of immunosuppression and the use of steroids generally showed poor and unstable results. Re-transplantation, however, is not contraindicated as long as the HCV infection as well as other risk factors have been eliminated. In this instance, the newly introduced oral anti-HCV agents, *e.g*., protease inhibitors and/or RNA polymerase inhibitors, should be considered before attempting renal transplantation[14].

Indications for retransplantation: The risk of recurrence is high in HCV carriers, active autoimmune disease, or in monoclonal gammopathy. Risk factors should be eliminated before retransplantation (Table 4).

Treatment of de novo PGNMID GN

There is no established therapy for *de novo* PGNMID[68]. However, a trial of rituximab, cyclophosphamide, plasmapheresis and high dose steroids have been introduced[63,65-67]. An observed reasonable response to rituximab and cyclophosphamide was reported with the recurrent disease, which was attributed by the authors to an early application of the protocol biopsy[63]. Multiple protocols have been tried by others including: High-dose steroids, RAS blocking agents, bortezomib, rituximab with and without steroids and plasmapheresis[78] (Table 3).

Rationale of rituximab use: B cells in PGNMID hypersecrete an abnormal IgG. The latter have the ability of self-aggregation and glomerular deposition. Rituximab, a monoclonal antibody has been widely used post renal transplantation for PTLPD, resistant antibody-mediated rejection and recurrent glomerular disease and as a prophylactic therapy for chronic antibody mediated rejection through inhibiting antibody production and hampering the B-cell immunity[120-127].

The recent advents of rituximab in PGNMID therapy have been shown to improve allograft function with better outcome[67,76,128]. Merhi *et al*[75] (2017), reported a unique results with the use of rituximab in two male

patients one *de novo* (with IgG3κ restriction) and the other is recurrent (with IgG1κ restriction). They reported better allograft function with continuous stability and return to basal creatinine level that have been continued for almost two years with persistent stable clinical and pathological response (Table 3). To declare the magnitude of benefits of rituximab, a clear insight on the pathogenesis of PGNMID depending in a wide scale of prospective controlled randomized trials should be accomplished. The role of allograft protocol biopsy in PGNMID in immunosuppressed patients is to be also declared[75].

Treatment of de novo non-collapsing FSGS

The early interference in the course of *de novo* FSGS by CNI withdrawal and introduction of MMF or mTOR inhibitors (mammalian target of rapamycin) may induce stabilization or even improvement of allograft function. One major drawback should be expected, *i.e.*, the increased risk of rejection, particularly so, if there is associated proteinuria or the CrCl was below 40 mL/min[129]. Allograft loss due *de novo* FSGS, however, does not preclude the attempt of retransplantation as long as the factors incriminated in the pathogenesis of FSGS would be eliminated. It will be also worthy to modulate the therapeutic strategies to decrease the risk of recurrence, *e.g.*, by CNI minimization and/or considering antiviral prophylaxis[14,129] (Table 3).

Retransplantation: In patients with *de novo* FSGS due to either CNI- or mTOR inhibitors-induced toxicity, there is no contraindication to retransplantation, however, the dose of the drug should be modified. If there was an associated AMR, the risk of recurrence would be high. Donors organs that are likely to trigger a repeat challenge by corresponding antigens leading to a rise in DSA should be excluded before retransplantation and, if feasible, desensitization be considered (Table 4).

Treatment of de novo CG

There is no particular therapy for *de novo* CG. With the presence of evidence of viral infection, antiviral agents may be suggested. Despite the unpredicted results, an attempt to use abatacept may be tried if there is B7-1 (CD80) expression in the podocytes[130]. In view of scarce data as regard re-transplantation in patients who lost their grafts due to *de novo* CG, there is no specific recommendation. However, an attempt to do re-transplantation in such a situation should be preceded by meticulous screening of antibodies to angiotensin II Type I receptors, in addition to an intensive course of antiviral therapy[14].

Retransplantation: The risk of recurrence of FSGS is potentially high. Antiviral therapy and/or clearance of the circulating antibodies are recommended in view of the potential role of viral infection and/or AMR in the first transplant (Table 4).

Treatment of de novo C3 glomerulopathy

Impact of therapy on glomerular morphology: Eculizumab has been reported to induce partial reduction in glomerular inflammatory activity as well as decline in deposits distribution[100]. On the other hands, other reports showed that eculizumab may be associated with EDD[131]. However, Nahm *et al*[95] (2016) used pulse steroids, ATG, rituximab, PE and IVIG to treat the associated AMR, with good response as regard normalization of serum creatinine and reduction of glomerular C3 deposition, but unfortunately the EDD persist. They speculate that C3 deposits may be masked at the locations that they were hard to wash out.

Follow up: Serial biopsies show more intensified tubular basement membrane deposits as compared to glomerular deposits. So, the E/M examination can declare these deposits more precisely as compared to the IF studies as shown by Hou *et al*[132] (2014), with IF pattern changes in about 43% of cases in repeated biopsies.

Rationale of eculizumab use: Eculizumab has been used in 11 cases of C3GN, with mostly but not always favorable results[101,102,133-141]. Eculizumab is a humanized monoclonal antibodies with a potent affinity to complement 5 and prevents the generation of serum membrane attack complex (sMAC) and release of a very potent inflammatory mediator C5a, giving an effective target of therapy[142]. So, it has been suggested that eculizumab administration could be effective in C3GN therapy if given early in cases with minimal fibrosis, short disease course and in patients with increased sMAC with accepted results[138]. These benefits were confirmed by Kersnik Levart *et al*[143] (2016). They reported clinical as well as laboratory improvement, in addition to normalization of the sMAC levels. Moreover, a quite evident decline in glomerular inflammatory activity was observed in the latest biopsies in the form of absent neutrophilic infiltration and necrotic lesions as well as reduced glomerular proliferation activity. Active cellular crescents get transformed into inactive fibrous crescents.

Decision to commence eculizumab therapy should not be attempted until all other differential diagnoses have been excluded and failures of other immunosuppressive measures have been proved[144]. This will work only if properly guided by serial allograft biopsies as well as the clinical features before commencing to use such an expensive drug with a prolonged-term therapeutic approach[143]. Renal function recovery and decline of proteinuria could be expected even in a patient with crescentic GN with a rapidly progressive course[140]. Furthermore, patient already commenced dialysis can quit RRT after only five months of eculizumab therapy[141]. Six months, however, should be elapsed prior to reporting the failure of eculizumab therapy[141,144]. Long-term sequalae of this drug is uncertain, however, it has been tried successfully in paroxysmal nocturnal hemoglobinuria

without evidence of appearance of proteinuria or decline in renal function[145]. Serial long-term biopsies follow up declared also the new observation of eculizumab binding to the renal tissues, an evidence with no harmful impact, despite the fact that eculizumab deposits are similar to that of the monoclonal Ig deposits[143].

Treatment of de novo MCD

A sustained remission of the nephrotic syndrome is usually expected with intensification of steroid therapy and other immunosuppressive agents[14]. A good renal function can be maintained after remission with or without minimal proteinuria (Table 3).

Retransplantation: Prognosis is quite favorable, there is no contraindication to retransplantation (Table 4).

Treatment of de novo IgA

For mild and moderate *de novo* IgA, no specific therapy is advised. However, Shabaka *et al*[111] (2017) reported that potentiation of immunosuppressive therapy with CNI and augmentation of RAS blockade can lead to a complete remission and better renal function. On the other hand, Carneiro-Roza *et al*[146] (2006) reported a better initial response in decreasing urinary protein level with no improvement in renal function. In patients presented with crescentic IgAN and a rapidly progressive course, pulse steroid, cyclophosphamide and PE may be tried with expected poor results[14].

Retransplantation: No contraindication to retransplant (Table 4).

CONCLUSION

The management of *de novo* GN diseases poses unique set of challenges. For a transplanting team, it is paramount to be armed with as much information as possible about the original disease of the native kidney when proceeding with renal transplantation. A lacunae in information would raise the risk of graft loss due to recurrent GN disease. Moreover, awareness of the pathogenesis of these diseases, their clinical features as well as their potential prognosis would help in improving both allograft and patient survival. One of the greatest obstacles hampering the achievement of these targets is the scarce number of the reported *de novo* GN diseases after renal transplantation. A world-wide cooperation between transplantation centers through multicenter randomized controlled trials would address many questions in regards to making a clear diagnosis and defining a robust management plan.

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REVIEW

Recurrence of primary glomerulonephritis: Review of the current evidence

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Abstract

In view of the availability of new immunosuppression strategies, the recurrence of allograft glomerulonephritis (GN) are reported to be increasing with time post transplantation. Recent advances in understanding the pathogenesis of the GN recurrent disease provided a better chance to develop new strategies to deal with the GN recurrence. Recurrent GN diseases manifest with a variable course, stubborn behavior, and poor response to therapy. Some types of GN lead to rapid decline of kidney function resulting in a frustrating return to maintenance dialysis. This subgroup of aggressive diseases actually requires intensive efforts to ascertain their pathogenesis so that strategy could be implemented for better allograft survival. Epidemiology of native glomerulonephritis as the cause of end-stage renal failure and subsequent recurrence of individual glomerulonephritis after renal transplantation was evaluated using data from various registries, and pathogenesis of individual glomerulonephritis is discussed. The following review is aimed to define current protocols of the recurrent primary glomerulonephritis therapy.

Key words: Recurrent glomerulonephritis; Renal transplantation; Primary glomerulonephritis

Core tip: Renal transplantation is the best-known therapy for end stage renal disease, with the glomerulonephritis represents a major aetiology for its prevalence. Unfortunately, recurrence of the glomerulonephritis (GN) disease after renal transplantation represents a real devastating impact on allograft survival. A clear understanding of their pathogenesis, will help not only in ameliorating GN recurrence, but also improves allograft survival.

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INTRODUCTION

The impact of glomerulonephritis (GN) recurrence varies widely from mild or negligible effect, *e.g.*, IgA nephropathy (IgAN), to a real detrimental impact on graft survival, *e.g.*, Focal Sclerosing Glomerulosclerosis (FSGS) and membranoproliferative GN (MPGN)[1]. Since it has been early recognized, the deleterious impact of the recurrent GN on allograft longevity, continuous efforts have been exerted to determine its real prevalence, clear pathogenesis and to tailor the best strategies for treatment and prevention[2]. Recently, several mechanisms have been postulated to address a clear pathogenesis of GN recurrence[1]. The prevalence of GN as an etiology of end-stage renal disease (ESRD) was reported to be exceeding 48% in China[3,4], 50% in Australian-New Zealand[2] and 30% according to USRDS 2015 report[5]. The frequent lack of kidney biopsy resulted in underestimation of the real prevalence of the GN recurrence[2]. Moreover, the distinction between recurrent GN and the *de novo* disease is not widely applied. Compared to an early (within the first year) post transplantation assessment of prevalence of about 4%, a value of 13% after 7.5 years[6], and 18% in other studies[7,8] have been recorded[2]. The reported wide variations in prevalence may be attributed to the variability in follow up periods of various studies[9].

The advent of the new immunosuppressive strategies in kidney transplantation have been reflected on the rates of acute and chronic rejection, but unfortunately has little (impact on the prevalence rates of GN recurrence as well as the *de novo* GN disease[10]). The expected improved allograft survival rate will be ultimately reflected in the future on the prevalence of the recurrent GN after kidney transplantation. It is noteworthy to mention that GN disease with a seemingly benign course, *e.g.,* IgAN is known to recur in 40% of patients but leads to graft loss only in 10%[11,12]. The magnitude of challenge, at times, seems insurmountable despite the progress in understanding the pathogenesis of certain recurrent GN, *e.g.,* permeability factors (suPAR in FSGS and ant-PLA2R AB in MN).

In this review, the authors have identified the most recent progress in understanding the pathogenesis of GN recurrence and its impact on the renal allograft survival. Further insights on the available strategies for treatment and prevention of GN recurrence, particularly so in the main primary GN is will be addressed.

GRAFT SURVIVAL IN RECURRENT GN DISEASE AFTER RENAL TRANSPLANTATION, GENERAL CONCEPTS

An assumed underestimation of the real prevalence of the GN recurrence has been proved By application of the "Protocol Biopsy" that defined as: biopsy at fixed time, with no relation to a clinical guide. Protocol biopsy delineates a higher incidence of GN recurrence (5%, 18%, 21%, 35%, 42% at 1, 3, 5, 8 and 10 years respectively)[5]. Many explanations have been postulated in this concept to shed the light on the reported discrepancy in prevalence of the GN recurrence: (1) absence of clear native kidney disease diagnosis; (2) absence of valid biomarker for GN recurrence; (3) difficulty in differential diagnosis from other pathological entities, *e.g.,* CAN and drug intoxication; (4) absence of clear stratification and characterization of GN recurrence nature in view of the advent of the new therapeutic approaches[13-15]; (5) the decision of biopsy is not always performed routinely whenever indicated (*e.g.,* proteinuria/hematuria, renal impairment); (6) IF/EM techniques are not routinely performed after each biopsy; (7) a wide discrepancy is found in certain diseases, *e.g.,* IgAN, between histopathologic characteristic changes and the appearance of clinical manifestations; (8) a trend to differentiate and isolate *de novo* disease from a true recurrent disease is usually not eventually attempted; (9) absence of basal data as regard etiology of ESRF and the native renal biopsy in many cases; and (10) data inconvenience may result in misdiagnosis of a recurrent disease as a *de novo* disease, which is in fact a true recurrence[2].

The detrimental impact of GN recurrence on allograft survival is irrefutable. The consideration of this impact relies on three points: (1) impact of recurrence of particular types of GN before transplantation on graft survival, *e.g.,* FSGS and MPGN Type I *vs* other types of GN. A significantly higher risk of graft failure in these types[9,16]. The proper evaluation should involve a fairly large number of patients studied and followed for an enough period of time[2]; (2) evaluation of the risk of graft failure in case of GN recurrence: The etiology of graft failure should be considered, membranous nephropathy (MN), for example, has high recurrence rate leading to hazardous effect on graft survival[17]; and (3) global allograft GN particularly recurrent disease and its relation

to the death censored allograft survival: As the time of recurrence is not constant, it should be considered a time-dependent variable for a better and proper evaluation[2].

As reported by Cosio *et al*[2] in the American Transplant Congress, 2015, Type Ⅰ MPGN and FSGS showed the highest rate of GN recurrence with subsequent increased risk of allograft loss, followed by IgAN. These data are supported by some studies[12], but not agreed by others[6,9]. It was assumed that 18%-22% of the death-censored kidney allograft losses was attributed to allograft GN (*de novo* and recurrent)[7], the second most common cause of death-censored graft losses[18] and third most prevalent cause of uncensored graft losses[9,16]. However, Mashaly *et al*[19] observed that the best allograft survival of kidney transplantation was noted in recipients whose end stage renal failure was due to polycystic kidney disease followed by those who had urologic disease and then those who had GN as the cause of renal failure. The recurrent GN disease has a wide variety of drawbacks deranging allograft function, which made it occupy the third most common etiology of allograft loss after death with a functioning graft and chronic allograft glomerulopathy, an assumption that was agreed by Fairhead and Knoll[20] (2010) who declared that the recurrent GN disease is a major determinant of the long term graft survival (Figure 1). On the other hand, Toledo *et al*[21] (2011) denied the presence of any difference between GN recurrence and other causes of allograft dysfunction as regard their influence on long term allograft survival. This discrepancy could be a statistical artefact attributed to the small number of patients in their study, racial impacts and the different immunosuppression strategies.

SIGNIFICANCE OF "PROTOCOL BIOPSY" FOR EARLY DIAGNOSIS OF RECURRENT GN

A full detailed map of allograft deterioration due to GN recurrence, can be obtained through a standard protocol biopsy, a widely applied strategy in many centers, so that the earliest changes in allograft histology can be discovered and the native GN disease recurrence can be early anticipated. An intraoperative basal kidney biopsy, at discharge, then after 3 wk, 3-6 mo, 12 mo and after 3 years biopsy is performed serially[22]. The importance of the protocol biopsy could be observed in identification of the early course changes in some transmitted GN diseases, *e.g.,* IgAN, which accounts for more than 90% of transmitted GN[23]. Early recurrence can be detected within 1-2 mo after transplantation. At that time and after the confirmation of recurrence in the third month, no hematuria/proteinuria could be observed; only histological recurrence can be titrated with the frequent specimens[22].

Japanese pathologists pioneered protocol biopsy to understand primary and secondary GN recurrence, *e.g.,*

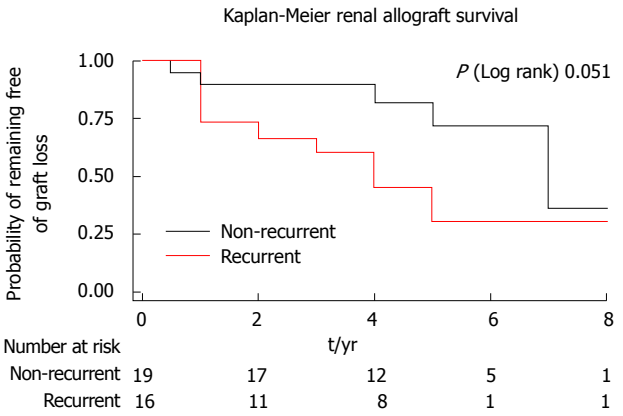

Figure 1 Kaplan Meier of allografts' survival in patients with membranoproliferative glomerulonephritis of immune complex mediated type as original disease (adapted from Alasfar *et al*[30] with permission).

FSGS[24,25], IgAN[26,27], atypical HUS[28] and light chain deposition disease[29].

Graft survival in MPGN type I recurrence

Green *et al*[23,24] (2015) reported that the risk of recurrence is higher in MPGN Type Ⅰ, with the following factors: (1) the HLA B49, HLA DR4; (2) previous transplantations; (3) acute tubular necrosis after transplantation; (4) shorter duration of dialysis before transplantation; and (5) Arab origin was all associated with decreased graft and patient survival[24].

A better allograft survival is expected in MPGN Type Ⅰ, with the following[24]: (1) unrelated living donors; and (2) absence of recurrence in the first year post transplantation.

The advent of the new concepts declaring the role of the alternative complement pathway in the pathogenesis of MPGN was addressed in appearance of the new classification of MPGN. It depends on the mechanism of glomerular injury instead of deposits distribution, which will be ultimately reflected on development of the new therapeutic policies (see therapy of GN recurrence) and its clinical interpretations[30]. So, MPGN will be immune complex mediated (ICGN), encompassing immune complexes and complement compounds, or complement-mediated (CGN) containing only complement, without immune complex (Table 1). Old studies were based on the old classification and data in this subject were very limited owing to the limited number of patients and short follow up durations. The highest prevalence rate has been observed with the previously named MPGN II[31,32].

Risk factors of MPGN recurrence

According to Alasfar *et al*[30] (2016) (Figure 2), the following risk factors have been proposed to be associated with more liability for MPGN recurrence: (1) preemptive renal transplantation[30]; (2) the living related donation[30]; (3) presence of monoclonal immunoglobulins[33]; (4) diminished complement levels[33]; (5) a higher level of proteinuria[32]; (6) human leukocyte antigen type: HLA B8, DR 3[34]; and (7) evidence of crescents in the original biopsy[34].

Table 1 The membranoproliferative glomerulonephritis new classification depends on the mechanism of glomerular injury instead of deposits distribution[30]

No	Type	Criteria	Prevalence
1	ICGN	Contains immune complexes + complement compounds	More common (most of the recurrent cases are ICGN)
2	CGN	Contains complement compounds only	Less prevalent (change from one type to another is possible)

ICGN: Immune complex-mediated glomerulonephritis; CGN: Complement-mediated glomerulonephritis.

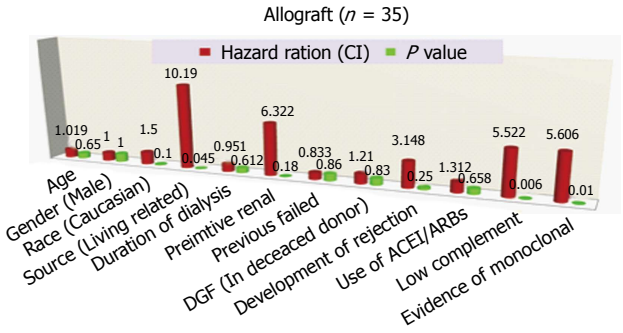

Figure 2 Variables associated with membranoproliferative glomerulonephritis immune complex mediated glomerulonephritis-type recurrence after kidney transplantation by univariate Cox analysis (adapted from Alasfar *et al*[30] with permission).

Impact of HLA typing on prevalence of MPGN recurrence: Green *et al*[24] (2013) concluded that the risk of recurrence is higher in MPGN Type Ⅰ, with certain human leukocyte antigen, *i.e.*, HLA B49, HLA DR4. Andresdottir et *al*[34] (1997) reported an increased risk of recurrence of MBGN Type Ⅰ was observed in patients with the HLA haplotype B8 DR3.

Graft survival in "recurrent MN"

The recurrence of primary MN after renal transplantation is obviously has deleterious impact on graft survival. For better evaluation of the death censored survival, timing of GN recurrence should be considered[17].

Anti-PLA2R autoantibodies in recurrent MN and graft survival: The pivotal role of anti-phospholipase A2 receptor (PLA2R) auto antibodies in the pathogenesis of primary MN before as well as after renal transplantation has an impressing popularity. The prevalence of anti-PLA2R antibodies in primary MN is approaching 70% and nearly the same percentage in RTR (70%-80%)[17,35,36], with about half of the patients are liable for recurrence after renal transplantation[17,37]. Patients with anti-PLA2R antibodies before transplantation have a 60%-76% chance of histologic recurrence, while absence of these autoantibodies decreases their risk of recurrence to less than 30%[17,36,38,39]. After transplantation the anti-PLA2R antibodies absorbed rapidly into the allograft and as a result of decreased antibodies production due to the immunosuppression medications leading to decline of their level in up to 50% of patients[36]. This decline is definitely associated not only by a lower risk of recurrence, but also by a slower rate of progression if MN does recur[36]. On the other hand, the significance of the anti-PLA2R post transplantation is greatly observed in their predictive value of recurrence and disease progression which is exceeding 80%[36], a high anti-PLA2R is usually accompanied by an increased risk of recurrence, rapid disease progression and probably more resistance to drug therapy[36,38].

Impact of anti-PLA2R on graft survival: Serial survey of the anti-PLA2R antibodies titer is of utmost importance for the following indications[2]: (1) evaluating the magnitude of recurrence risk; (2) determining the rate of disease progression; (3) prediction of the response to treatment[2]; and (4) differential diagnosis of proteinuria in recipients with native primary MN.

Non-anti-PLA2R MN recurrence: Not all the patients with primary MN express anti-PLA2R antibodies, 30% of these patients are negative to these antibodies. Instead, few patients have been reported to have antibodies against other types like cationic bovine serum albumin and thrombospondin type Ⅰ[40] but data, however, concerned with the real significance of these mediators are still deficient[40-42].

Of note that if the anti-PLA2R antibody titer is negative, we should search for the "glomerular PLA2R" staining, in such a case there is associated anti-PLA2R MN with negative anti-PLA2R serum level, which is present in 30% of cases[36,43].

Graft survival in recurrent "primary focal segmental"

Primary focal segmental (FSGS) is proved to be one of the highest glomerulonephritis (GN) in recurrence rate after kidney transplantation (KTx), with a percentage of prevalence exceeding 30% in the most recent series[2], with an expected very poor graft survival rate[43]. It can recur immediately post-transplantation, or recur lately, where its diagnosis is usually masked by the secondary FSGS resulting from the reduced total nephron mass, or due to other causes, *e.g.*, iatrogenic[20,44]. Of all causes of the FSGN, "genetic" subtype showed the least incidence of recurrence[19,45,46]. On the other hand, podocin mutations did not show a decreased risk of recurrence[45]. However, revising the recent series, there is consensus about certain clinical parameters that is considered the paramount risk factors for FSGS recurrence: (1) White race[43]; (2) higher level of proteinuria[46,47]; (3) rapid progression to ESRD (< 3 years); (4) younger age (< 15 years old) at time of diagnosis[46]; and (5) the most

reliable risk factor for recurrence is recurrence in a previous graft[2].

By far, the most reliable risk factor for recurrence is recurrence in a failed allograft, which will be ultimately reflected on allograft survival. Losing of allograft due to recurrent FSGS is associated of an 80% liability of recurrence of the original disease[2].

Graft survival in "recurrent IgAN"

The reported incidence if recurrent IgAN is quite variable according to the considered diagnosis and period of follow up. IgAN can remain silent for 5 years before it became clinically evident. So, an average incidence of 30% has been reported[48]. The histologic recurrence is by far more prevalent and discovered earlier before the disease became clinically evident. Rarely, crescentic disease with a rapidly progressing course can occur, which ultimately is associated with poor prognosis[48-50].

A growing body of evidence that three markers of an active disease indicates a great liability for recurrence: (1) galactose-deficient IgA1; (2) IgA-IgG immune complexes; and (3) lower levels of IgA-soluble CD89 circulating complexes, the myeloid cell receptor for IgA[51]. The only defect in considering these components is that they were considered on a clinically evident base of IgAN recurrence, therefore, silent disease - a quite common IgAN behavior - will be definitely missed, which means an easily missed diagnosis of IgAN recurrence[2].

Risk factors of IgAN recurrence include: (1) young RTR; (2) aggressive course of the disease before transplantation; (3) living *vs* deceased donation[52,53]; (4) polymorphisms in IL-10[54,55]; (5) HLA-B8-DR3 haplotype[56]; (6) steroid-free regimen[57,58]; and (7) impact of histological classification: could have prognostic implications[18,59,60].

Despite the reported excellent outcome after renal transplantation and the better graft survival in comparison with other GN diseases[61-63], recurrent IgA disease - on the other hand - have been proved to be detrimental to the allograft. So, definitely, patient with recurrent IgAN have a higher risk of losing their grafts in comparison with patients without recurrence[18,48,64,65].

ROLE OF IMMUNOSUPPRESSION

A definite role of immunosuppression on recurrent GN prevalence was previously denied by the early reports[13]. However, recently, some explanations were given to argue that immunosuppressive therapy could cure or at least modulate the recurrent GN course[2]: (1) certain GN recurrences show a diminished rate of recurrence[20,66-68]; (2) an increased rate of recurrence has been observed with steroid free regimen in pediatrics as well as in IgAN[57,58,64], but not in FSGS patients[69,70]; and (3) an observed decline in antibody level (anti-PLA2R), one of the essential effects that observed once the immunosuppressive agents have been commenced[36].

GENERAL RECOMMENDATIONS (EDTA DATABASE) FOR RECURRENT GN THERAPY

On behalf of the EDTA database, Floege *et al*[10] tried to shed the light on the most vital recommendations in dealing with a RTR with an underlying glomerular disease as follows: (1) defining the original native glomerular disease in RTR will help prevent its recurrence; (2) with "living-related" kidney donation, and expected familial GN such as IgAN, renal biopsy should be considered. Floege *et al*[10] accept living related donation for RTR with MN, MPGN Type Ⅰ, IgA and anti-GBM disease; (3) sharp limiting roles should judge the living related donation pool. A deep discussion with a patient with dense deposit disease (DDD) and a child with FSGS should be instituted; (4) the list of recipients with high risk of recurrence includes advanced mesangiocapillary alterations in renal biopsy, age of less than 15 years and short duration between established diagnosis and ESRD; (5) a benefit/risk ratio should be balanced properly between proceeding to kidney transplant and surviving on dialysis accordingly; (6) etiology of graft loss in a previously failed transplant is better to be elucidated; (7) avoid living donation in case of a previously failed transplant due to GN recurrence, the risk of recurrence and subsequent allograft loss will be enhanced in presence of the recurrence risk factors[71]; (8) the impact of modification of immunosuppression protocols still questionable by some authors; and (9) robust battery of investigations is required including renal biopsy with its related studies, *e.g.,* LM, IF, E/M and immune-studies should be accomplished with every renal biopsy, so that a perfect differential diagnosis from other possible lesions, *e.g.,* chronic allograft glomerulopathy could be established.

TREATMENT OF RECURRENT MPGN

The advent of a new classification of MPGN including the classic morphology as well as the other features enables not only a better understanding of the course of this disease, but also delineates the best tools of prevention and therapy of recurrence, which will be ultimately reflected on the allograft survival[72,73]. This fact is evolved from the observed wide discrepancy in the behavior of each subtype (see below) as regard the incidence and the intensity of recurrence as well as its impact on allograft survival[2].

One of the largest series about post-transplant MPGN recurrence in the literature was admitted by Alasfar *et al*[30], it was the first study that applied the new MPGN classification in evaluating post-transplant MPGN recurrence (Table 1, Figure 3). Despite the absence of worse survival in the recurrent cohort of Alasfar *et al*[30], the rate of allograft loss was higher (Figure 1).

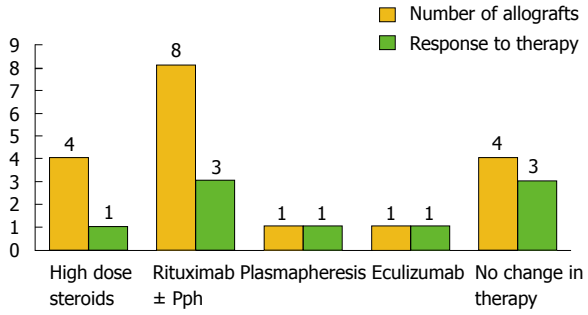

Figure 3 Response of post-transplant membranoproliferative glomerulonephritis recurrence to different treatments (response to therapy defined by improvement in GFR and no subsequent graft loss). Adapted from Alasfar *et al*[30] with permission.

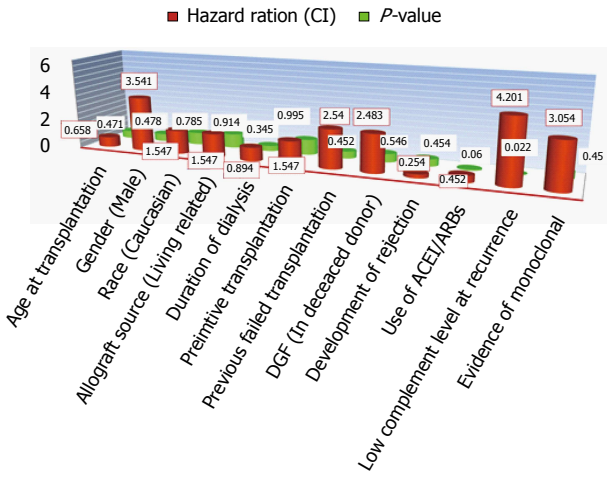

Figure 4 Variables associated with allograft loss among patients with membranoproliferative glomerulonephritis immune complex mediated glomerulonephritis-type recurrence after KTx by univariate Cox analysis (*n* = 16). Adapted from Alasfar *et al*[30] with permission.

They explained this discrepancy by the small sample size. Unfortunately, the response to immunosuppressive therapy in this study was poor, as less than 50% of their patients treated by high dose steroid therapy, rituximab and/or plasmapheresis, or eculizumab could attain allograft function stability and prevent graft loss (Figure 4). An assumed benefit of ACEi/ARBs therapy in prevention of graft loss was suggested by this study, which should be considered cautiously regarding the small number of cases[30]. Alasfar *et al*[30], however, showed that 43% of their patients who developed MPGN recurrence were of the immune complex-mediated GN (ICGN) type and were complicated by graft loss. On the other hand, one of the two patients with GN recurrence and subtyped as complement-mediated GN (CGN) developed graft loss. The average time of graft loss was 6.5 mo (2-18 mo). Interpreting these results showed non-significant results between recurrent and non-recurrent groups, despite the presence of tendency to worse survival[30]. Also, no significance could be detected with other factors, *e.g.,* (age, race, gender, mismatching degree, graft source, pre-emptive transplantation and degree of proteinuria). In contrary to other factors and despite of non-significance, ACEi/ARB therapy could ameliorate the tendency of graft loss (Figure 4). For more specified specific therapy, all the old biopsies before the advent of the new classification, should be reclassified. The CGN is generally less prevalent, on the other hand, ICGN is more common (Table 1) and most of the native as well as the recurrent MPGN appear to be classified as ICGN. It is noteworthy to remind that some of the reclassified cases may change their microscopy by time. Unfortunately, the latter change could be difficult to differentiate from a *de novo* GN disease, which will be ultimately resulted in a difficulty on choosing the mode of therapy[30].

Impact of the new classification on therapeutic options

MPGN with Ig deposits: We should focus in suppression of the antibody production, but there are no controlled trials.

MPGN with monoclonal Ig deposits: The anti-CD20 antibodies are proved to be effective in uncontrolled trials in native as well as in allograft recurrence[74]. Monoclonal deposits are proved to be associated with a higher rate of recurrence[75]. This association may suggest they have their role in the pathogenesis of MPGN, consequently, two important steps have been suggested: (1) meticulous screening for "monoclonal gammopathy" during preparation of a patient with MPGN for renal transplantation; and (2) a "hematologist consultation" may be advised with strict follow up in such situation for long periods[30].

MPGN C3GN: The use anti C5 monoclonal antibodies, eculizumab, is shown to be effective with mixed results[76-80], depending on the success of this drug in preventing the recurrence of atypical HUS, which own a similar pathogenesis[80-83].

Impact of subtype's behavior on therapeutic options

MPGN with polyclonal Ig deposits: Usually presented late, within the first 5 years, with a relatively benign course as regard low risk of recurrence and slow progression. Interestingly, the morphology of the lately recurred MPGN with polyclonal Ig deposits is difficult to be differentiated from the *de novo* GN which can behave similarly as regard the late presentation post transplantation as well as the presence of polyclonal Ig deposits[18]. The former group has C4d deposits in their glomeruli, fortunately help in differential diagnosis. Also, a higher risk of recurrence could be expected with the presence of reduced complement level (C3 and C4) level[74] (Figure 5).

C3GN: C3 glomerular deposits are abundant with absence or minimal Ig deposits[84,85]. The risk of recurrence in C3GN is very high, exceeding 70%, can be presented early with a very aggressive course that ultimately ends by graft failure in nearly half of the

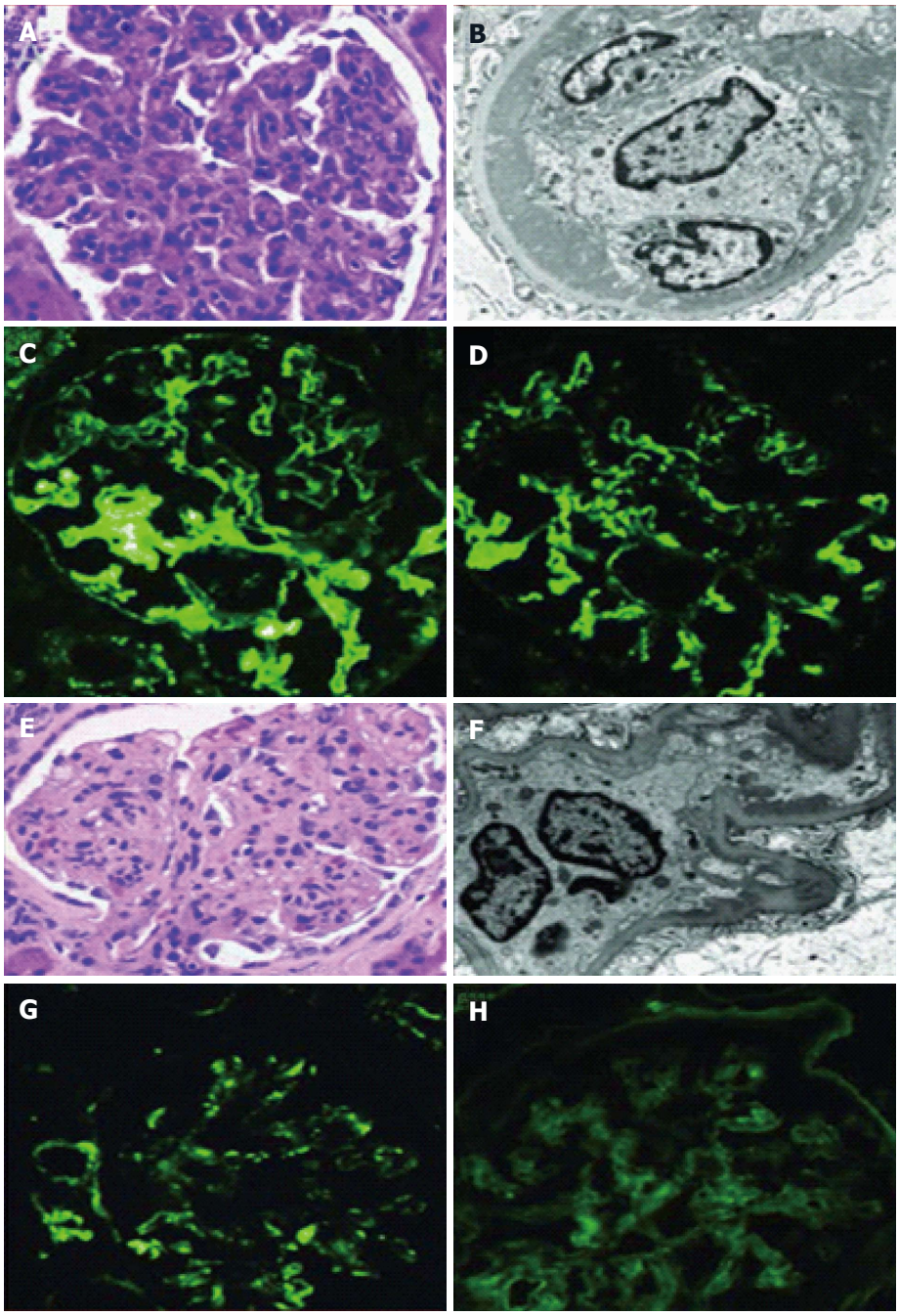

Figure 5 Histological changes of membranoproliferative glomerulonephritis in kidney transplant biopsies. Typical LM, EM and IF finding in cases previously classified as MPGN. First panel shows a case reclassified as ICGN with C3 abnormalities, including (A) the classic MPGN pattern GN on LM (B) large sub-endothelial electron dense deposits on EM and granular mesangial and capillary wall staining for both (C) IgG and (D) C3 on IF. Second panel shows a case reclassified as a C3 glomerulopathy, with (E) a similar MPGN pattern on LM, (F) smaller sub-endothelial deposits on EM and granular mesangial and capillary wall staining for (G) C3, but no significant staining for (H) IgG. Adapted from Alasfar *et al*[30] with permission. LM: Light microscopic; EM: Electron microscopy; IF: Immunofluorescence.

patients[86]. There is no established treatment for C3GN. For complement dysregulation in the pathogenesis of this disease, a supply of "normal plasma" has been suggested[87]. Recently, a new therapy targeting an alternative complement pathway using the anti-C5 AB[88-90] and soluble CR1 (a potent regulator of complement activity) has been reported[91]. However, controlled trials regarding the efficacy of these therapies have not yet been conducted.

Dense deposit disease subtype: The rate of recurrence of this subtype is extremely high (80%-90%), leading to reduced graft survival[32,92]. Two criteria characterize this subtype: It is usually slowly progressive with minimal or absent clinical manifestation, and the timing of recurrence is mostly delayed[92,93]. Both DDD and C3GN usually express an alteration in the alternative pathway with resultant overproduction of the activated C3[94,95]. Recently, polymorphism of the complement

regulating proteins, especially in alternative pathway are found to be propagated mostly in all subtypes of MPGN, with a possible alterations related to renal outcome were assumed[96]. In DDD and other C3 glomerulopathies: Eculizumab or anti-auto antibodies activating complement cascade therapy have been suggested[97].

"Monoclonal gammopathy with renal significance": Both C3 GN and DDD lack C4d, indicating alternative pathway activation[98]. Any MPGN subtype associated with monoclonal Ig deposits usually complicated by GN recurrence in 66% of cases and expressing a very aggressive course often complicated by allograft failure[99]. Interestingly, 70% of these cases do not express monoclonal IG either in serum or in urine, without any evidence of plasma cell dyscrasia in bone marrow and with low risk of progress into multiple myeloma[100,101].

Monoclonal proteins: Monoclonal proteins are present in 30% of cases with MPGN with monoclonal Ig deposits have serum monoclonal proteins[100] despite absence of any evidence of multiple myeloma. A subtype name of this group of patients called "monoclonal gammopathy with renal significance"[102,103], which obviously will express a very high risk of recurrence[104].

Stem cell transplantation: It is noteworthy to declare that in monoclonal gammopathy, stem cell transplantation can reverse the renal dysfunction through elimination of the light chain and immunoglobulins, with an expected general improvement. The observed link between C3GN and monoclonal and the complement (alternative pathway) activation by λ-light chain has been recorded in previous reports[105-107].

Recommendations for a better management

Extrapolating the aggressive behavior of these recurrent diseases, especially in the presence of monoclonal deposits and C3GN, rigorous precautions should be considered to strive against its activity. A prophylactic protocol to guard against MPGN with monoclonal deposits recurrence utilizing an anti-CD20 AB before transplantation is currently under evaluation by Cosio *et al*[2], with promising preliminary results. It is assumed that the C3GN remains silent until they exposed to a certain event*, e.g.,* ischemia/reperfusion injury of transplantation that results in dysregulation of complement activation with evolution of the pathological events associated to its aggressive course[108-110]. So, it is essential to reclassify the MPGN based on the recent MPGN classification, which will help not only in designing a therapeutic protocol, but also in instituting a prophylactic policy. It is noteworthy mentioning that the clinical course of MPGN pre- and post-transplantation are not the same, *i.e.,* slow preoperative course is not necessarily applied to the post-transplantation behavior[2].

TREATMENT OF RECURRENT MN

RTR with recurrent MN are better to be under RAAS-blockade as well as symptomatic therapy in the form of diuretics, statins and anticoagulants. Other lines include were listed below.

CNI

Referring to its efficacy in MN in the native kidney disease, many RTR with recurrent MN are utilizing CNI therapy relevant to the recent advances in understanding the pathogenesis of MN recurrence[111].

Corticosteroid/alkylating agents (cyclophosphamide or chlorambucil) combination

Again effective in both native and recurrent MN disease[112]. Unfortunately, leukopenia could be quite troublesome, so, holding MMF while commencing the alkylating agents' therapy is advised[112].

Anti-CD20 antibody

Rituximab is also successful in treating the native as well as the recurrent MN disease[113-117]. More than 80% of cases could achieve partial or complete remission, while 40% of cases could express subendothelial deposits resolution[17,117]. Despite the increased risk of infection with anti-CD20 therapy[17,113], rituximab is generally safe, effective, simpler to utilize and more tolerated as compared to alkylating agents. So, the anti-CD20, rituximab, is recommended as a primary line in treating MN recurrence, without alterations in the immunosuppression protocol and regardless the anti-PLA2R antibody level[2].

Resistant cases

Alternative therapy between rituximab and alkylating agents is suggested, once one of them failed, then shift to the other line[17,115]. As the level change of anti-PLA2R antibodies titre precedes the decline of proteinuria after rituximab therapy, serial follow up of the antibody titre can be used to anticipate the magnitude of response to therapy as well as the possibility of relapse[113].

Timing of therapy

Early intervention-in contrary to native MN[116] - with anti-CD20 therapy is recommended, exactly when the proteinuria approaching one gram per 24 h. A very high rate of success would be expected[17], which will be ultimately reflected on reduction of the rate of death censored allograft failure related to MN recurrence (45%).

Prophylaxis history

The anti-CD20 was used effectively by Cosio *et al*[2], to prevent MN recurrence in two patients with a previous allograft loss due to MN recurrence, with serial follow up through a protocol biopsy.

Prevention

The use of ant-CD20 few months pre-transplantation may be applied in an attempt to prevent recurrence through the reduction of the ant-PLA2R antibody titer. However, two reasons may prevent the application of this maneuver in a wider scale: (1) the ant-PLA2R antibody titer already declines soon after transplantation, which will decrease the chance of recurrence[36]; and (2) the expected high rate of success achieved by the anti-CD20 in case of early recurrence has been documented[17,117,118].

TREATMENT OF RECURRENT FSGS

The recent progress in understanding the pathogenesis of FSGS recurrence was unfortunately not supported by evidence-based controlled trials.

Plasmapheresis

In 1985 treating FSGS with plasmapheresis (PE) sessions has been commenced with variable success[119]. Plasmapheresis has the ability to induce remission in 70% of children and 63% of adults as reported by Ponticelli *et al*[120]. An overestimation of these reports is postulated due to retrospective nature of the study, short follow up period and lack of controlled design. Once the disease recurrence become clinically evident, we can extrapolate a satisfactory response with commencing the PE sessions early after transplantation. PE is usually prescribed as one to two times plasma volume exchanges, three times per week, with total 8-12 treatments until remission has been established. An intensified course for longer period was suggested by other researchers[121].

Prophylactic PE

Gohh *et al*[122] has admitted preoperative PE for eight sessions in ten patients. In case of living donation, the recipient received PE one week before and one week after the operation. In case of deceased donation, PE was only given 24 h preoperatively. No one case of FSGS recurrence has been diagnosed in the high risk group and only half of his patients has had their allograft failed. They concluded these results were less than previous reports[122], while others denied any benefits for the prophylactic PE[121,123]. A combination of PE and immunosuppressive agents has been proposed with limited data[124,125].

Higher dose of CyA

Only the intensified dose of CyA can reduce the proteinuria level, in contrary to the standard dose that can do nothing for FSGS recurrence[126]. Relevant to its lipophilic criteria, CyA has the ability to bind the LDL receptors on the cell surface of the peripheral lymphocytes. As a result of the rich lipid content (LDL cholesterol) in the nervous system, blood level of the drug is reduced, which could only be overcome through a higher dose augmentation. At this base, *i.v.* CyA 3 mg/kg/d for 3-4 wk, followed by oral route aiming at preserving the blood level at 250-350 ng/mL, have been successful in induction of remission[127]. However, this policy has been hampered by the multiple untoward effects of the high dosage.

Rituximab

An anti-CD20 chimeric monoclonal antibody depleting the B cells with a direct protective effect on the podocytes. It has the ability to abort the downregulation of sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b) protein and the acid sphingomyelinase (ASMase), both of them were documented to be present in the podocyte exposed to the sera of recipients with recurrent FSGS[128]. In 2006, beneficial benefits of rituximab in treating the recurrent FSGS post transplantation was suggested[126]. A remission rate of 64%, either partial or complete, has been reported with rituximab therapy[129]. A better response is expected with a normal albumin serum level, and fewer administered infusions as well as in young age recipients[130]. It is not well-proved if titrating rituximab dosage will be the best policy to deplete the B-cell or not. The typical published dosage of rituximab is 375 mg/m^2/dose/2-6 doses, with 1-2 wk apart.

PE and rituximab combination

An augmented benefit was assumed to be expected with the combined therapy including PE in addition to rituximab[131,132]. Tsagalis *et al*[131] utilized one gram rituximab per dose, in two doses with two wk apart with PE not performed before 72 h. Two of his patients commenced complete remission and the other two have a partial remission with a stable renal profile and absence of severe complications for 18-60 mo of follow up.

While the resolution of recurrent FSGS was assumed to be possible[2] through the use of the anti-CD 20 AB, rituximab[133], this efficacy, unfortunately, is not consistent but rather limited to certain subtypes. The use PE proved to be effective in removing the circulating permeability factors[134]. For instance, we cannot rely only on this effect in case of recurrent FSGS disease. On the other hand, rituximab was proved in a small pediatric group with recurrent FSGS to be effective in achieving PE independence successfully. The variability in response of recurrent FSGS to both PE as well as anti-CD 20 AB (rituximab) therapy is widely spread[135-137], which indicates a variable response that varied according to different subtypes. Despite the absence of well-designed randomized prospective studies, some trials attempted to prove an effective response of removing a putative permeability factor through PE sessions to guard against FSGS recurrence, which was not confirmed by others. A new strategy has been tailored by Cosio *et al*[2] to evaluate the ability of the anti-CD25, rituximab, before transplantation to prevent/decrease FSGS recurrence rate has been commenced with encouraging early results.

Renin-angiotensin system blockade

Few case reports have proved the efficacy of renin-

angiotensin system blockade on reducing proteinuria in recurrent FSGS[138,139], which shed the light on the fact that the recurrent FSGS is not completely pure immunological in origin, but additional factors including the primary as well as the adaptive form of FSGS have been incorporated.

Ability of "galactose infusion" therapy

In ameliorating the toxicity of the circulating permeability factor has been shown in one case series. Galactose therapy has been proposed by Savin *et al*[139] as a non-toxic agent for treatment of the FSGS-associated nephrotic syndrome. The focal segmental permeability factor (FSPF) has a high affinity to galactose. The latter has the ability to inactivate and clear FSPF from the circulation. In addition, the FSPF-galactose complex has a high liability to uptake and catabolism[139].

Cyclophosphamide

In addition to its untoward toxic manifestations with prolonged use, conflicting results have been determined with cyclophosphamide therapy. Kershaw *et al*[140] used a high dose of cyclophosphamide in three pediatric patients with recurrent FSGS, two achieved complete remission and the third one have had partial response. Cochat *et al*[141] reported sustained remission through a regimen composed of pulse steroid, cyclophosphamide and plasmapheresis. Cheong *et al*[142] reported sustained remission only in two of six patients with recurrent FSGS through a similar protocol. Dall'Amico *et al*[143] achieved sustained remission in seven of eleven pediatric patients through utilization of steroid pulse-free protocol composed of cyclophosphamide and PE only. Three major toxicities hampered the widespread use of cyclophosphamide, the immunosuppression burden, gonadal toxicities and the risk of malignancy[144].

Resistant recurrent FSGS to PE and rituximab therapy

A case report recorded a complete remission using the T-cell costimulatory protein B7-1 blocker abatacept, which was not confirmed by others[145-147].

TREATMENT OF IGAN

There is no recommended specific therapy in treating the recurrent IgAN. Treatment of recurrent IgAN is similar to that in native disease in non-transplant patient[1]. However, the following maneuvers have been reported.

ATG induction

The use of ATG as induction therapy is shown to be associated with less risk of IgA recurrence[148].

Low-dose steroids after transplantation

A protective impact against IgAN recurrence was reported[57,58].

"Tonsillectomy"

As advocated by the Japanese, a better prognosis post-tonsillectomy could be expected[149-151].

ACE inhibitors

The use of ACEi is proved to be of no benefit in improving the allograft survival[152]. Only the anti-proteinuric effect could be beneficial to the allograft[153,154]. All patients of the study of Floege *et al*[152] received ACEi with graft failure occurred in more the half of them.

Methylprednisolone pulse

In of the study of Floege *et al*[152], only 20% of patients received steroid pulses; again more than half have had their graft lost.

Maintenance immunosuppression

No benefit could be expected with any alterations on the immunosuppressive policy in regard to improvement of graft survival[155]. However, Moroni *et al*[12] assumed that immunosuppressive protocols including less than three agents is an independent risk factor of recurrence, however, this theory is still debatable. The choice of immunosuppressive strategy members has nothing to do with IgAN recurrence after renal transplantation[152].

CONCLUSION

One of the most challenges for renal allograft survival is the GN recurrence after renal transplantation. With improving long-term renal allograft survival, recurrent disease has increased prominence as a significant contributor to late graft loss. Knowledge on the risk factors for recurrence, onset time and impact on graft function is prerequisite to informed decisions. There are minimal data on the risk of recurrent disease with new immunosuppressive agents. The early recognition would slow down deterioration of renal function even if it may not slow down the course of progression of GN. Each of the GN types has a very unique natural history in renal allograft. With more advancement in understanding its pathogenesis in the future, prophylactic treatment for prevention of GN recurrence might be effective.

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MINIREVIEWS

Hepatocyte transplantation: Consider infusion before incision

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Abstract

Human hepatocyte transplantation is undergoing study as a bridge, or even alternative, to orthotopic liver transplantation (OLT). This technique has undergone multiple developments over the past thirty years in terms of mode of delivery, source and preparation of cell cultures, monitoring of graft function, and use of immunosuppression. Further refinements and improvements in these techniques will likely allow improved graft survival and function, granting patients higher yield from this technique and potentially significantly delaying need for OLT.

Key words: Hepatocye; Transplantation; Cell therapy; Liver; Graft; Orthotopic

Core tip: Further human studies involving humans are needed, however, the current collectively suggest progress in terms of improved effectiveness of human hepatocyte transplantation (HTx). With improvements in optimizing delivery technique and assessing proper recipients of livers, monitoring graft function, as well as recognizing and treating graft rejection, HTx may be able to be used more widely in metabolic liver disease and potentially delay necessity of orthotopic liver transplantation.

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INTRODUCTION

Human hepatocyte transplantation (HTx) is being studied as a potential future alternative and currently use as a bridge to orthotropic liver transplantation (OLT). Over the last 10 years it has been noted that the number of patients requiring transplant as well as total transplants being performed has been stable (NIHMS). Given the inadequate supply of donor organs in relation to patients who would benefit from transplantation, continued research into alternate therapies for treatment or to prolong time before transplantation becomes necessary is timely. HTx is a technique which has been refined over the past three decades which seeks to improve liver function *via* transplantation of donor hepatocytes directly, rather than transplanting an entire organ. While a number of disorders have been evaluated for efficacy of therapy with this technique, individuals with inborn errors of metabolism appear the greatest benefit[1,2]. Sustained benefits have not been observed, however, refinements in the practice may lead to greater temporal benefits. While this review aims to summarize use of HTx in studies, it also seeks to highlight potential shortcomings of previously utilized technique and focus on areas of future study which may lead to improved yield of HTx.

PREPARATION OF HEPATOCYTE CULTURES

While many consider avoidance and delay of surgery appealing when considering HTx compared to OLT, it should be noted that the source of hepatocytes utilized for HTx generally come from livers deemed unsuitable for OLT[1-4]. The most common reason for rejection of a liver for OLT being steatosis, which is associated with both lower cell viability and yield[5-7]. Ischemic damage to livers is also a common reason for rejection, similarly affecting the yield and viability of extracted hepatocytes[8]. That stated, there is evidence that high quality hepatocytes may be obtained from cardiac death donors with prolonged warm ischemia, though prolonged episodes of ischemia predictably decreases viability[9,10]. While a current argument in favor of expanding research and use of HTx is that one is able to utilize cells from a larger pool of donor organs, one suspects that use of hepatocytes for HTx cultured from livers deemed suitable for OLT would likely result in greater success of this therapy. Beyond simple increased efficacy, multiple recipients could benefit from a single donor liver. Admittedly, there are concerns regarding evaluating the fitness of a recipient to receive donor hepatocytes. For instance, the cytochrome P450 enzyme is involved metabolism of drugs and steroids, bile synthesis, cholesterol synthesis, and vitamin D production. This enzyme system has been noted to have different levels of expression and function within humans, however, and this variability may be partially responsible for variant viability of HTx[11-13]. Not every person may be fit to receive any donor hepatocyte culture due to pre-existing chronic condition or associated medication they take, however, increasing the donor pool would still likely increase overall access to HTx. Furthermore, cell cultures can be cryopreserved and stored until needed, whereas there is a finite amount of time a whole liver can be stored before it is no longer viable for OLT[14].

CLINICAL INDICATIONS FOR HEPATOCYTE TRANSPLANTATION

Further discussion of refinement in technique warrants first discussing potential clinical indications for its use. As previously noted, congenital metabolic disorders appear to hold the greatest promise for use of HTx as metabolism of substrates in questions occur almost exclusively in hepatocytes. The cases reviewed below demonstrate HTx as a successful bridge to OLT.

Crigler-Najjar syndrome (CN) Type Ⅰ is an autosomal recessive condition with complete absence of a uridine diphosphate glucuronosyltransferase (UDPGT) enzymes, resulting in life threatening unconjugated hyperbilirubinemia with long term risk of kernicterus. While phototherapy can be an effective treatment, its effectiveness has been observed to decrease with increased age[15]. The first hepatocyte transplantation was performed in a rat model deficient in UDPGT enzymes[16-18]. A minimal percentage of liver mass comprised of engrafted cells (0.2%), resulted in a 40% decrease in unconjugated bilirubin levels[18]. Humans with CN Type Ⅰ have subsequently undergone HTx with marked improvement in unconjugated bilirubinemia, however, all patients subsequently required OLT anywhere from 4 to 20 mo after HTx due to either loss of graft or insufficient improvement in symptoms[19-23].

Urea cycle disorders, comprising a group of disorder due to deficiencies in one of six different enzymes in the urea cycle, are another group seemingly optimally situated to benefit from HTx. These deficiencies collectively result in hyperammonemia with significant neurologic sequelae. Most patients present as neonates, with current therapy involving protein restriction, hemodialysis, or hemofiltration. Hyperammonemia is still noted despite these treatments, however, with OLT being the only current definitive treatment. Humans have successfully undergone HTx as a bridge to whole organ transplantation, with stabilization of ammonia metabolism noted between 4-13 mo before OLT became necessary[24,25].

Familial hypercholesterolemia (FH) is caused by absence of the low density lipoprotein receptor (LDLR) resulting in early onset severe coronary artery disease. Low density lipoprotein (LDL) apheresis or OLT are the only current treatments, however, a rabbit model of FH undergoing HTx was noted to have decreased levels of serum cholesterol by 30%-60% for 100 d[26-28]. In 1995, 5 patients between the ages of 7 and 41 underwent HTx, demonstrating up to a 20% reduction in LDL in three of the patients, the other 2 not responding to therapy[29].

Glycogen storage disease Type Ⅰ (GSD-I) is an

Table 1 Summary of hepatocyte transplantation reports in human patients

Ref.	Indication	No. of patients	Infusion site	Outcome
Ambrosino *et al*[21]	Criggler-Najjar Type I	A 9-year-old boy	Portal vein	Decreased bilirubin approximately 4 mo, underwent OLT
Lysy *et al*[22]	Criggler-Najjar Type I	A 9-year-old girl	Jejunal vein	Decreased bilirubin approximately 6 mo, underwent OLT
Lysy *et al*[22]	Criggler-Najjar Type I	A 1-year-old girl	Splenic vein	Decreased bilirubin approximately 4 mo, underwent OLT
Zhou *et al*[53]	Criggler-Najjar Type I	2 (4-mo-old boy and newborn boy)	Portal vein	Decreased bilirubin approximately 3-4 mo with subsequent OLT
Meyburg *et al*[24]	Urea cycle disorders	4 (1-d to 3-year-old)	Portal vein	Stable 4-13 mo before OLT, 1 death at 4-mo
Grossman *et al*[29]	Familial hyper-cholesterolemia	5 (7-year-old to 41-year-old)	Portal vein	Three patients with approximately 40% reduction in LDL lasting 4 mo
Lee *et al*[31]	Glycogen storage disorders	A 8-year-old kid	Portal vein	Followed for 7 mo, on tacrolimus and able to fast for 7 h without hypoglycemia
Muraca *et al*[1]	Glycogen storage disorders	47-year-old, female	Portal vein	Followed for 9 mo, on tacrolimus and able to fast for 7 h without hypoglycemia
Sokal *et al*[33]	Refsum disease	4-year-old girl	Portal vein	16 mo improvement
Dhawan *et al*[36]	Hemophilia A	2 (3-mo-old and 35-mo-old)	Portal vein	6 mo with 70% reduction in Factor VII requirements
Hansel *et al*[42]	A1AT deficiency	A 52-year-old	Portal vein	A1AT levels did increase before OLT available 2 d later
Soltys *et al*[52]	Phenyl-ketonuria	A 27-year-old female	Portal vein	7 mo of unrestricted diet

A1AT: Alpha 1 antitrypsin; OLT: Orthotopic liver transplantation.

autosomal recessive metabolic disorder resulting from deficiency of the hepatic enzymes glucose-6-phosphatase (Ia) or glucose-6-phosphate transporter (Ib), resulting in deficiency in glucose production with noted severe hypoglycemia, lactic acidosis, hyperlipidemia, growth retardation, hyperuricemia, and renal dysfunction. While many patients can be treated with consumption of starch, some are unresponsive to dietary therapy and require OLT to correct the underlying defect[30]. Two patients, 18 and 47 years old, underwent HTx with subsequent ability to maintain unaltered diet for up to 7-9 mo[1,31].

Infantile Refsum disease is an autosomal recessive disorder characterized by impaired peroxisome function, resulting in accumulation of very long chain fatty acids and branched chain fatty acids which are normally degraded in peroxisomes. Patients present with severe neurologic defects and rarely survive beyond age 10, with treatment generally centering around supportive care[32]. One 4 years old female patient underwent HTx, demonstrating significant biochemical improvement for more than 16 mo[33].

HTx has been suggested as a treatment for Hemophilia A and B; with murine models demonstrating in 5%-10% increase in factor Ⅷ and 1%-2% increase in factor Ⅸ[34,35]. These increases do result in decreased bleeding time and do provide a therapeutic benefit. In one 2004 study, a 3 mo and 35 mo old patients underwent HTx with 70% reduction in factor Ⅶ requirements noted after 6 mo, however, both patients eventually underwent OLT[36].

Progressive familial intrahepatic cholestasis (PFIC) encompasses a group of autosomal recessive liver diseases presenting in infancy and childhood with progressive cholestasis of hepatocellular origin, with three subtypes noted involving different components of bile metabolism[37]. Murine models of this disease process demonstrated improved bile metabolism using intrasplenic HTx[38]. Two children have been treated with HTx, however, both required OLT after 5 and 14 mo. Biopsies of the livers demonstrated extensive fibrosis and no donor cells on pathology before transplantation, the conclusion made that existing fibrosis likely impaired engraftment[39].

Phenylketonuria (PKU) is one of the most common inborn errors of metabolism, a deficiency of the enzyme phenylalanine hydroxyls (PAH) resulting in toxic concentrations of phenylalanine, the only current treatment involving phenylalanine restricted diet[40]. Murine models demonstrate significant improvement in PAH levels[41].

Alpha 1 antitrypsin (A1AT) deficiency - in 1997, a 52 years old patient underwent HTx as a bridge to transplant, with wild type A1AT levels were noted to increase in the interval between OLT, which occurred 2 d later[42].

This list is not exhaustive, however, it serves to illustrate the potential for this route of therapy in a large number of disorders mediated by hepatocyte dysfunction and subsequent metabolic derangements. While temporary improvements have been noted, others suspect the temporal benefits of HTx could be extended with transplantation of adequate cell mass, improved stock and implantation of transplanted hepatocytes, evaluating the ideal route of delivery, and improved and more accurate monitoring of graft function with emphasis on timely detection of rejection (Table 1).

METHODS OF DELIVERY

Regarding adequate transplantation, two issues warrant discussion. One, culturing of hepatocytes from livers deemed unsuitable for OLT, has been previously discussed. Another issue regarding viability deals with transplantation of "fresh"

vs cryopreserved hepatocytes. Fresh hepatocytes do demonstrate higher viability, with cryopreserved hepatocytes observed to have mitochondrial respiratory chain alterations and decreased ATP production[43]. Furthermore, protein synthesis has been noted to be impaired in cryopreserved cells relative to fresh hepatocytes[44,45]. A 2013 cohort study compared viability of freshly isolated hepatocytes against cryopreserved hepatocytes at 24, 48, and 72 h[46]. Freshly isolated hepatocytes demonstrated mean viability of approximately 81%, while means viability was approximately 61% at 24 h, 52% at 48 h, and 48% at 72 h. There was no noted increased caspase activity, an enzyme involved in apoptosis, though there did appear to some mild derangement in Cytochrome activities, previously noted above to be involved in hepatic metabolism of many different substrates.

Hepatocytes have been transplanted into the liver, spleen, and peritoneal cavity, with intraportal injection being the preferred and most physiological site for clinical transplantation[14,42]. This site may be accessed *via* percutaneous trans-hepatic puncture, cannulation of the umbilical vein, or open cannulation of a mesenteric vein[14]. Shear stress from catheterization can have an effect on viability, however, it has been demonstrated that catheters as small as 4.2 F are associated with acceptable viability[47]. Portal hypertension and any thrombosis are associated with lower engraftment levels, however, use of heparin infusion has been proposed as a potential mechanism to improve engraftment[48]. In cases of known portal hypertension, the spleen may be used as an alternate engraftment site, however, there are cases of splenic necrosis after injection into the splenic artery[8,49]. The peritoneal cavity is another alternate site, however, engraftment levels and long term viability of the graft have been observed to be significantly lower than portal vein infusion[50,51]. There are studies comparing efficacy of any method of delivery against another, and proper determination of the relative efficacy of each would be invaluable toward design of future studies evaluating HTx.

MONITORING GRAFT FUNCTION

Beyond just the method of delivery, appropriate pre-treatment of the recipient has been evaluated to improve efficacy of HTx. A 2017 case series details use of pre-operative liver-directed radiation[52]. Preoperative liver-directed irradiation has been noted to demonstrate complete correction of the bilirubin conjugation defect noted in rat models of Criger-Najjar syndrome Type I following HTx[53]. This case series demonstrated improved function of HTx from porcine hepatocytes comparing primates receiving hepatic pre-irradiation *vs* those who did not. Function was assessed by measuring levels of porcine albumin after HTx; pre-irradiated subjects demonstrated significantly higher levels of this protein than control subjects. Using immunohistochemical staining, spatial analysis of stained recipient liver tissue post HTx demonstrated level of engraftment to be approximately 11.8% in experimental subjects *vs* approximately 5% in control subjects. Survival of the graft appears improved in the pre-treated group appears improved as well, with no evidence of infiltrating T cells or macrophages noted in cells of the experimental group. Given the promising nature of the above results, two children with urea cycle defects were subsequently infused after undergoing the irradiation preconditioning protocol. One child was 4 mo of the age, the other underwent HTx shortly after birth. Regarding the patient receiving HTx shortly after birth, at 26 h, cell viability was noted to be approximately 63% with ammonia metabolism noted at normal levels[52]. One patient was noted to have intermittent episodes of hyperammonemia, however, it was noted that goal tacrolimus levels post-transplant were not sustained. This patient did eventually undergo OLT at 3.5 mo of age. The other patient maintained normal levels of ammonia for approximately 40 d, however, was not to have intermittent episodes of hyperammonemia after this point. On day 84 acutely increased levels of ammonia, glutamine, and urinary orotic acid suggested graft failure.

This same case series included a 27 years old female patient with PKU also undergoing HTx after irradiation pretreatment, doing well for 7 mo on an unrestricted diet before demonstrating evidence of rejection. Tacrolimus levels were again noted to be below goal level, and the patient was treated with corticosteroids and augmented immunosuppression protocol with phenylalanine tolerance returning. Phenylalanine levels remained normal for over one year, however, the patient's follow became inconsistent and adequate monitoring of immunosuppression was not performed. Her brother, also afflicted with PKU, was used a control agent. At this point in the study, the graft was assumed to be rejected and immunosuppression discontinued without any adverse effects noted. All three cases demonstrated improved length of graft function after HTx has taken place, and suggests pre-operative irradiation may serve as standard pretreatment to improve HTx efficacy.

Also evident in the above case series, however, is the issue of recognizing and treating graft rejection. The case series did detail how rejection was diagnosed, however, there remains no consensus on pretreatment to reduce risk of rejection, graft monitoring, and treatment once rejection is recognized or suspected. This case series chose to utilize monitoring for CD154+ T-cytotoxic memory cells, previously demonstrated to be sensitive for acute rejection in pediatric liver or intestinal implants[53-56]. Increasing concentration of this T cell was noted to generally correlate with suspected decreased function of the hepatocytes, however, the authors do note that significant daily variance of measured total bilirubin in the CN Type I patients and phenylalanine levels in the PKU patient[52]. Reviewing the aforementioned cases and use of immunosuppression, it

appears tacrolimus is an acceptable immunosuppressive agent, however, that closer monitoring of drug levels may be necessary to ensure continued appropriate function of the transplanted hepatocytes[1,31,52]. Further prospective cohort studies utilizing different monitoring intervals or alternate immunosuppressive therapy is likely necessary to ensure sustained and optimized graft function.

CONCLUSION

Further human studies involving humans are needed, however, the above collectively suggest progress in terms of improved effectiveness of HTx. With improvements in optimizing delivery technique and assessing proper recipients of livers, monitoring graft function, as well as recognizing and treating graft rejection, HTx may be able to be used more widely in metabolic liver disease and potentially delay necessity of OLT.

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MINIREVIEWS

Elderly donor graft for liver transplantation: Never too late

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Abstract

The definitive treatment for end stage liver disease remains a liver transplant and hence livers are needed for these patients along with cases of acute fulminant liver failure. Hence livers are a scarce and highly valuable commodity in the current time. By extending the pool of donors to include the elderly livers, it allows for increased availability of donors and reduces the mortality that is associated with the waiting list itself. There is an increasing prevalence of end stage liver disease due to conditions like chronic hepatitis B and C, non-alcoholic steatohepatitis, alcoholic liver disease. Many studies show non-inferior outcomes when elderly livers are used as a vigorous selection process is implemented. The process takes into account the characteristics of the donor, graft and recipient allowing for appropriate donor-recipient coupling. To meet the increasing demands of livers, elderly donors should be utilized for liver transplantation. The aim of this review article is to describe the aging process of the liver and the outcomes associated with use of elderly livers for transplantation.

Key words: Liver transplantation; Donor age; Elderly; Age; Outcome; Success

Core tip: There is an increasing demand of livers for transplantation. Several studies showed successful results with elderly donors. We reviewed the aging process of the liver and the transplant outcomes of elderly donors. We highlight that elderly donors can be utilized given the extensive screening process allowing for risk factor analysis and appropriate allocation. Hence they should be used to allow for treatment of

liver disease globally and help mitigate the shortage of hepatic grafts.

Chela H, Yousef MH, Albarrak AA, Romana BS, Hudhud DN, Tahan V. Elderly donor graft for liver transplantation: Never too late. *World J Transplant* 2017; 7(6): 324-328 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/324.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.324

INTRODUCTION

Orthotropic liver transplantation is an area of hepatology that is under continuous evolution. It is the definitive treatment for end stage liver disease as well as cases of acute fulminant liver failure. The fact that livers are a paucity and in high demand all over the world has forced the medical community to evaluate livers from marginal or extended criteria donors thus allowing the pool of donors to enlarge. The term marginal donors or extended criteria donors encompasses a group of criteria that allows for the enlargement of the donor pool. These comprise of elderly donors (aged > 60 years), steatosis > 30%, grafts with cold ischemia time > 12 h, hypernatremia, hepatitis B and C viral infections, split-liver grafts, donors who are living relatives, donors from cardiac arrest patients[1]. The need for livers greatly exceeds the supply and hence leads to prolonged waiting time and the associated subsequent mortality while awaiting transplant. The availability of younger donors is decreasing given the advances in medicine overall less motor vehicle accidents. The purpose of this review is to evaluate the impact of age on the liver and the implications that it carries on the outcome of the transplantation. Genetic and environmental factors also influence the aging process of the liver itself[2]. Multiple series of studies have revealed that the stigma that aging livers carry is not completely viable. The fear associated with elderly donors is that the increased risk of complications due to concern for impaired function and lack of a robust response to external and internal stressors as compared to younger livers[2]. There is a potential of transmission of occult malignancies as well as concern of overall decreased survival of the recipient and the graft itself[1,2]. Numerous variables are taken into account with regards to the donor, the recipient and the graft itself. Balancing these and carefully selecting the correct donor-recipient pair yields good outcomes which are comparable to those obtained from transplanting donor livers.

Impact of age on the liver

Similar to all organs in the human body, the liver undergoes many age related changes. Though as compared to other organs, the liver possesses the capacity to regenerate, abundant vascularity, as well as superior functional reserve[1]. Two of the main changes in the liver include a decrease in the overall hepatic mass and the blood flow[1]. Understanding the changes in the structural, morphological and functional changes that occur as the liver ages can help in making appropriate decisions regarding the use of older livers for transplantation.

Macroscopic and microscopic changes

As the liver ages, it tends to shrink in size and undergoes a process brown atrophy. Grossly, it acquires a brownish colored appearance and is due to the deposition of lipofuscin which are insoluble proteins[1-5]. The Glisson's capsule also acquires a fibrous thickening[1-5]. Microscopically, there is reduction of the number of hepatocytes though the cell volume increases[1-2]. There is increased variation in the cell size and the nuclear size increases as well as the amount of nuclear DNA along with aneuploidy[1]. Similar to the cells themselves, the mitochondria undergo a process of acquiring increased volume but reduction in the overall number of mitochondria[1,2,6]. These alterations reflect that the cells and their organelles are attempting to overcome the reduction in the overall number[1]. The cells are vulnerable to reperfusion injury due to the reduced mitochondrial adenosine-triphosphate content[2]. There is reduction of smooth endoplasmic reticulum and buildup of lysosomes[2]. Hepatic sinusoids exhibit increased thickness of the endothelial lining and reduction in the fenestration in the endothelial cells[2]. There is increased thickness of the hepatic arteriolar walls as well[2]. Reduction in the secretion of bile acids as well as reduced bile flow is also reported[1,2].

Vascular changes

Along with reduction in liver mass there is a substantial reduction in the hepatic blood flow with age especially after the age of 30 years[1,2,4]. This can significantly impact the clearance of medications hence leading to the potential for complications to arise[1]. Age related atherosclerotic changes affect the vascular tree and its branches. The branches of the abdominal aorta is predominantly impacted in the proximal and mid proximal regions, however in cases where there is occlusive pathology of the distal portions there can be involvement of the hepatic artery[1]. This predisposes to vascular complications and can hence impact the graft survival and overall outcome post-transplant.

Functional change

The overall synthetic function of the liver declines with age especially with regards to protein synthesis as well as synthesis of clotting factors[1]. The levels of serum bilirubin, alkaline phosphatase and transaminases are not impacted by age and instead are a measure of liver damage and not to the functional capacity of the liver[1]. It appears that overall age does not have a major effect on function of the liver itself but alters the response to stressors (especially external) including states of increased metabolic need or disease processes[1]. There is also report of diminished phase Ⅰ metabolism of

drugs and increased production of pro-inflammatory cytokines[2]. There is increased predisposition to the development of diseases due to decreased rates of DNA repair, decreased expression of growth regulatory genes and the impact of oxidative stress[2]. Regenerative capacity of the liver is not impaired but the rate of regeneration is decreased as a consequence of aging[1].

Evaluating the aging liver

Diligent and through assessment of the graft as well as the donor is required for selection for transplant. Marginal or extended criteria donors are associated with increased risk of complications including initial poor function and primary non-function[1]. Initial poor function is an aspartate transaminase (AST) value more than 2000 IU/L, ammonia level > 50 μmol/L, prothrombin time > 16 s on post-transplant days 2-6[1]. Primary non-function is defined as graft failure in the first week post-transplant or require a re-transplant for survival[1]. There are many variables associated with the donor that can predict failure of the graft and increased mortality of the recipient[1,2]. By use of Cox regression analysis there are seven major factors that are independently associated with graft failure[1,2,7]. These include donors aged > 40 years (especially > 60 years), prolonged warm ischemia, split/partial grafts, prolonged cold storage > 10 h, length of ICU stay > 5 d, decreased donor height, cerebrovascular accident, black race[1,2]. Scores like the marginal liver score have also been formulated to aid in identifying the higher risk factors associated with poor graft survival and overall outcomes[1]. The factors include donor age > 60 years, cold ischemia time > 13 h, length of intensive care unit (ICU) stay > 4 d, hypotensive episodes < 60 mmHg for > 1 h, alanine transaminase (ALT) > 170 U/L. AST > 140 U/L, dopamine dose > 10 mg/kg, serum sodium > 155 mEq/L, bilirubin > 2.0 mg/dL[1]. Each factor has a score of 2 and an overall score of 3 or above predicts poor survival of the graft[1]. When livers are being prepared for harvesting, caution has to be exercised to ensure adequate circulation to avoid ischemia, hypovolemia, hypoxemia as well as avoiding infection[1]. Dopamine is commonly used in cases of hypotension to augment renal and mesenteric circulation, however doses exceeding 10 mcg/kg per minute can result in acute tubular necrosis and doses beyond 15 mcg/kg per minute have been associated with graft preservation injury[1]. Hence a delicate balance exists and must be maintained to ensure adequate perfusion and oxygenation of the liver.

Increased length of stay in the ICU especially > 4 d can affect the post-transplant function of the liver due to the use of vasopressors and the resultant effect on hormonal status, hemodynamics and nutritional status[1,2,8]. Another factor that is recognized to have a negative impact is hypernatremia which leads to cell swelling and worsens ischemia reperfusion injury and graft dysfunction[1,2]. However increased transaminases in elderly donors were considered as marginal criteria in the past, however these are not commonly elevated in elderly donors that are used in successful transplants and this is indicative of the rigorous selection technique[2,9].

An ultrasound of abdomen is recommended to evaluate the donor liver for steatosis, tumors of the liver and other intra-abdominal malignancy or abscess[1,2]. Many experts also recommend obtaining a liver biopsy as well to assess for fibrosis, steatosis, hepatitis, cholestasis[1,2,9]. Microsteatosis may be linked to early allograft dysfunction, however macrosteatosis > 30% increases the risk of reperfusion injury and is a strong predictor of poor outcome especially when combined with prolonged cold or warm ischemia[1,2]. Some studies have revealed that microsteatosis may not impose any challenges regardless of the severity as opposed to macrosteatosis in which the outcome is negatively influenced with increasing severity of fat infiltration[10]. Prevalence of steatosis does increase with age and is linked to malnutrition, obesity, type Ⅱ diabetes, chronic alcohol intake[2].

Prolonged cold ischemia time leads to ischemia reperfusion injury which is a type of microvascular injury and leads to increased risk of rejection of the graft and morbidity[1,2,11]. There are 4 stages of injury: Pre-preservation, cold preservation, rewarming and reperfusion[1]. The chances of this injury and the severity are affected by various factors which can potentially be controlled hence minimizing the risk of injury and improving the outcome of transplant. Older livers are more vulnerable to this form of injury hence extra caution must be exerted to keep the cold ischemia time to a minimum in them[1,2]. Increased warm ischemia time also has deleterious effects and should also be kept minimized[1,2].

Overall outcome of using elderly donors

Underlying condition of the recipient does influence survival of the graft, however recurrent diseases are a major cause of graft failure[2]. Cirrhosis secondary to hepatitis C is a major cause of liver failure requiring transplantation and has exceedingly high recurrence rates[2]. Graft fibrosis after transplantation was linked to the organ age and hence elderly livers are avoided in such cases, however this may change with the advancements in antiviral therapies or hepatitis C reducing the risk of recurrence[2]. With time post transplantation there is occurrence of chronic hepatitis and eventual fibrosis and hence elderly livers are avoided for transplant in the pediatric population[1,2].

Due to the decreased number of hepatocytes and the alteration in the regenerative capacity of older livers there has been concern to use them for transplant due to fear of early allograft dysfunction and primary non function[2]. Due to the increased prevalence of advanced atherosclerotic disease in the elderly there is increased concern for vascular complications developing post-transplant when elderly donors are used[2]. Though the elderly have increased arteriosclerosis in the celiac axis, it appears that the hepatic arteries are not significantly

impacted by this and more distal portions of the hepatic arterial system is used for transplant[2]. Arteriosclerosis affects the graft by two methods: Decreased blood supply at time of organ harvesting due to stenosis of celiac axis ostium causing poor graft preservation and increasing chances of primary non-function. The second method is effect on the vascular reconstruction process if the donor arteries are diseased by arteriosclerosis leading to early as well as delayed difficulties[2]. Hepatic artery thrombosis is one of the major causes of graft failure and has increased prevalence with increasing age of the donor[1,2,11]. The major causes of mortality in recipients of elderly donors are medical complications, cirrhosis due to hepatitis C recurrence and *de novo* tumors[1]. In a study performed by Zhao *et al*[11], that involved the use of elderly brain-dead donors, it was found that there was no primary non functions or need for re-transplant in the patients receiving the elderly (> 60 years) livers. They also found that early graft function was similar between the elderly and the younger donor group[11]. If careful selection and risk stratification is performed then acceptable and even at times comparable outcomes can be achieved with elderly donors. For example, using high risk donors for low risk recipients so that the risk of the donor is offset by the lower risk of the recipient to achieve more favorable outcomes and to avoid the waiting list mortality[1]. Using marginal or extended criteria donors requires that multiple factors be taken into account to match the appropriate donor with the corresponding recipient. By detailed assessment of the graft characteristics and taking into account factors that will augment each other negatively, appropriate donors can be selected[10]. Evaluating the recipient's ability to accommodate high risk donor grafts allows appropriate matching to occur without yielding a negative outcome[10].

In a retrospective study performed by Zhao *et al*[11] in which they evaluated 106 donor liver transplants which were harvested from cadavers. They were used in total of 98 patients and 7 of these patients were recipients of elderly donor livers (age > 60 years). The patients were divided into two groups. Group Ⅰ received livers from elderly donors > 60 years, and Group Ⅱ received livers from donors < 60 years. They accounted for risk factors like age of the donor, body mass index, the etiology of death, duration of stay in the ICU, gender, blood pressure and the amount of vasopressor used[11]. There were no significant differences overall with regards to parameters like bilirubin levels, transaminases at one week post-operatively[11]. Outcome with regards to recipient and graft survival as well as complications like primary non-function, biliary complications, hepatic artery thrombosis, need for re-transplantation. Zhao *et al*[11] supported the use of elderly donors for liver transplantation.

A study conducted in Birmingham (United Kingdom) revealed that mortality due to primary non function was comparable between the donors less than 70 years (1.3%) and more than 70 years (2.0%)[2]. They noted that recipients of aged livers experienced fewer arterial complications such as hepatic artery thrombosis and though it was not significant at the statistical level but did reflect that elderly livers should not be associated with worse outcomes[2]. Other centers around the world have experienced similar outcomes hence promoting the use of elderly liver donors.

Another study conducted by Rodríguez González *et al*[12] using 100 liver allografts from elderly donors aged > 60 years also supported the use of aged donors > 60 years. They emphasized that elderly donors can be used as long as a comprehensive risk assessment and evaluation is performed. Assessing the pre-transplant conditions like the liver function tests, length of ICU stay, cold ischemia time, hemodynamic status, whether vasopressors were used or not[12]. Factors like cold ischemia time of less than 6 h ideally as well as macrovesicular steatosis < 30% were very important contributors to a favorable outcome when using the elderly donors in their study[12]. Hence their study showed favorable outcomes when elderly donors were used as long as pre-operative risks were assessed and minimized as much as possible[12].

A study conducted by Thorsen *et al*[13] focused on liver from decreased donors aged > 75 years and though they noted an increased rate of biliary complications, they did not see overall worse outcomes with regards to mortality rates in recipients or graft survival.

CONCLUSION

The use of elderly donors is becoming more favorable and helping to reduce the mortality associated with the waiting list itself. The liver is a remarkable organ that possesses several unique qualities though like other organs in the human body it is also subjected to the process of aging. Though advancing age is not an advantage for the process of transplantation, it should not preclude the use of elderly livers for transplant. A careful and meticulous selection process can be carried out allowing to risk stratify the donor, the graft and the recipient. Factors in the graft like the gross as well as microscopic appearance are evaluated to exclude donors with obvious abnormalities like tumors or significant macroscopic steatosis. An ultrasound is recommended along with a liver biopsy to evaluate for occult tumors along with pathologies like fibrosis, steatosis. A thorough evaluation of the donor should be performed including detailed medical history, intra-operative exploration of the abdominal as well as thoracic cavities to exclude malignancies. Recipients should be evaluated as well and accordingly matched to appropriate donors hence achieving optimal outcomes. With this rigorous selection process it has been shown in several studies that elderly donors are comparable to younger donors and have successful outcomes. We would like to emphasize that elderly donors can be utilized given the extensive screening process allowing for risk factor analysis and appropriate allocation. Hence they should be used to allow for treatment of liver disease globally and help

mitigate the shortage of hepatic grafts.

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MINIREVIEWS

Polyoma virus nephropathy in kidney transplantation

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Abstract

BK virus (BKV) is a polyomavirus that is able to cause renal dysfunction in transplanted grafts *via* BK virus-associated nephritis (BKVAN). This condition was mis-diagnosed in the past due to clinical and histopthological similarities with acute rejection. Due to the prevalence of the virus in the population, it is an important pathogen in this context, and so it is important to understand how this virus functions and its' relationship with the pathogenesis of BKVN. Screening for BKV often reveals viruria and/or viremia, which then manifests as BKVN, which can be asymptomatic or result in clinical features namely renal dysfunction. The pathogenesis of BKV infection is still unclear and needs to be further investigated; nevertheless there are a variety of hypotheses that indicate that there are a host of factors that play important roles. Treatments for BKVAN include a reduction in immunosuppression, the use of antiviral therapy or the combination of both treatment options.

Key words: Polyoma; Kidney; Transplant; Infection; Virus

Core tip: Prior to its recognition as a separate entity, kidney transplant infection with the polyoma virus, BK virus (BKV), and the ensuing viral nephropathy (BKVN) portended a poor prognosis. But with the advent of heightened clinical suspicion and improved diagnostics the prognosis has improved considerably. Blood and urine polymerase chain reaction testing allows invasive investigation (*i.e.*, transplant biopsy) to be selective and appropriate. Peripheral blood assays of anti-BKV cell mediated immunity offers potential for refining risk stratification. While conventional antiviral agents have failed to show utility to date, reduction of immunosuppression currently represents the most effective and proven treatment for BKVN.

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INTRODUCTION

BK virus (BKV) was first isolated from the urine of a patient with transplant ureteric stenosis[1] in 1971. BKV is a member of the *Polyomaviridae* family, falling into the *Betapolyomavirus* subcategory with JC virus and Simian virus 40 (SV40)[2]. BKV and SV40 have approximately 70% similarity within their respective genomes[3,4]. This similarity between the genomes of these viruses enables SV40 to be a marker for immunohistochemical staining, which is vital in diagnosis of BKV-associated nephropathy (BKVAN)[4]. BKV primary infection occurs in early childhood and is asymptomatic in the majority of cases[5,6]. Transmission of BKV is thought to involve respiratory and oral routes[7], and results in a seroprevalence of 82% in adulthood[8]. BKV is a latent infection, which can lie dormant in tissues, the kidney being the most notable. Heritage *et al*[9] showed that BKV was present in 50% of kidneys based on DNA sequencing of BKV in renal samples. The virus is able to reside in renal tubular epithelial cells and in the uroepithelium. Therefore, another "artificial" route of BKV transmission is through kidney transplantation, particularly on the background of the immunosuppression required in this context to prevent organ rejection. Moreover, although primary infection in non-transplanted patients is generally asymptomatic, viral infection in the context of transplantation and immunosuppression may result in viral replication within epithelial tissues (in this case renal tubules), and the development of inflammation, BKVAN, which resembles other forms of tubule-interstitial nephritis and transplant rejection. If left untreated, these processes progress to result in allograft dysfunction and failure[10]. Indeed, North American and European series suggest that although not the leading overall cause of graft failure, it represents an important and potentially treatable (even preventable) cause in many[11,12]. For many years, BKVAN was mis-diagnosed as rejection, and therefore inappropriately treated, resulting in graft failure in many patients. Nevertheless, greater understanding of BKV has resulted in clinical advances in the field. In this literature review the virology of BKV, the mechanism of BKVAN pathogenesis, and advances in therapeutic strategies will be addressed.

VIROLOGY OF BKV

BKV is a member of the *Polyomavirus* genus of the *Polyomaviridae* family. These viruses are 40-45 nm in diameter[1,13] comprising of an icosahedral capsid surrounding double-stranded DNA, which is able to replicate in the host cell nucleus[14]. The BKV genome contains 5153 base pairs that can be translated bi-directionally[6,15,16]. However, recent analysis of BKV from a kidney transplant patient observed a genome size of 5141 base pairs[17]. The BKV genome is divided into three regions: Early, late and regulatory (non-coding control region or NCCR) regions. The early stage encompasses regulatory proteins such as small tumour antigens (tAg) and large tumour antigens (TAg) as well as late structural capsid proteins - viral protein (VP) 1, VP2, VP3 and the agnoprotein[18]. However, these late proteins are produced after the genome of the virus has be replicated[19]. VP1 is the most common protein found on the outer layer of the capsid and contains a small groove used for host cell receptor binding[20]. The NCCR encodes transcriptional control elements, such as the origin of replication and promoters for genes encoded within the early and late regions[11,21]. BKV enters cells *via* VP1 binding to sialic acid residues of glycoprotein receptors[22,23]. After receptor binding BKV is internalised *via* a caveolae-mediated endocytosis pathway, the virus then travelling to the cell nucleus to launch either a latent or acute infection[13,24,25]. According to Jin *et al*[26], four serotypes (Ⅰ-Ⅳ) of BKV are recognised based on the differences between amino acids 61-83 in the region coding for VP1, with the similarity of this region between different serotypes at 61%-70%[27]. Serotype Ⅰ is the most common within the worldwide population (80%), followed by type Ⅳ (15%)[28]. However, Sharma *et al*[29], using a phylogenetic whole-genome approach, suggested a classification system of BKV which contain serotypes V and Ⅵ.

PATHOGENESIS OF BKVAN

There are many proposed factors relating to the pathogenesis of BKVAN as shown in Figure 1: Source of the viral infection; host cellular immunity to BKV; the influence of immunosuppression; HLA matching; recipient and donor blood group matching.

Source: Donor vs recipient

BKVAN within transplanted kidneys arises from either primary infection from the transplanted kidney itself, or following reactivation from latency in the patient's native urinary tract. Epidemiological work has striven to understand the predominant mechanism in this context. Andrews *et al*[30] were the first to show that recipients of transplants from seropositive donors (either deceased or living), was associated with increased rate of BKV infection within the transplant kidney, thereby suggesting the importance of donor-derived infection in the pathogenesis of BKVAN. This was supported by data from Bohl *et al*[31] who observed BKV infection in 25 of 54 (46%) recipients of kidneys from seropositive donors compared with 4 of 27 (15%) recipients of kidneys from seronegative donors (P = 0.007). These authors also noted that the rate at which BK viruria occurred was faster in patients receiving kidneys from seropositive *vs* seronegative donors (median onset 45 d *vs* 370 d

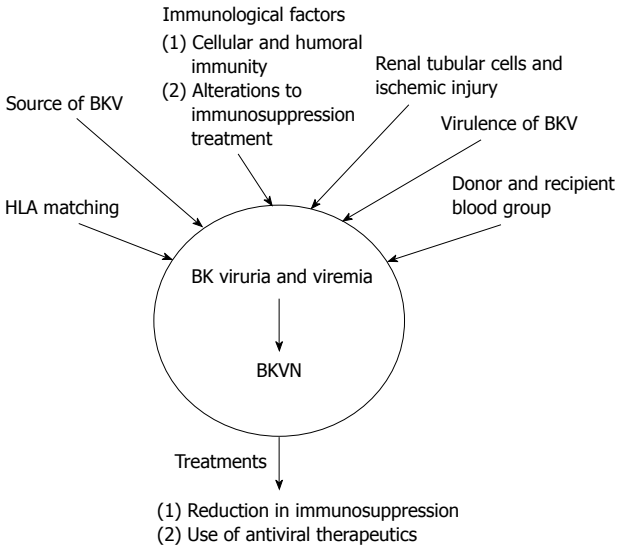

Figure 1 Proposed mechanisms for the pathogenesis of BK virus-associated nephritis after BK virus infection has occurred resulting in BK viruria or BK viremia. These mechanisms include immunological factors, such as alterations to immunosuppressive therapy and cellular and humoral immunity, the source of BKV, either from the recipient or the donor, HLA matching, donor and recipient blood group. The two main treatment options for BKVN are a reduction in immunosuppression and the use of antiviral therapies. These treatments can also be used for BK viruria and viremia in order to prevent progression to BKVAN. BKV: BK virus; BKVAN: BK virus-associated nephritis.

respectively; $P < 0.001$). The duration of BK viruria was also longer in the context of seropositive donors (median duration 157 d *vs* 7 d, $P = 0.009$). The authors also recorded that none of the 27 recipients from seronegative donors developed viremia or sustained viremia, whereas these numbers were 7 and 4 respectively in recipients from seropositive donors. In addition, this study demonstrated donor BKV antibody titre inversely correlated with the time to onset of post-transplant viruria ($P = 0.001$), and was positively correlated with duration of viruria ($P = 0.014$) and peak urine viral titres ($P = 0.005$). These studies did not evaluate the importance of recipient BKV serostatus, but this was done in a study of paediatric recipients[32], which suggested the importance of recipient BK seronegativity. In this study, all patients developed BKV viruria, with recipient seronegativity strongly associated with the development of nephropathy ($P = 0.01$). Finally, Saundh *et al*[33] studied 112 renal transplant patients before and after transplantation, and conducted a phylogenetic analysis of VP1 sequences and serotypes. Twelve patients developed BKV viremia, and 8 had a sufficiently high viral load to allow amplification of VP1. Based on this analysis the authors concluded that donor-derived infection was responsible for the majority of cases of BKV infection. A single patient had two differing VP1 subgroups present (Ib-1 after 6 mo followed by Ia after 12 mo post transplantation), perhaps suggesting a potential switch between donor and recipient strains, and means that cases of BKV infection due to reactivation from the recipient may be a real phenomenon. However, the burden of evidence from this, and the other aforementioned studies, is that donor-derived infection represents the major risk confronting the kidney transplant recipient.

Cellular and humoral immunity

Humoral and cellular immunity is thought to be implicated with the pathogenesis of BKVAN, and both $CD8^+$ and $CD4^+$ T cells are involved in the recognition and clearance of viruses such as BKV. The lack of BKV specific IgG may be important in the development of BKVAN[12]. As mentioned above, there is a greater risk for patients that are BKV seronegative at the time of their transplant, as they will have no BKV specific antibody; patients with previous exposure and who have developed immunity to BKV, may not develop the infection[34]. Yet the presence of a BKV-specific antibody response is clearly not protective, and it is likely that cellular immunity plays the central role in viral control. Certainly, patients with BKV specific antibodies remain at risk of developing BKVAN[35]. Comoli *et al*[36] showed that patients that had BKVAN had fewer BKV-specific lymphocytes that secreted interferon-γ (IFN-γ), with the mean frequency of BKV specific, IFN-γ being 151×10^6 cells. This was approximately 10 times less than other viruses related to transplantation, such as EBV. The researchers concluded that, based on their data that there is reduced BKV immunity, which in turn would increase the rate of active BKV infection.

Immunosuppression burden

Immunosuppression is required to ameliorate rejection of the transplanted kidney by the host immune system. However, with developments in immunosuppressive drugs such as mycophenolate mofetil (MMF) and tacrolimus (Tac), the reduction in rejection rate has been inversely paralleled by an increased incidence of BKV infection. Calcineurin inhibitor (CNI) based therapies have been shown to increase the risk of BKVAN and subsequent nephrotoxicity following renal transplant[37]. However, a recent study by Jacobi *et al*[38] observed no significant change in the number of patients with BKV infection ($n = 352$) when using CNI of either tacrolimus or cyclosporine A (CyA). This led to the conclusion that CNI immunosuppressants were not associated in BK viremia.

Mengel *et al*[39] suggested particularly increased risk of BKV nephropathy with a combination of Tac and MMF. Similarly, Brennan *et al*[40] showed that viruria was most common with a drug combination of Tac-MMF (46%) and lowest with cyclosporine-MMF (13%). These authors also confirmed the association between viruria and subsequent BKV viremia. In addition, when surveillance for BK viremia was undertaken for the purposes of this study, a reduction in immunosuppression in response to detectable viremia resulted in reductions in viral load in 95% of patients, and without increased risk of rejection. A more recent randomized study also supports the role of immunosuppression in this context. In a study of 682 patients, the combination of Tac and MMF was associated with greater rates of viremia at 6

and 12 mo post-transplantation than the combination of cyclosporine and MMF (16.3% *vs* 10.6%, P = 0.048 and 12.1% *vs* 4.8%, P = 0.004 respectively). Cumulative steroid dose up to month 3 was also a risk factor for viremia in this study, highlighting the role of overall immunosuppression burden in this disease[41]. Clinical observations of campth therapy were made by Kayler *et al*[42], they administered campth to two patients with BKV viruria and one with nephropathy. In all cases, there was increased viral replication and one of the patients with viruria developed nephropathy. The authors concluded that campth treatment does not permanently remove immune cells that are able to respond against BKV and that the therapy does not prevent stop the clearance of BKV from the blood.

Recent attention has focused on the role of lytic antibody induction, and particularly the role of alemtuzumab (anti-CD52 monoclonal antibody; "Campath") which is undergoing more recent widespread usage. A large study ("3C study") demonstrated double the incidence of BKV infection with alemtuzumab compared with basiliximab induction. However, absolute incidence was low (8% *vs* 4%), and this difference was driven by BK viremia (7% *vs* 3%) rather than BKV nephropathy, the rates of which were very low in both alemtuzumab and basiliximab arms of the study (1% and 2% respectively)[43]. Conversely, it has been shown that alemtuzumab did not remain significant risk factor after the adjusted hazard ratio for each variable had been calculated[44].

A United States OPTN database review showed that there was an increased risk of BKV infection with an induction therapy using thymoglobulin (P < 0.0001)[44]. In a study by Ott *et al*[45] renal transplants patients, under either basiliximab (n = 22) or thymoglobulin (n = 27) treatment regimens, were assessed for complication in a mean follow up period of three years. Of the 27 patients treated with thymoglobulin, two developed BKVAN whereas no patients had a BKV infection when treated with basiliximab. However, CMV infections were observed in both patient cohorts, with four and three patients infected for basiliximab and thymoglobulin therapies, respectively. This indicated that treatments using thymoglobulin carry a greater risk of BKV infection to renal transplant patients post transplant.

Effect of HLA matching

The adaptive immune response to viral infection is dependent on T-cell recognition of viral antigen presented in the context of self-MHC. In other transplant settings, it has been shown that immune responses to viral antigen presented in the context of donor-derived MHC (in this case cytomegalovirus) do not develop[46]. The donor-derived nature of BKV may therefore impair the magnitude or timing of effective immune clearance. In keeping with this concept, a study by Lee *et al*[47] used a mouse model to show ineffective clearance of BKV in the context of MHC mismatching. Clinical data also supports this concept of increased HLA mismatch as a risk factor for BKVAN[48-50]. However, in contrast, a clinical study from Drachenberg *et al*[51] found lesser degrees of HLA matching was actually associated with maintained graft function in patients with established BKVAN. This led to the proposal that even though reduction in HLA matching would decrease the recipient's ability to mount an effective immune response to BKV, there would be less tissue damage, thus reduced risk of graft loss. This in turn raises questions in regard to the mechanism of viral clearance in this context, and whether this is dependent on the T-cell response or other viral elimination mechanisms such as NK and NKT cell activation. Clinical data from other cohorts may also serve to clarify the current understanding.

Aside from HLA matching, the question arises as to whether BKV infection and nephropathy is associated with specific (donor or recipient) HLA alleles. Awadalla *et al*[50] found no such association(s), but another study from Bohl *et al*[31] found a possible link between HLA C7 and the severity of BKV infection. Although there was no association between BK viremia and either donor or recipient HLA-A, -B or -DR type, all 11 transplant recipients with persistent BK viremia received kidneys from HLA C7 negative donors, and 10 of these 11 recipients also lacked HLA C7. The possible mechanism underlying this observation is unclear. However, if confirmed, this raises implications for the relevance of HLA-C typing in transplantation, which is not currently recommended or undertaken, but which may identify individuals at greater risk of refractory infection.

Donor and recipient blood group

A fundamental feature of transplantation is the matching of blood groups of donor and recipient in order to avoid the risk of hyper-acute rejection. Nevertheless, with intensified preconditioning and antibody removal, blood group-incompatible transplantation is now commonplace[52]. However, Sharif *et al*[53] suggest a high rate of BKVAN in such patients. In a study of 62 blood group incompatible transplantations between 1998 and 2010, the risk of BKVAN was 17.7% (compared to 3% risk among blood group compatible patients). This data has been replicated in a different cohort by Bentall *et al*[54]. While we may infer this is due to desensitization and/or heightened immunosuppression for incompatible patients, the authors actually observed a lower risk among a cotemporaneous group of 221 HLA antibody incompatible transplants (5.9%, P = 0.008) who also underwent intensified preconditioning and received stronger induction therapy (ATG) compared to either blood group incompatible or compatible patients (Basiliximab). Therefore, pre-conditioning or heightened immunosuppression cannot be the sole explanation for this observation. Interestingly, the authors identified the lack of a typical accommodation-like phenotype (defined as C4d deposition in the absence of any micro-circulation inflammation) among blood-group incompatible transplant recipients with BKVAN

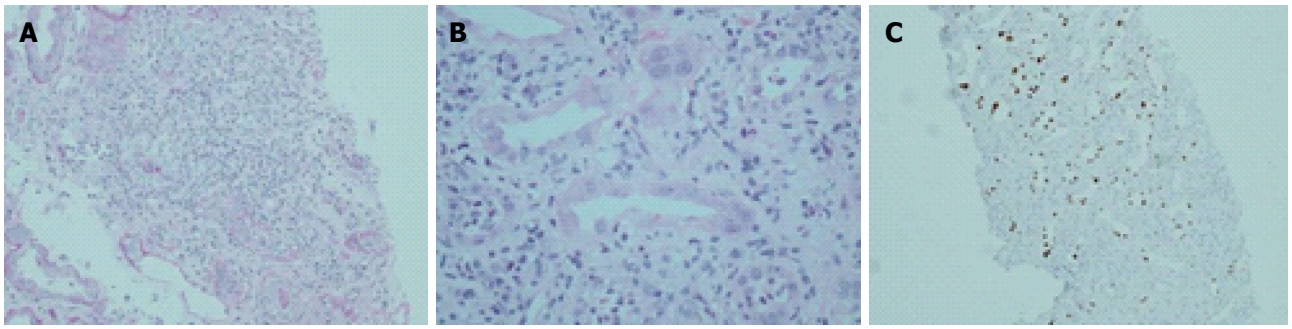

Figure 2 Histological features of BK virus nephropathy by light microscopy. A: Tubule-interstitial infiltrate and tubulitis classical for BKVN, but also compatible with any other form of interstitial nephritis such as acute cellular rejection; B: Higher power view of same biopsy sample, with characteristic viral inclusions seen within epithelial cells (circled); C: Positive SV40 immunoperoxidase staining on same specimen, confirming diagnosis of BKVN. BKVN: BK virus nephropathy.

compared to those without BKVAN (40.0% *vs* 75.8% respectively, $P = 0.04$). However, it is unclear from this data whether lack of accommodation-like phenotype development increases the risk for BKVAN or whether blood-group incompatible patients with BKVAN lose their accommodation-like phenotype but further studies are warranted to research this further.

OTHER RISK FACTORS

There are also a number of risk factors between the donor and recipient that can increase the risk of a BKV infection, including gender, race, age, diabetes mellitus or where the organ was sourced from a deceased donor[39,44,55,56].

CLINICAL FEATURES AND DIAGNOSIS OF BKVAN

The median time to clinically apparent BKVAN is within the first year after transplantation[57,58]. The recipient is characteristically asymptomatic, with the infection presenting as progressively worsening renal function, usually in the absence of significant or new-onset proteinuria[59]. This presentation generally results in a "for cause" biopsy, which shows the characteristic features of BKVAN. A number of histological grading systems have attempted to classify BKVAN[60-63], and whilst differences exist between these alternative systems, recurring themes are:

The separation into stages of BKVAN depending on the presence of viral infection in the absence of inflammation or significant chronic damage (Grade A), with inflammation dominating over chronic damage (Grade B), and with chronic damage (fibrosis and tubular atrophy) as a notable component, with or without inflammation (Grade C).

That prognosis is correlated with these stages of BKVAN, and especially with the presence of significant chronic damage (Grade C nephropathy).

In simultaneous biopsy cores, discordant findings (*i.e.*, the lack of evidence of BKVAN in one of the cores) was found in around a third of cases. Of note, in the core without evidence of virus, interstitial inflammation and/or acute tubular injury were frequent findings

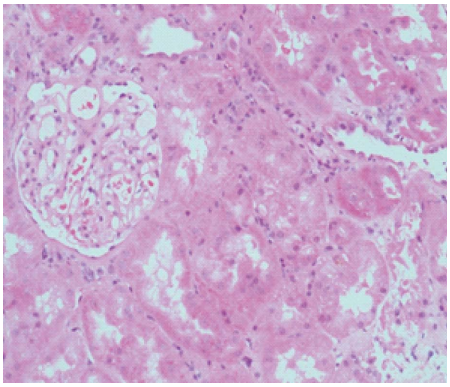

Figure 3 Kidney with preserved tubular architecture, without significant chronic damage or interstitial inflammation, but with BK virus nephropathy confirmed by virtue of positive SV40 staining (as shown in insert taken from immunoperoxidase sample from same biopsy specimen).

(approximately 80%), raising implications for the clinical interpretation of kidney biopsy specimens where only one core is retrieved, where the sampling is inadequate, and where there is collateral evidence that BKVAN may be a diagnosis[64].

Whilst concurrent viremia is almost universal with the finding of BKVAN on microscopy, the magnitude of circulating viral load seems to have little or no relationship with the extent of nephropathy[64]. Representative examples of the histological appearances of BKVAN are shown in Figures 2-4.

For many years, BKVAN was confused with acute rejection, as both have the appearance of an "interstitial nephritis". With more widespread recognition of BKVAN, the availability of blood and urine testing for viral load, and the utility of SV40 staining on biopsy samples, the pathological diagnosis of BKVAN has become more straightforward. However, acute rejection and BKVN are not mutually exclusive, and particularly in the period following BKVAN treatment (see below), the two may coincide, and it may be unclear which represents the dominant process. Despite efforts, there remains no consensus in regard to the most accurate way to separate these entities, although the presence of macro- or micro-vascular inflammation points to

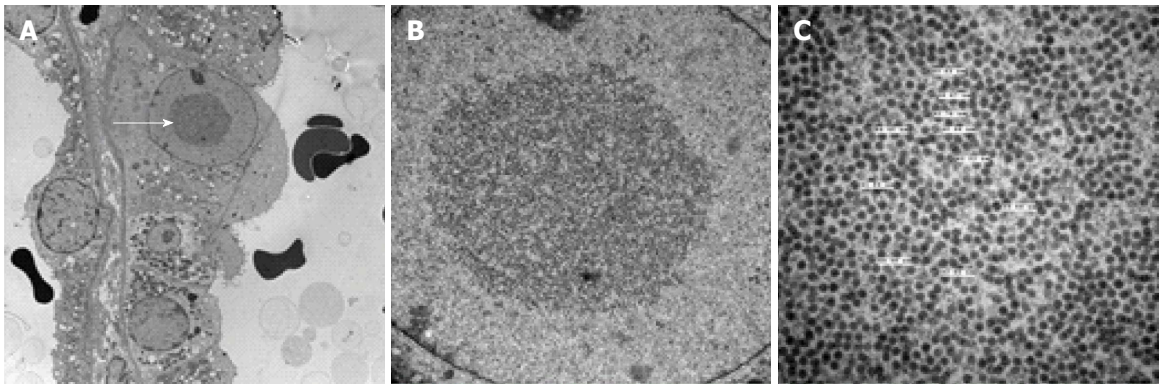

Figure 4 Histological features of BK virus nephropathy by electron microscopy. A: Electron microscopy evidence of viral inclusions (arrow) within epithelial cells, equivalent to those seen and circled in the light microscopy sample shown in Figure 2B; B: Higher power magnification of epithelial viral inclusions; C: Highest magnification demonstrating characteristic appearance and size (labelled) of BK virions.

rejection as a component at least.

Aside from the classical presentation described above, BKVAN may present in a "subclinical" manner, as is seen with other forms of transplant-related renal injury including "subclinical rejection". Recent clinical studies have used protocol renal biopsies to test for the presence of BKV in this setting. Buehrig *et al*[65] concluded that allograft biopsy allowed earlier detection of BKVAN, and the potential for this to enable earlier treatment, although this proposed approach has not yet been evaluated. Whether such a strategy translates into improved overall clinical outcome (and justifies the risk, inconvenience and cost of the biopsy) remains to be seen.

SCREENING FOR BKV INFECTION

Established BKV screening methods include testing urine for decoy cells, viral particles by electron microscopy, and viral DNA by PCR. However, plasma PCR for detection of viremia remains a more common approach to screening[66]. It has also been suggested that circulating viral loads above certain thresholds (approximately > 4 log copies/mL) can be considered presumptive of nephropathy even in the absence of histological evidence (see above). Whilst inter-laboratory standardization of such PCR assays is awaited, such discrete values remain subject to interpretation by individual centres.

A more recent study carried out by Singh *et al*[67] investigated whether the qualitative detection of three-dimensional aggregates of polyomavirus (Haufen crystals) within a patient's urine could be used as a diagnostic test for patients BKVAN. Of 21 patients known to have BKVAN 77 of the 143 samples taken contained Haufen. During follow up, the presence or absence of Haufen matched the course of renal disease. All control samples (194) were negative. The predictive values of Haufen for BKVAN were 97% for positive and 100% for negative. This leads to the conclusion that Haufen testing in urine is a more accurate approach than detection of viral DNA in urine or plasma, although the reproducibility and generalizability of these findings requires further clarification.

Finally, the role of urine profiling for VP1 (BK capsid protein) mRNA is under investigation, with exploratory[68] and validation studies[69] suggesting potential utility in predicting nephropathy, albeit in small patient numbers Further generalisation may yet provide additional important information in this regard.

CURRENT AND FUTURE TREATMENTS FOR BKV INFECTION

The first step in the treatment of BKV infection is reduction in immunosuppression. Certainly, this approach is not disputed for cases of nephropathy, although evidence guiding the order with which the component immunosuppressant are withdrawn is lacking. Less clear is whether immunosuppression should be altered in the face of viremia and in the absence of overt nephropathy. This question was addressed in a large and important study of 200 patients by Brennan *et al*[40], where reduction in immunosuppression in response to detectable viremia on protocolised plasma samples resolved 95% cases of viremia with no signal towards graft rejection, dysfunction or loss. Other smaller studies also support this approach of "pre-emptive" therapy[70]. The efficacy of this strategy is also supported by Saad *et al*[71]. In this study, MMF and/or Tac doses were reduced for the patients, who in this study were not restricted to those with viremia (24 patients: 66% BKVN, 34% Viremia). Overall, a decline in BKV viral load was seen. However, three patients developed acute cellular rejection, albeit with successful treatment with intravenous bolus steroids. One patient experienced BKVN relapse during pregnancy and lost the graft. Seventeen patients maintained or improved their graft function following this reduction in immunosuppression. In summary, the evidence from these studies support decreasing immunosuppression as the first line of treatment for patients that present with BKVAN, and possibly with detectable viremia, although clearly the risk of rejection needs careful consideration. Controlled studies are required to solidify these findings, although

in the meantime the recommendation from Kidney Disease: Improving Global Outcomes (KDIGO) expert panel is to reduce immunosuppression when plasma viral loads exceed a certain threshold (10000 copies per mL, whilst accounting for inter-laboratory variation)[72].

BKV interacts with the AKT/mammalian target of rapamycin (mTOR) pathway[73]. Everolimus and sirolimus are examples of mTOR inhibitors (mTOR-i)[73-76]. Everolimus was observed by Polanco *et al*[75] to increase renal function in BKVAN positive patients that had their treatments converted from tacrolimus to everolimus, with a suspension of mycophenolate. This study involved 15 patients, all presenting with BKVAN of which 9 underwent the immunosuppressant conversation. The serum creatinine of these patients decreased from 2 (± 0.21) mg/dL at the time of conversion to 1.6 (± 0.39) mg/dL at the final follow up. BK viremia became negative in 5 of the 9 patients and the remain 4 had a > 95% decrease in BKV. This decrease in BKVAN is also seen in conversion to sirolimus. In a recent single centre retrospective study by Tohme *et al*[77], patients were either placed on a tacrolimus or sirolimus based immunosuppression therapy. If the patients were < 62 years old they were converted from tacrolimus to sirolimus. Clinically significant BK viremia fell when converting from tacrolimus (P = 0.04) to sirolimus (P = 0.02), 17.9% to 4.3%, respectively. However, the hazard ratio for the male gender was also associated with the incidence of BK viremia (P = 0.03). Discontinuation of the sirolimus treatment occurred in 34% of patients due to various side effects. Thus, the use of mTOR-i as a treatment option of not only provides immunosuppression, reducing the risk of acute rejection, but also due to its behaviour as a metabolic pathway inhibitor for BKV it can also aid in the reduction in viral load, hence a lower risk of developing BKVAN.

The next question is whether detection of virus in urine (rather than waiting for it to appear in plasma) might represent a more efficient screening and intervention biomarker. In this regard, the clinical data is less optimistic. Specifically a series of retrospective studies have suggested increased rates of (or episodes of) acute rejection in the presence of viruria, even in the absence of viremia[78-80]. Whilst a proportion of these episodes were likely a response to immunosuppression weaning, there were clearly others which were unrelated, and which may potentially be a manifestation of low grade viral reactivation and inflammation inciting a secondary alloimmune response. However, irrespective of the mechanism, these observations (although limited by study design and interpretation) suggest that immunosuppression weaning in the context of viruria should not be recommended until and unless further information comes to light.

Even with successful treatment with immunosuppression reduction, the timeline of viral clearance is variable, although the reported median time to complete plasma clearance is 9 mo[81]. Serial renal histopathology following treatment is interesting, although reports are limited due to the nature of such studies. Of relevance though, the report from Menter *et al*[81] suggests that a self-limited "interstitial nephritis" is common during the phase of viral clearance and that this may represent an appropriate antiviral response rather than alloimmunity.

Specific antiviral therapy is generally used as a secondary line of treatment for BKVAN, and although an attractive approach, the role(s) of multiple agents remain unproven and unclear. Although better recognized as antibacterial agents, the quinolone antibiotics do display *in vitro* activity against polyoma viruses. Arroyo *et al*[82] retrospectively investigated the effects of ciprofloxacin on patients with BK viruria and viremia, after clinical failure with prior reduction in immunosuppression. The study showed that there were no adverse effects of ciprofloxacin and that out of the nine patients that received the treatment, three showed complete clearance of the virus and another three had the viral load in the plasma reduced by ⩾ 50%. Unfortunately, a subsequent randomized controlled trial of 3 mo levofloxacin (from post-operative day 5) in 154 kidney transplant recipients showed no effect on the development of BKV viruria compared with the control group (29% *vs* 33%)[83]. In addition, an increased incidence of antibiotic resistance to bacterial isolates, and also a signal towards increased tendonitis was seen in the levofloxacin treated arm. Observational data also comes from Jung *et al*[84], this time studying the effect of leflunomide on biopsy-proven BKVN in paediatric patients. Tac dosage was reduced and leflunomide and intravenous immunoglobulin treatment was instituted. Viral load then decreased and remained below 100 copies/mL over an 18 mo period with no loss of renal function, from a value of 474140 copies/mL of BKV viral load in patient serum. Intravenous immunoglobulin in the absence of adjunctive antiviral agents has also been reported as a treatment for BKV infection[85], and observational data supports a potential role for another antiviral agent, cidofovir[86]. Yet in the absence of more robust data, few conclusions can be drawn; it is also relevant to highlight the conclusion of a 2010 systematic review, which found no evidence of an effect for either leflunomide or cidofovir in treating this infection[87]. Adoptive cell therapy in the context of transplant-associated infections is perhaps best known in the context of EBV and post-transplant lymphoproliferative disease. Whilst no data exists for this strategy in the setting of BKV infection, it is possible this approach might hold promise. In the context of infection with the related polyoma virus, JC virus, a report describes a positive clinical response to this form of therapy in a patient following hematopoietic cell transplantation[88]. Intuitively, the same approach may be worthwhile for BKV infection.

CONCLUSION

This review focuses on the pathogenesis, risk factors, presentation and treatment of BKV infection in the setting of kidney transplantation, which remains clearly the most common scenario in which this polyoma virus

infection is encountered. Whilst important understanding has accumulated over recent years, and has certainly led to improved recognition of this infection and clinical management of patients, there is much more to be discovered and studied. We believe the most important tasks at hand are now to: (1) more accurately risk-stratify patients prior to (and also following) transplantation, with aim of individualizing immunosuppression and reducing the risk of (or duration/consequences of) BKV infection. This may include developing understanding of, and then monitoring strategies for, cell-mediated immune responses to this virus, which can then be interpreted in combination with peripheral blood and renal biopsy measures of viral load and (admittedly currently unavailable) standardised assays of alloreactivity to garner a more "holistic" understanding of the overall and antigen-specific immunosuppressive burden; and (2) to enhance the sizeable observational experience of treatment strategies with controlled studies of immunosuppression weaning and/or adjunctive antiviral agents. It is not unconceivable that with such refined approaches BKV infection (whilst not eradicated) may present a far less sinister complication for kidney transplant patients in the future.

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MINIREVIEWS

Human leukocyte antigen typing and crossmatch: A comprehensive review

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Abstract

Renal transplantation remains the best option for patients suffering from end stage renal disease (ESRD). Given the worldwide shortage of organs and growing population of patients with ESRD, those waitlisted for a transplant is ever expanding. Contemporary crossmatch methods and human leukocyte antigen (HLA) typing play a pivotal role in improving organ allocation and afford better matches to recipients. Understanding crossmatch as well as HLA typing for renal transplantation and applying it in clinical practice is the key step to achieve a successful outcome. Interpretation of crossmatch results can be quite challenging where clinicians have not had formal training in applied transplant immunology. This review aims to provide a worked example using a clinical vignette. Furthermore, each technique is discussed in detail with its pros and cons. The index case is that of a young male with ESRD secondary to Lupus nephritis. He is offered a deceased donor kidney with a 1-0-0 mismatch. His complement dependent cytotoxicity (CDC) crossmatch reported positive for B lymphocyte, but flow cytometry

crossmatch (FCXM) was reported negative for both B and T lymphocytes. Luminex-SAB (single antigen bead) did not identify any donor specific antibodies (DSA). He never had a blood transfusion. The positive CDC-crossmatch result is not concordant with DSA status. These implausible results are due to underlying lupus erythematosus, leading to false-positive B-lymphocyte crossmatch as a result of binding immune complexes to Fc-receptors. False positive report of CDC crossmatch can be caused by the underlying autoimmune diseases such as lupus erythematosus, that may lead to inadvertent refusal of adequate kidney grafts. Detailed study of DSA by molecular technique would prevent wrong exclusion of such donors. Based on these investigations this patient is deemed to have "standard immunological risk" for renal transplantation.

Key words: Human leukocyte antigen typing; Cytotoxic crossmatch; Flow cytometry crossmatch; Virtual crossmatch; Human leukocyte antigen null alleles

Core tip: Understanding crossmatch for renal transplantation and applying it in clinical practice is the fundamental step to achieve a successful outcome. At times, interpreting an ambivalent report of crossmatch can be very challenging for clinicians since they have not been trained formally in applied transplant immunology. While there are several published reviews, this is presented as a worked example and is aimed to discuss immunological risk stratification by using an example of an index case.

Althaf MM, El Kossi M, Jin JK, Sharma A, Halawa AM. Human leukocyte antigen typing and crossmatch: A comprehensive review. *World J Transplant* 2017; 7(6): 339-348 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/339.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.339

INTRODUCTION

Renal transplantation is the best option in suitable and fit patients who have end stage renal disease (ESRD) as a result of lupus nephritis. Several studies have shown that five- and ten-year allograft survival are similar to that of recipients with other causes of ESRD[1-3]. It is also worth noting that lupus nephritis can recur in the allograft. The risk of recurrence of clinically apparent disease in renal transplantation is between 2%-30% of cases[1,4,5]. Systemic Lupus Erythematosus and its complications predominantly occur in women. However, it is worth noting that clinical manifestations are slightly different in men who have poorer outcomes[6,7]. Understanding crossmatch for renal transplantation and applying it in clinical practice is the key step to achieve a successful outcome. At times, interpreting an ambivalent report of crossmatch can be very challenging for clinicians since they have not been trained formally in applied transplant immunology. The following review is aimed to discuss an immunological risk stratification by using an example of an index case.

CLINICAL VIGNETTE

We are presented with a 30-year-old male patient who had been on maintenance haemodialysis for five years. His primary disease was Systemic Lupus Erythematosus which was complicated with lupus nephritis which eventually progressed to end-stage renal disease. He was offered a kidney from a deceased donor with a 1-0-0 mismatch. His complement dependent cytotoxicity (CDC) crossmatch reported positive for B lymphocyte, and flow cytometry crossmatch (FCXM) was reported negative for both B and T lymphocytes. His Luminex-SAB did not identify any donor specific antibodies (DSA). He never had a blood transfusion.

HLA TYPING

HLA typing is a crucial step in renal transplantation, as recognition of foreign HLA by recipient T lymphocytes would trigger an immune response. T lymphocyte activation initiates a cascade of mediators that direct the immune system against the allograft[8]. HLA laboratories currently perform serologic as well as molecular typing methods.

Serological typing

In this approach, a tray containing sera with antibodies to a multitude of known HLA alleles is used. These are commercially available. For typing, recipient lymphocytes are introduced into the tray wells contacting sera, complement and dye. In tray wells where antibodies can bind to the antigens on the surface of lymphocytes; complement is activated. This results in complement pathways triggered resulting in cell death, ultimately allowing the dye to enter the cell. Tray wells with significant cell death are then identified under phase contrast microscopy. Through a process of comparison and elimination of positive wells the HLA type is assigned. The key benefit of serologic typing is that results are available in a short period. This is particularly important in deceased donor renal transplantation. Quick results mean less cold ischemia times. This method also offers the ability to differentiate HLA alleles that have identifiable DNA sequences with molecular typing but with no cell surface antigen expression. These alleles termed "null" HLA alleles are of less immunological significance[9]. The downside of this method is the lack of sera with antibody specificities that are capable of identifying the ever-growing number of HLA alleles[10]. The HLA-Cw, DQ, and DP antigen may have clinically significant effects on the outcomes of allografts. However, serologic assays are scarce for these loci. Furthermore, serologic methods do not readily detect differences in HLA protein small amino

acids. These may be antigenic enough to trigger potent immunological responses[11,12]. With more advanced methods of typing currently available serological typing has fallen into disuse.

Molecular typing

Sequence-specific primer polymerase chain reaction: In this approach extracted DNA from the subject is amplified in several wells. Each well has primers that are complementary to specific HLA alleles. In wells where DNA probes are complementary to the specific sequence of the HLA molecule, an amplification product is formed. This is then instilled into an agarose gel and undergoes electrophoresis where they appear as a band. HLA typing is then allocated by matching the primers of the amplification product to DNA sequences of several candidate alleles.

Sequence specific oligonucleotide probes: Amplified DNA is mixed with oligonucleotide probes that are complementary to specific segments of the DNA of different alleles. Unique HLA alleles are then identified using fluorescent tags. For a particular gene of interest, the precise order of nucleotides is determined through sequencing. HLA type is then assigned using available HLA allele sequences[10].

Direct DNA sequencing: This method determines the precise order of nucleotides in the gene of interest. Using published HLA allele sequences, HLA type is subsequently assigned by comparison.

Molecular typing regardless of the method can clearly identify differences in HLA antigen between donor and recipient. Often with detail to the amino acid level that can provide insight to the risk accompanying mismatched donor-recipient antigens, epitopes and amino acid[12,13]. HLA typing based on polymerase chain reaction (PCR) is highly specific where specific alleles are identified with no cross-reactivity. However, a gene may occur in two or more forms called alleles. Cross-reactivity is the identification of an allele which is essentially similar to the allele of interest. While this feature is a key advantage of this method its acts as a double-edged sword. The disadvantage it poses is that new alleles not currently on the HLA sequence databank will fail to be identified. Primers used in HLA-typing are constructed on an HLA sequence databank that contains alleles available when the databank was designed[14].

HLA typing of the donor kidney and our patient revealed a 1-0-0-mismatch that corresponds to the pair of alleles mismatched, respectively, at HLA-A, HLA-B and HLA-DR. These three antigens are the considered as the most important ones in kidney transplantation. Logically the fewer the mismatches; the better the match between donor and recipient resulting in a successful transplant outcome. The dissimilarity in the HLA antigen reflects the alloimmune burden that a donor kidney presents to the recipient. In this case, there is 1 HLA mismatch which is that of HLA-A. Mismatch for different HLA antigens does not have equal weight. We know from the initial Collaborative Transplant Study (CTS) analysis that HLA-DR and HLA-B antigens offer the most alloimmune burden with less so from HLA-A[15]. Eurotransplant and old United Kingdom transplant data suggest that HLA-DR matching has a far greater effect than HLA-A or HLA-B[16,17]. Interestingly one study demonstrated that the influence of HLA-DR mismatching had the most effect during the first six months post-transplant while the maximal effect of HLA-B mismatching occurred two years post-transplant[18]. Data from the United Network for Organ Sharing (UNOS) registry further highlighted the significance of paying attention to having the least number of mismatches. They looked at quantifying the risk of transplant failure with HLA mismatch in patients who had their first adult kidney allografts from deceased donors. This study revealed that having six HLA mismatches translated to a 64% higher risk while the risk was down to 13% with just one HLA mismatch. Furthermore, these results were independent of locus[19]. Another study identified seven specific HLA mismatch combinations that were associated with decreased renal allograft survival. These were termed "taboo mismatches". A taboo mismatch translated to 81% one-year survival and 50% five-year survival[20].

In recent times, the HLA mismatching in deceased donor kidney transplants is of lesser significance due to the use of more potent immunosuppression and better identification of non-immunological determinants of transplantation[21]. Nonetheless, HLA matching continues to have a significant impact on allograft survival.

HLA ANTIBODY SCREENING

Almost a third of patients who are waitlisted for transplantation may have a degree of anti-HLA antibodies detected. The usual route for sensitisation towards HLA antigens occurs in three instances; pregnancy, post blood transfusion and prior transplantation. Preformed antibodies increase the chances of immunological failure of the allograft by causing positive crossmatches and, thereby, result in the exclusion of donors[9]. The index patient did not have a history of prior transplantation or blood transfusions. Both sensitive and specific detection of anti-HLA antibodies is crucial. Where crossmatch is negative, even low titres of DSA can lead to early as well as late antibody mediated rejection[22,23]. For sensitised patients, successful transplantation is possible by employing strategies such as desensitisation, paired exchange and acceptable mismatching[13,24,25]. There are different methods used for HLA antibody screening as shown below.

Cytotoxic (cell-based) antibody screening

A set of cell donors are randomly selected to be representative of a population. This should be representative of the population of potential deceased donors. Each panel consists of around 30 to 40 different donor lymphocytes. The method is similar to that of serologic typing however

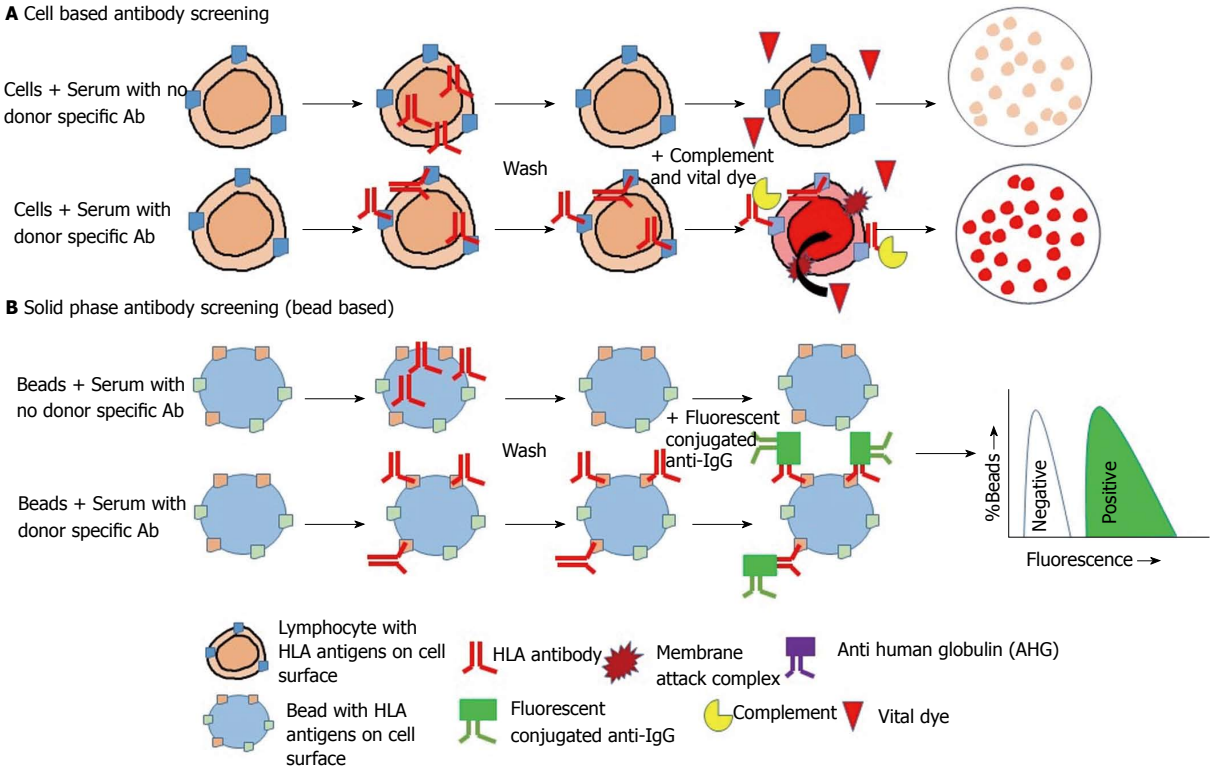

Figure 1 Schematic diagram of lymphocyte based antibody screening and solid phase (bead based) antibody screening. A: Cell based antibody screening; B: Solid phase (bead based) antibody screening.

here recipient serum is mixed with "cell donor" lymphocytes in individual wells along with complement and dye. Where the serum contains antibodies that bind to the cell surface with adequate density complement pathways are activated which results in cell death and uptake of the dye (Figure 1A). The degree of cytotoxicity is expressed as percentage PRA (panel reactive antibody). It is a tool that can be employed to approximate the risk of a given recipient of having a positive crossmatch. This is to a likely organ donor taken from a similar population.

The limitations of this method are that PRA percent can be different numerically without a corresponding change in the type or amount of antibody. This largely depends on the cell panel used which are commercially produced and may not truly represent the population. HLA frequencies and racial differences need to be factored in but cannot be done. Moreover, significant false positive results can be produced due to non-HLA antibodies, autoantibodies and nonspecific IgM antibodies. Similarly, false negative results are possible as this is purely complement dependent that requires higher antibody titres to be activated[26-28]. The lack of a complement activation simply due to low titres allows a true antibody to be hidden[29]. Precise, complete lists of antibody specificities and unacceptable antigens cannot be identified using this method as there are several antigens in each well[9].

Solid phase antibody screening

This method employs soluble or recombinant HLA molecules instead of lymphocytes targets - as lymphocytes present both HLA as well as non-HLA molecules (Figure 1B). The variants of these methods are:

Enzyme-linked immunosorbent assay platform: In this method, purified HLA molecules are applied to enzyme-linked immunosorbent assay (ELISA) platforms and will bind individually to HLA antibody after the addition of recipient serum[30,31]. Enzyme conjugated antibodies to IgG (human) is then added to detect the presence of HLA antibody in the serum which is bound to the antigen. Detection is performed by optical density reading.

Microbead platform/single-antigen beads: Pooled panel beads with several different class Ⅰ or Ⅱ HLA antigens on a bead yield a positive or negative result and are utilised for screening[32]. The phenotype or also known as ID beads are individually coated with class Ⅰ or Ⅱ HLA antigens of an individual patient-derived cell line. Microbead that is fluorescent dye conjugated is then added to detect the presence of HLA antibody in the serum which is bound to the antigen. Fluorescence detection can be done traditionally using a flow cytometer (Flow PRA®) or *via* the single-antigen beads (SAB) Luminex® platform. These estimate PRA by the proportion of positive beads. SAB are individually coated with a single HLA antigen and yield a list of distinct antibody specificities[33]. Specificities are subsequently compared with HLA frequencies in the donor population

to determine the calculated panel-reactive antibody (cPRA)[34]. This yields the best estimate of the likelihood of a positive crossmatch/donor specific antibody to a randomly selected donor[35,36].

It is important to understand the difference between PRA and cPRA. A high traditional PRA value translated to a high probability of a positive crossmatch. cPRA is based on unacceptable HLA antigens - those that the patient has been sensitized to. Furthermore, if these were present in a donor, would represent an unacceptable risk to the potential recipient or organ transplantation program. cPRA is calculated from HLA antigen frequencies among approximately twelve thousand kidney donors in the United States during the period between 2003 and 2005. This, therefore, represents the proportion of actual organ donors who express one or more of the unacceptable HLA antigens[36]. cPRA is useful in the allocation of kidney and pancreas transplants. cPRA estimates the proportion of donors with whom a particular recipient would be incompatible. An offer for a recipient with a high cPRA is a high probability of a positive crossmatch. Formerly, the same highly sensitised potential recipient would be higher on the list of each match run for donors with their blood group. Renal transplant programs were hesitant to set up final crossmatches for more highly sensitised patients for fear of not allocating the kidneys[37-39].

We are able to better discriminate immunologically relevant positive crossmatches from false-positive results when traditional cell based methods are complemented with solid phase assays[40]. Microbead assays (both Flow PRA® and Luminex®) are ten percent more sensitive for lower titre antibody than ELISA. ELISA is ten percent more sensitive compared to anti-human globulin (AHG) enhanced cytotoxicity based assays. Being in control of the antigens places on the beads, these assays are specific for anti-HLA antibodies. SAB assays are rapid with results available in 3-4 h. The assay is also quite efficient in a single reaction chamber up to one hundred unique antigen beads can be tested. Its additional multiplexing ability permits testing many patients simultaneously[41]. Results from SAB enable virtual crossmatching (VXM) to identify DSA pre-transplant, thereby enabling organ allocation and risk stratification[37]. SAB assays permit identification of anti-HLA antibodies for all common and numerous rare antigens and alleles. Its range of identification is up to eleven HLA loci[33].

Despite the fact that solid phase antibody screening addresses most of the short comings with cellular assays they have limitations as well. They detect both complement and non-complement binding simultaneously. Being too sensitive they can detect antibody that is below the threshold associated with a positive crossmatch. The detected antibody may not always have clinical implications but can preclude a potential donor. Non-HLA antibodies are also increasingly being recognised as clinically relevant predictors, and these cannot be accounted for utilising this method solely[42,43]. With the ever-growing list of HLA alleles, the complete spectrum of unique HLA antigens cannot be fully presented on solid phase assays.

The SAB - Luminex® assay has been shown to be susceptible to an artefact known as the prozone phenomenon[44]. This phenomenon is recognised when sera with high titer anti-HLA antibodies give negative results when tested neat, however, react strongly positive after 1:10 dilution[45,46]. The complement-mediated prozone effect is most likely caused by complement component 1 (C1) by competitively displacing the detection antibodies in the confined spaces between antibodies bound to HLA molecules. This in turn prevents HLA antibody binding to the HLA antigen on the bead. A similar scenario can arise with the binding of IgM antibodies or other serum factors to the beads. This can be resolved by treatment with dithiothreitol (DTT) and serum dilution. It is worth noting that nonspecific binding by serum proteins as well as drugs such as intravenous immunoglobulin (IVIG) could also interfere with the specific binding of anti-HLA antibodies to the HLA antigens on beads. Another cause for a false negative result is epitope sharing. Different HLA antigens on different beads share mutual antibody binding epitopes leading to the binding of an anti-HLA antibody to more than one bead. This leads to a reduction in the mean fluorescence intensity (MFI) on a single bead[41].

CROSSMATCHING (XM)

The cytotoxic assay was implemented as the requisite test prior to transplantation when it was shown that recipients with DSA had significantly higher rates of allograft failure due to hyperacute rejection as well as primary failure[47,48]. The presence of donor-specific cytotoxic antibodies depicted as a positive crossmatch was a contraindication to transplantation. With PRA that identifies several antibodies to a potential cluster of donors, the crossmatch will identify if a recipient had antibodies to a specific donor of interest. Despite the obvious benefits of testing the T cell cytotoxic crossmatch had a twenty percent false positive rate and a four percent false negative rate. Therefore, it is insufficient to identify all relevant antibodies, and in addition to that, it may needlessly exclude patients from transplant. The solid-phase antibody test should be used together with crossmatch results to identify those that are immunologically relevant[49].

Complement-dependent cytotoxicity crossmatch

Similar to cytotoxic assay the complement-dependent cytotoxicity crossmatch is interpreted as positive if a considerable number of lymphocytes are destroyed after the incorporation of complement (Figure 2A). This suggests that a significant DSA has been bound to the cell surface. Complement-dependent cytotoxicity crossmatch (CDC-XM) can be done for B and T lymphocytes. Sensitivity is limited if the relevant antibody is in low titres, but this can be overcome by increasing the incubation

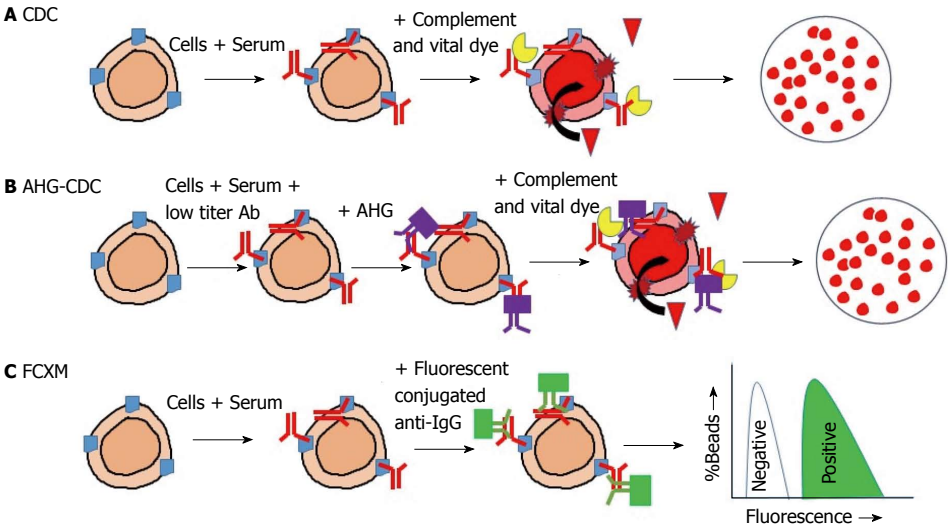

Figure 2 Schematic diagram of complement-dependent cytotoxicity crossmatch antibody to human immunoglobulin complement-dependent cytotoxicity crossmatch flow cytometry crossmatch. A: CDC; B: AHG-CDC; C: FCXM. CDC: Complement-dependent cytotoxicity; AHG: Antibody to human immunoglobulin; FCXM: Flow cytometry crossmatch.

time, the use of the AHG-enhanced method as well as additional wash steps[26,27]. The complement fixing antibody to anti-human immunoglobulin (AHG) will bind to any DSA present on lymphocytes (Figure 2B). This increases the chances of activating complement and thus raises the sensitivity of the test.

The antibodies that are present in lower titres are clinically significant as a negative test has an 18% graft loss in 1 year compared to a positive test that is associated with 36%[28]. Similar to cytotoxic PRA this method could miss low titre antibody resulting in false negatives. CDC-XM can also give false positives by detecting autoantibody, IgM/IgG HLA or non-HLA.

Flow cytometry crossmatch

Flow cytometry crossmatch (FCXM) detects DSA independent of complement fixation. It precisely detects the presence or lack of IgG DSA on donor lymphocytes. In this method, recipient serum is mixed with donor lymphocytes and then tagged with a fluorochrome-conjugated anti-IgG antibody. Several antibodies with separate fluorochromes particular to B and T lymphocyte surface proteins can be added (Figure 2C). With the use of flow-cytometry, B and T lymphocytes can be readily identified and have their DSA individually interrogated. Compared to complement-dependent cytotoxicity crossmatch this offers greater sensitivity[9]. Different laboratories use different methods, and this can result in a difference in the results between them[50]. However, this approach is not widely available, and its role in assessing immunological risk is still unclear.

Virtual crossmatching

In virtual crossmatch (VXM), both donor HLA typing and solid phase antibody screening are utilised together. It is not precisely a crossmatch in the sense of mixing serum and lymphocytes. The data is used to forecast the actual *in vitro* crossmatch results by "mixing" identified antibody specificities of recipient serum with donor HLA antigens[9]. The use of VXM can lead to shorter wait times and improved outcomes for sensitised transplant recipients. The speed of results generated allows a VXM to be performed at the time of donor identification owing to the fact that there is progressively sensitive and specific flow cytometry technology. VXM permits transplant physicians to consider donor organs that would not otherwise be available by means of a prospective crossmatch strategy, and thereby, allows to consider a potentially positive crossmatch a risk factor for donor selection[51,52].

Titres, specificities, and presence or absence of antibodies could significantly vary over time. Thus, the use of antibody specificity from historical serum sample (earlier than six months) could not predict a crossmatch with certainty. Other factors that can influence antibody specificities should be considered, and these include pregnancies, transplants and blood transfusions. The VXM should, therefore, be done considering all available serum results including at least one recent within less than 3-6 mo for a given patient. False positive results of VXM may arise where there are significantly low titre and/or non-complement binding antibodies, thereby, resulting in the wrong exclusion of potential donors[9]. The VXM can also give false negative results due to the fact that the list of all potential HLA donor antigens have been classed differently and, therefore, can not be correctly represented[53]. The results from VXM are not a hundred percent accurate and current practice mandates an actual crossmatch be performed as well[37]. Furthermore, VXM does not identify the HLA "Null" alleles. Null HLA alleles are ones have identifiable DNA sequences with molecular typing but do not express HLA products on the cell surface. In excess of 190 null alleles have been identified across HLA class Ⅰ and Ⅱ. There is a significant risk where a null allele is misidentified for its fully expressed

Table 1 Summary of the pre-transplant risk assessment of immunological challenge

Donor crossmatch result	Crossmatch method	Current or historical	Antibody screening results	Interpretation of immunological risk
Positive T and B lymphocyte	CDC (DTT)	C	IgG HLA class Ⅰ DSA	High risk[1] Hyperacute rejection (veto to transplantation)
Positive B lymphocyte	CDC (DTT)	C	IgG HLA class Ⅱ DSA	High risk[1]
Positive B lymphocyte	CDC (DTT)	C	Weak IgG HLA class I DSA	Intermediate risk[2]
Positive T and B lymphocyte	FCXM (CDC neg)	C	IgG HLA class Ⅰ DSA	Intermediate risk[2]
Positive B lymphocyte	FCXM (CDC neg)	C	IgG HLA class Ⅱ DSA	Intermediate risk[2]
Positive T and B lymphocyte	CDC (DTT)	H	IgG HLA class Ⅰ DSA	High risk[3]
Positive B cell	CDC (DTT)	H	IgG HLA class Ⅱ DSA	High risk[3]
Positive B lymphocyte	CDC (DTT)	H	Weak IgG HLA class I DSA	Intermediate risk[2]
Positive T and B lymphocyte	FCXM (CDC neg)	H	IgG HLA class Ⅰ DSA	Intermediate risk[2]
Positive B lymphocyte	FCXM (CDC neg)	H	IgG HLA class Ⅱ DSA	Intermediate risk[2]
Positive T and B lymphocyte	CDC (neg DTT)	C or H	IgM HLA class Ⅰ DSA	Standard risk
Positive B lymphocyte	CDC (neg DTT)	C or H	IgM HLA class Ⅱ DSA	Standard risk
Positive T and B lymphocyte	CDC (neg DTT)	C or H	IgM non-HLA (often autoreactive)	Standard risk
Positive B lymphocyte	CDC (neg DTT)	C or H	IgM non-HLA (often autoreactive)	Standard risk
Negative T and B lymphocyte	FCXM	C or H	IgG HLA class Ⅰ or Ⅱ DSA (detected by Luminex SAB alone)	Standard risk
Positive T and/or B lymphocyte	CDC and/or FCXM	C or H	Negative (Luminex Ab detection and/or SAB)	Standard risk (IgM/IgG non-HLA, often showing *in vitro* autoreactivity)
Positive T; Nnegative B lymphocyte	CDC and/or FCXM	C or H	Positive (Luminex SAB-not donor-specific) or negative	Standard risk (results suggest antibody is not HLA-specific)
Negative T and B lymphocyte	FCXM	C or H	Positive (Luminex SAB) not donor HLA-specific	Standard risk
Negative T and B lymphocyte	CDC and/or FCXM	C or H	Negative (Luminex Ab detection and/or SAB)	Standard risk
Donor crossmatch result	Crossmatch method	Current or historical	Antibody screening results	Interpretation of immunological risk
Positive T and B lymphocyte	CDC (DTT)	C	IgG HLA class Ⅰ DSA	High risk[1] Hyperacute rejection (veto to transplantation)
Positive B lymphocyte	CDC (DTT)	C	IgG HLA class Ⅱ DSA	High risk[1]
Positive B lymphocyte	CDC (DTT)	C	Weak IgG HLA class Ⅰ DSA	Intermediate risk[2]
Positive T and B lymphocyte	FCXM (CDC neg)	C	IgG HLA class Ⅰ DSA	Intermediate risk[2]
Positive B lymphocyte	FCXM (CDC neg)	C	IgG HLA class Ⅱ DSA	Intermediate risk[2]
Positive T and B lymphocyte	CDC (DTT)	H	IgG HLA class Ⅰ DSA	High risk[3]
Positive B lymphocyte	CDC (DTT)	H	IgG HLA class Ⅱ DSA	High risk[3]
Positive B lymphocyte	CDC (DTT)	H	Weak IgG HLA class I DSA	Intermediate risk[2]
Positive T and B lymphocyte	FCXM (CDC neg)	H	IgG HLA class Ⅰ DSA	Intermediate risk2
Positive B lymphocyte	FCXM (CDC neg)	H	IgG HLA class Ⅱ DSA	Intermediate risk2
Positive T and B lymphocyte	CDC (neg DTT)	C or H	IgM HLA class Ⅰ DSA	Standard risk
Positive B lymphocyte	CDC (neg DTT)	C or H	IgM HLA class Ⅱ DSA	Standard risk
Positive T and B lymphocyte	CDC (neg DTT)	C or H	IgM non-HLA (often autoreactive)	Standard risk
Positive B lymphocyte	CDC (neg DTT)	C or H	IgM non-HLA (often autoreactive)	Standard risk
Negative T and B lymphocyte	FCXM	C or H	IgG HLA class Ⅰ or Ⅱ DSA (detected by Luminex SAB alone)	Standard risk
Positive T and/or B lymphocyte	CDC and/or FCXM	C or H	Negative (Luminex Ab detection and/or SAB)	Standard risk (IgM/IgG non-HLA, often showing *in vitro* autoreactivity)
Positive T; negative B lymphocyte	CDC and/or FCXM	C or H	Positive (Luminex SAB-not donor-specific) or negative	Standard risk (results suggest antibody is not HLA-specific)
Negative T and B lymphocyte	FCXM	C or H	Positive (Luminex SAB) not donor HLA-specific	Standard risk
Negative T and B lymphocyte	CDC and/or FCXM	C or H	Negative (Luminex Ab detection and/or SAB)	Standard risk

[1]High immunological risk: Hyperacute rejection is unlikely (reported only in cases with very high titre HLA-DR antibodies) but donor-specific HLA class II antibodies are increasingly recognised as being associated with refractory humoral rejection and poor transplant prognosis; [2]Intermediate immunological risk: Transplantation should be avoided if reasonably possible (*i.e.*, short waiting time, easy to avoid unacceptable mismatches) but may be undertaken with appropriate clinical caution; consideration for enhanced immunosuppression, proactive use of clinical intervention strategies and post-transplant antibody monitoring; [3]Risk of anamnestic secondary T and/or B lymphocyte response: Need to consider high risk immunosuppression strategy, the duration, titre and priming source of antibody and repeat mismatches (pregnancy or regraft). Historical positive crossmatches caused by cross-reactive alloantibodies (avoiding the main specificity and priming stimulus) constitute intermediate immunological risk and are less likely to be associated with refractory T or B lymphocyte responses. CDC: Complement dependent cytotoxicity; DTT: Dithiothreitol; HLA: Human leukocyte antigen; DSA: Donor specific antibodies; FCXM: Flow cytometry crossmatch; SAB: Single-antigen beads.

counterpart in stem cell transplantation. However, the risk is slightly lower in solid organ transplantation. A recipient will have the risk of developing DSA for the mismatch where the null allele is misidentified as a fully expressed product and, therefore, transplanted with a donor bearing the expressed antigen. This mismatch is not life threatening but can affect future transplantation. In contrast, where a donor null allele is misidentified as a fully expressed product and subsequently transplanted into a recipient bearing the expressed antigen results in no humoral rejection and is well tolerated[54].

DEFINING RISK

Gebel *et al*[49] stratified the prospective renal transplant patients into various categories according to immunological risk in renal transplantations. On the basis of this with further additions the principles of risk assessment are as follows:

High immunological risk

At the time of transplantation, there are high titres of circulating antibodies specific for mismatched donor HLA (DSA). This can lead to hyperacute rejection. The presence of DSA precludes transplantation. However, there are reports of innovative pre-transplant desensitisation regimens to reduce this risk.

Intermediate immunological risk

At the time of transplantation, there is a low titer of DSA, and historic DSA is not detectable. It may be acceptable to consider intensified immunosuppression as well as immunological monitoring in the post-transplant period.

Standard immunological risk

Where there is no evidence of donor directed sensitisation to HLA. Refer to Table 1 that gives a summary of the immunological risk assessment pre-transplant based on donor crossmatch and antibody screening outcomes[55].

RISK ASSESSMENT OF OUR CLINICAL VIGNETTE

Our patient had CDC-XM reported positive for B and T lymphocytes but FCXM was reported negative for both B and T lymphocytes. His Luminex-SAB did not identify any DSA. These results can be risk stratified as "standard immunological risk", and we can proceed with transplantation. Positive CDC-XM result is not in accordance with DSA status. These implausible results are due to underlying lupus erythematosus, leading to false-positive B- lymphocyte crossmatches as a result of binding immune complexes to Fc-receptors.

CONCLUSION

Interpretation and clinical application of transplant immunology are crucial steps to a successful outcome. Understanding of crossmatch results and the caveats of individual tests can be quite challenging where clinicians have not had formal training in applied transplant immunology. This case illustrated a common scenario and detailed the approach to testing and its interpretation. If we were to rely simply on the CDC-XM, we would have made an erroneous conclusion. It is crucial to realise that false positive report of CDC-XM can be due to autoimmune diseases where type Ⅲ hypersensitivity occurs such as in Systemic Lupus Erythematosus. The false-positive B-lymphocyte crossmatch result from immune complexes binding to Fc-receptors[56,57]. Such a result may lead to inadvertent refusal of adequate kidney grafts. It has been previously reported that false positive CDC-XM could also be a result of medications such as Isoniazid and Hydralazine[58,59]. Detailed study of DSA by molecular technique would prevent erroneous exclusion of such donors. This can eventually lead to improved organ allocation and shorter waiting times in transplant lists.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Risk factors and clinical indicators for the development of biliary strictures post liver transplant: Significance of bilirubin

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Abstract

AIM

To identify risk factors associated with the formation of biliary strictures post liver transplantation over a period of 10-year in Queensland.

METHODS

Data on liver donors and recipients in Queensland between 2005 and 2014 was obtained from an electronic patient data system. In addition, intra-operative and

post-operative characteristics were collected and a logistical regression analysis was performed to evaluate their association with the development of biliary strictures.

RESULTS

Of 296 liver transplants performed, 285 (96.3%) were from brain dead donors. Biliary strictures developed in 45 (15.2%) recipients. Anastomotic stricture formation (n = 25, 48.1%) was the commonest complication, with 14 (58.3%) of these occurred within 6-mo of transplant. A percutaneous approach or endoscopic retrograde cholangiography was used to treat 17 (37.8%) patients with biliary strictures. Biliary reconstruction was initially or ultimately required in 22 (48.9%) patients. In recipients developing biliary strictures, bilirubin was significantly increased within the first post-operative week (Day 7 total bilirubin 74 μmol/L *vs* 49 μmol/L, P = 0.012). In both univariate and multivariate regression analysis, Day 7 total bilirubin > 55 μmol/L was associated with the development of biliary stricture formation. In addition, hepatic artery thrombosis and primary sclerosing cholangitis were identified as independent risk factors.

CONCLUSION

In addition to known risk factors, bilirubin levels in the early post-operative period could be used as a clinical indicator for biliary stricture formation.

Key words: Biliary stricture; Liver transplantation; Bilirubin; Anastomotic stricture; Ischemic type biliary lesion; Magnetic resonance cholangiopancreatography

Core tip: Biliary stricture formation post liver transplantation is a frequent cause for patient morbidity and mortality and is referred to as the Achilles' Heel of transplant. Strictures can be anastomotic or non-anastomotic depending on their number and anatomical location. Early stricture identification is key to providing successful treatment options. Known risk factors for biliary stricture formation include surgical technique, bile leak, hepatic artery thrombosis, primary sclerosing cholangitis, donation after circulatory death donors and increased cold ischemic time. This study identifies risk factors and clinical indicators for the development of biliary strictures post liver transplantation. It also discusses the importance of bilirubin and its potential role when implementing surveillance tools for biliary stricture formation post-transplant.

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INTRODUCTION

Orthotopic liver transplantation is currently the gold-standard treatment for patients with end-stage liver disease[1,2]. Post-operative biliary complications, in particular biliary stricture formation, are a frequent cause for patient morbidity and mortality and is often referred to as the Achilles' heel of liver transplantation. Hospital re-admissions and clinical interventions used to treat biliary complications post-transplant are also a significant cost to health systems[3]. Despite advances in treatment techniques for biliary strictures post liver transplant, including non-surgical methods, formation is still observed in approximately 5%-32% of recipients[2,4]. Biliary tract complications post liver transplant include anastomotic strictures (AS), non-anastomotic strictures (NAS), bile leaks, stone formation, sludge and sphincter of Oddi dysfunction. It is important to note however, that biliary complications are often sub-clinical and studies have showed approximately 19% of the total number are clinically relevant[4].

Usually manifesting 5-8 mo post-transplant, AS occur when there is a narrowing of the anastomosis between the donor and the recipient bile ducts[4,5]. With a reported incidence of 4%-9%, the development of AS are thought to be associated with biliary ischemia, provoking a localized fibrotic response[5]. In symptomatic patients, treatment options include balloon dilatation or stenting using endoscopic retrograde cholangiography (ERCP) or placement of a biliary drain using percutaneous trans-hepatic cholangiography (PTC). In 16%-32% of patients, surgical interventions including re-operation of the biliary anastomosis or re-transplant are used[6,7]. Published literature indicates that risk factors for AS formation are mainly due to suboptimal surgical technique or the presence of a bile leak in the post-operative period[8].

In contrast, NAS are a narrowing of the biliary duct system at any site outside of the biliary tree and proximal to the biliary anastomosis, this can be both extra-hepatic and intra-hepatic. The pathophysiology remains largely unknown, however, fibrosis following injury to the biliary epithelium is the proposed pathological process for the development of NAS. Macroangiopathy and microangiopathy are two proposed etiologies of NAS. Those NAS occurring within the first year of transplant are thought to be associated with hepatic artery thrombosis (HAT), those that occur without HAT are often referred to as ischemic type biliary lesions. The incidence of NAS is varied, with 1%-20% incidence reported in the literature[9]. NAS are complex due to their location, often occurring in multiples and are longer in length. Due to complicated management issues, morbidity and mortality related to NAS is higher compared with AS[10]. Known risk factors for NAS formation include hepatic artery thrombosis, chronic ductopenic rejection, ABO incompatibility, primary sclerosing cholangitis (primary pathology), donation after circulatory death donors (DCD), prolonged use of vasopressors, older age of

donor, preservation injury and prolonged cold and warm ischemia times[5,11]. Endoscopic treatment methods, including ERCP with balloon dilatation and stenting, are also used to treat NAS, however, patients often require multiple treatments with a reported 50%-75% success rate[5,12]. Secondary graft loss is common with up to 50% of patients experiencing graft loss and either requiring re-transplant or succumb to their illness whilst waiting for a life-saving re-transplant[13-15].

The aim of this study was to identify risk factors and clinical indicators associated with the formation of biliary strictures post orthotopic liver transplantation in the state of Queensland, Australia over a 10-year period. In addition to this, the study aimed to investigate potential post-transplant surveillance methods that could be used to identify patients at risk of biliary stricture formation.

MATERIALS AND METHODS

Study population

We retrospectively analyzed all adult liver transplant recipients in the state of Queensland, Australia between 1st January 2005 and 31st December 2014 with studied follow up until 30th June 2015. Transplants analyzed consisted of varied graft types, including whole liver, right split and heart-liver-lung (HLL). HLL graft types were excluded from this study as these transplants were performed and followed up at a different transplant center within the state of Queensland (n = 5). Of the transplants studied, 25 were repeat liver transplants. De-identified donor and recipient data was collected from internal hospital records. The study protocol was approved by the Human Research Ethics Committee for the state of Queensland, Australia (HREC/13/QPAH/382) as well as the University of Queensland Ethics Committee (2015001248). In addition, approval was obtained from the Queensland Government to access confidential patient information, held by Queensland Health, for the Purpose of Research under the provision of section 280 of the Public Health Act 2005.

Organ retrieval process

To prevent coagulopathy, the organ retrieval process routinely involved a 25000 IU flush of Heparin into the donor. The dopamine antagonist Chlorpromazine was used in addition to Heparin as per the discretion of the retrieval surgeon. Rapid cooling of organs was achieved by the instigation of a 2 L cold saline flush, followed by University of Wisconsin cold storage solution (UW Solution) through aortic and portal vein cannulas. Organs were transported in static cold storage prior to transplantation.

Transplant procedure and post-operative care

Orthotopic transplants at our center was exclusively performed using the piggyback technique. Right split grafts were transplanted using either the piggyback or venovenous bypass technique if indicated. A 1 L saline flush of the liver was infused during the inferior vena cava (IVC) anastomosis. Prior to reperfusion, 500 mL of blood was vented from the IVC. Hepatic artery anastomosis was performed after reperfusion had occurred. Right split liver transplant was performed using the piggy back. Venous-arterial extension grafts were used when required for both whole and right split liver transplantation. The biliary anastomosis was performed using one of two methods, Roux-en-Y hepaticojejunostomy or end-to-end choledochocholedochostomy. Use of each method was based on consideration of the patient's past medical history and surgeon preference. Early in our cohort, T-tubes were routinely inserted to drain bile in transplant recipients, however, these were later replaced by silicon stents, used at the surgeons' discretion. The macrolide calcineurin inhibitor, Tacrolimus, was used in conjunction with oral corticosteroids and Azathioprine post-transplant. Tacrolimus dose was titrated based on blood levels, a therapeutic level of > 8 but < 10 μg/L was considered optimal. Patients were initially followed up daily in an outpatient clinic post hospital inpatient discharge, following this twice weekly if three week's post-transplant, weekly if two months post-transplant, monthly if three months post-transplant and finally with third monthly blood tests if 12-mo post-transplant.

Data collection

Donor and recipient demographic data associated with the formation of biliary strictures was collected for this study. For recipients, this included age, gender, body mass index, reason for transplant, previous transplant and follow-up period. Donor demographic data included age, gender, body mass index, cause of death, donor type and cause of death. In addition to these parameters, intraoperative data was collected, including cold ischemic time (CIT), warm ischemic time (WIT), hepatic artery warm ischemic time, time of portal vein anastomosis, type of biliary anastomosis performed and the use of *T*-drains. Post-operative Day 0 to Day 7 liver functions tests, including total bilirubin were also collected.

Complications, treatments and outcomes

Biliary stricture formation and the time frame that this occurred post-transplant were classified into the following categories; anastomotic stricture, ischemic type biliary stricture (ITBS) and recurrence of primary sclerosing cholangitis. For continuity of diagnosis, biliary stricture identification was made by an experienced transplant surgeon (JF) in our center using patient records and visualization of radiological imaging and reports. A stricture was defined as a narrowing of the bile duct with dilatation of the proximal biliary duct. No strict diameter cut-offs were used to define the structure. Routine post-operative magnetic resonance cholangiopancreatography (MRCP) was not performed at our center. Instead, imaging is guided by patient symptomatology. For the purposes of this study, a

Table 1 Baseline donor and recipient characteristics *n* (%)

Characteristics	Overall (*n* = 296)	Biliary strictures (*n* = 45)	Nil biliary strictures (*n* = 251)	*P* value
Donor characteristics				
Age (yr)	42 (28-54)	48 (40-57)	42 (27-54)	NS
Gender (male)	165 (55.7)	25 (55.6)	140 (55.8)	NS
BMI (kg/m^2)				NS
< 18.5	5 (1.7)	0 (0.0)	5 (2.0)	
18.5-24.9	137 (46.3)	18 (40.0)	119 (47.4)	
25-29.9	117 (39.5)	20 (44.4)	97 (38.6)	
> 30	37 (12.5)	7 (15.5)	30 (12.0)	
Donor type				NS
Donation after brain death	285 (96.3)	44 (97.8)	241 (96.0)	
Donation after circulatory death	11 (3.7)	1 (2.2)	10 (4.0)	
Cause of death				NS
Stroke	154 (52.0)	29 (64.4)	125 (49.8)	
Hypoxia	43 (14.5)	6 (13.3)	37 (14.7)	
Accident	37 (12.5)	3 (6.7)	34 (13.5)	
Other	62 (20.9)	7 (15.6)	55 (21.9)	
Recipient characteristics				
Age at transplant (yr)	52 (45-57)	53 (40-58)	52 (45-57)	NS
Gender (male)	207 (69.9)	31 (68.8)	176 (70.1)	NS
BMI (kg/m^2) (*n* = 267)	267	44	223	NS
Underweight (≤ 18.5)	5 (1.9)	0 (0.0)	5 (2.0)	
Normal weight (18.5-24.9)	94 (35.2)	17 (38.6)	77 (30.7)	
Overweight (25-29.9)	93 (34.8)	15 (34.1)	78 (31.1)	
Obese ≥ 30	75 (28.1)	12 (27.3)	63 (25.1)	
Reason for transplant				NS
Viral hepatitis (B, C) ± hepatocellular carcinoma	124 (41.9)	19 (42.2)	105 (41.8)	
Hepatocellular carcinoma without hepatitis	22 (7.4)	1 (2.2)	21 (8.4)	
Alcohol	33 (11.1)	6 (13.3)	27 (10.8)	
Biliary[1]	29 (9.8)	8 (17.8)	21 (8.4)	
Non-alcoholic steatohepatitis	14 (4.7)	3 (6.7)	11 (4.4)	
Acute/fulminant liver failure	12 (4.1)	2 (4.4)	10 (4.0)	
Complications first transplant	21 (7.1)	3 (6.7)	18 (7.2)	
Other	41 (13.8)	3 (6.7)	38 (15.1)	
Previous transplant	25 (8.5)	5 (11.1)	20 (8.0)	NS

[1]Biliary - Primary sclerosing cholangitis, primary biliary cirrhosis. BMI: Body mass index; NS: Not significant.

biliary stricture was considered a true stricture only in those requiring intervention. The requirement for post-operative ERCP and percutaneous drainage was recorded. In addition to this, we examined the number of patients who required re-transplantation and those who ultimately died as a result of biliary complications. The incidence of hepatic artery thrombosis was also established.

Statistical analysis

All continuous variables are expressed as median (interquartile range) and all categorical variables as frequency (percentage)[16]. Multivariable logistical regression analysis was performed to determine the risk factors for biliary strictures following transplantation[17]. All factors with a *P*-value of 0.1 or less in univariable regression were included in the model. A *P*-value of < 0.05 was considered statistically significant. Statistical analysis was performed using IMB SPSS Statistics for Macintosh, Version 23.0 (IBM Corp. IMB SPSS statistics, Armonk, NY). No external statistical review was obtained.

RESULTS

Population characteristics

Demographic data on donors and recipients are shown in Table 1. Between 1st January 2005 and 31st December 2014, a total of 296 patients underwent liver transplantation (Table 1). The average age of liver donors that developed biliary complications was higher than those that did not (48 and 42 years, *P* = 0.10). Most donors either had a normal BMI or were overweight, 137 (46.3%) and 117 (39.5%) respectively. Of the 11 DCD donors in the cohort, the majority did not develop biliary strictures (*n* = 10). Stroke was the leading cause of death in both groups, but was more substantial in the biliary stricture group (64.4% *vs* 49.8%, *P* = 0.28).

Liver transplant recipients had a median age of 52 years, the majority were male. Overweight or obese patients accounted for 93 (34.8%) and 75 (28.1%) of the recipient population respectively. The indication for transplantation did not differ between the two groups, with viral hepatitis B, C with or without hepatocellular carcinoma accounting for approximately

Table 2 Transplant procedure characteristics *n* (%)

Characteristic	Total (*n* = 296)	Biliary strictures (*n* = 52)	Nil biliary strictures (*n* = 244)	*P* value
Cold ischemic time (min)	415 (308-520)	414 (319-530)	415 (307-520)	NS
Warm ischemic time (min)	27 (23-32)	28 (23-33)	27 (23-32)	NS
Time until hepatic artery anastomosis (min)	74 (61-88)	70 (60-86)	74 (61-89)	NS
Time between portal vein and hepatic artery anastomosis (min)	47 (35-60)	41 (36-57)	48 (35-60)	NS
Anastomosis used			246	NS
Duct to duct	213 (72.0)	29 (64.4)	184 (73.3)	
Roux-en-Y	78 (26.4)	16 (35.6)	62 (24.7)	
T-drain used (*n* = 231)	23 (7.8)	3 (6.7)	19 (8.0)	NS

NS: Not significant.

Table 3 Interventions required per type of biliary stricture *n* (%)

Complication	Total frequency (*n* = 45)[1]	Requiring intervention (ERCP/PTC) (*n* = 29)[1]	Reoperation of biliary anastomosis (*n* = 22)[1]	Retransplant (*n* = 12)[1]
Anastomotic stricture	25 (55.6)	17 (58.6)	15 (68.2)	2 (16.7)
< 6 mo	15 (33.3)	10 (34.4)	8 (36.4)	1 (8.3)
> 6 mo	10 (22.2)	7 (24.1)	7 (31.8)	1 (8.3)
Right split graft	3 (6.7)	1 (3.4)	2 (9.1)	1 (8.3)
Ischemic type biliary stricture[2]	10 (22.2)	7 (24.1)	6 (27.3)	6 (50.0)
< 6 mo	8 (17.8)	5 (17.2)	5 (22.7)	5 (41.7)
> 6 mo	2 (4.4)	2 (6.9)	1 (4.5)	1 (8.3)
DCD donor	1 (2.2)	1 (3.4)	1 (4.5)	1 (8.3)
Right split graft	3 (6.7)	2 (6.9)	1 (4.5)	1 (8.3)
PSC recurrence	8 (17.8)	3 (10.3)	2 (9.1)	1 (8.3)
Ischemic cholangiopathy due to HAT	3 (6.7)	3 (10.3)	0 (0.0)	3 (25.0)
Total patients[1]	46 (100.0)	0 (100.0)[3]	23 (100.0)	12 (100.0)

[1]One patient had two complications; [2]Excluding hepatic artery thrombosis, including primary sclerosing cholangitis and ischemic type biliary stricture with anastomotic stricture; [3]Some patients were represented more than once as they underwent two interventions for biliary stricture formation. PSC: Primary sclerosing cholangitis; HAT: Hepatic artery thrombosis; DCD: Donation after circulatory death; ERCP: Endoscopic retrograde cholangiography; PTC: Percutaneous trans-hepatic cholangiography.

40% of transplants. Recipients demographic data overall did not differ when comparing those with or without biliary complications. Of the parameters analyzed for this study, 98.5% of the data was available.

Transplant procedure characteristics

Table 2 presents the data on the transplant procedure characteristics, comparing those with and without biliary complications. Overall, no significant difference were found between the two groups.

Biliary stricture formation and treatment

A total of 45 (15.2%) recipients developed biliary strictures throughout the study period. One patient developed two complications. Anastomotic stricture formation was the commonest complication with 15 (33.3%) of these occurring within 6-mo of transplantation (Table 3). Anastomotic stricture formation was the leading cause for intervention with ERCP/PTC and reoperation of the biliary anastomosis (17, 58.6% and 15, 68.2% respectively) (Table 3). The development of ITBS accounted for 22.2% of all biliary stricture formation. ITBS were the primary indication with five (41.7%) patients undergoing an additional transplant within the first six-months of initial graft placement. Some patients were represented more than once as they underwent two interventions for biliary stricture formation. Serum Tacrolimus levels on post-operative Days 1-7 were found not to be significantly associated with the development of biliary strictures.

Risk factors for biliary stricture formation

In recipients developing biliary strictures, total bilirubin was significantly increased within the first post-operative week (Day 7 total bilirubin 74 μmol/L *vs* 49 μmol/L, P = 0.012) (Figure 1). In both univariate and multivariate regression analysis, Day 7 total bilirubin > 55 μmol/L was associated with the development of biliary stricture formation (Table 4) with an odds ratio of 2.54 (1.22-5.29), P = 0.013. In addition, hepatic artery thrombosis and primary sclerosing cholangitis were identified as independent risk factors for biliary stricture formation (OR = 25.23, $P \leqslant 0.001$ and OR = 3.10, P = 0.028, respectively). The sensitivity and specificity

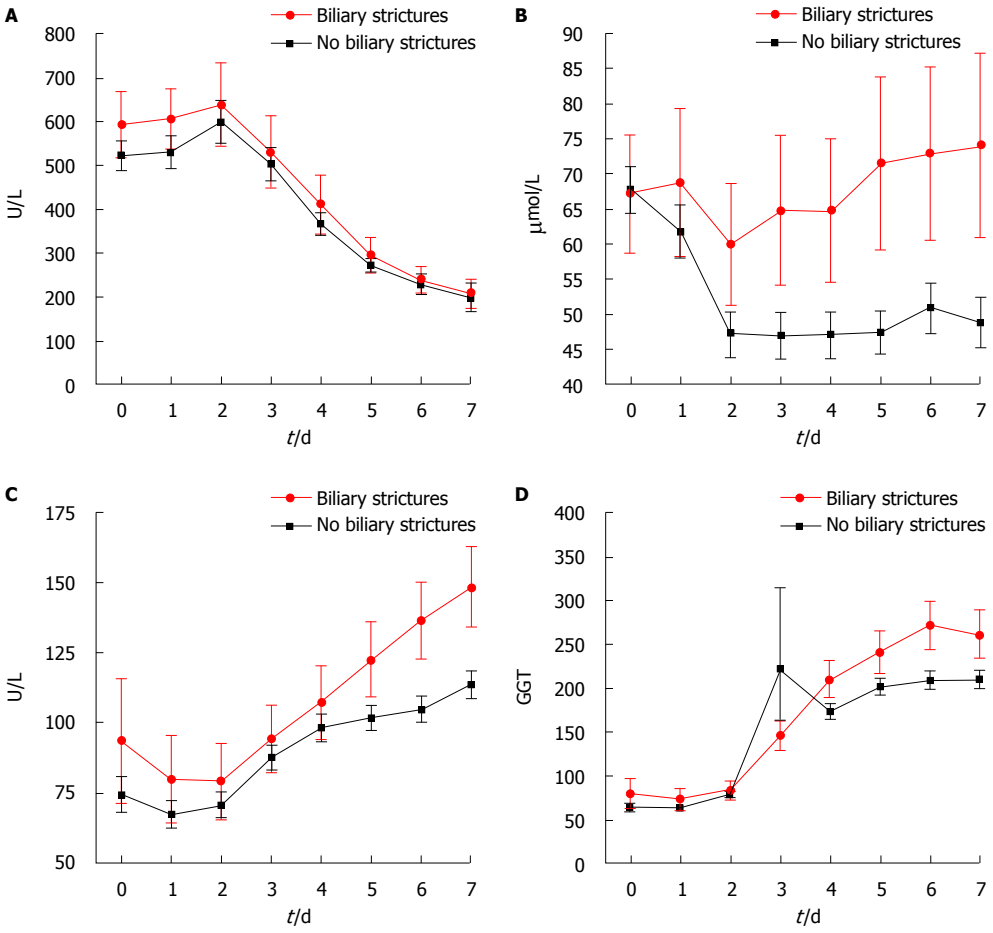

Figure 1 Post-transplant Days 0-7 liver function tests and serum total bilirubin. This graph demonstrates the trends in A: Serum alanine transaminase; B: Serum total bilirubin; C: Serum alkaline phosphatase; D: Serum γ-Glutamyl transferase (biliary strictures: Day 7 total bilirubin 74 μmol/L and nil biliary stricture: 49 μmol/L, $P = 0.012$) in recipients with biliary strictures (red line) and recipients without biliary strictures (black line) over post liver transplant Day 0-7.

Table 4 Risk factors associated with biliary stricture formation

Characteristic	Univariate regression		Multivariate regression	
	Odds ratio (95%CI)	*P* value	Odds ratio (95%CI)	*P* value
Biliary strictures ($n = 45$)[1]				
Donor age > 55 yr	1.54 (0.80-3.00)	NS		
Cause of death - stroke	1.88 (1.00-3.52)	0.049	1.49 (0.72-3.08)	NS
Donation after circulatory death	0.55 (0.07-4.39)	NS		
Split *vs* whole graft	1.09 (0.43-2.79)	NS		
Primary sclerosing cholangitis as primary indication for transplant	2.40 (0.82-7.07)	0.11	3.10 (1.13-8.51)	0.028
Hepatic artery thrombosis	22.93 (4.59-114.52)	< 0.001	25.23 (4.73-143.64)	< 0.001
⩾ 2 prior transplants	1.44 (0.51-4.07)	NS		
Day 7 total bilirubin > 55 μmol/L	2.19 (1.11-4.30)	0.024	2.54 (1.22-5.29)	0.013

[1]Inclusive of anastomotic, ischemic type biliary strictures, primary sclerosing cholangitis and ischemic cholangiopathy due to hepatic artery thrombosis. NS: Not significant.

for Day 7 total bilirubin > 55 μmol/L was 38.6% and 77.6%, respectively.

DISCUSSION

In this study, we analyzed the risk factors associated with biliary stricture formation post liver transplant in an Australian cohort. Total bilirubin in the direct post-operative period was found to be associated with the development of biliary strictures, especially at post-operative Day 7.

The development of post-operative biliary strictures is still regarded as the Achilles' heel of liver transplantation, causing significant patient morbidity and mortality.

This study supports this statement, as it demonstrated an overall incidence of biliary stricture formation post deceased liver donor transplantation in an Australian center at 15%. This is comparable to other reported literature globally, with a 5%-15% incidence reported in deceased donors and a 28%-32% incidence reported in living liver donors[18]. These comparable incidence rates of biliary stricture formation are of interest, as unlike other overseas hospitals, our center does not routinely screen for biliary stricture formation with modalities such as MRCP. Instead, regular ultrasounds are used in the immediate inpatient setting. After this period, our center only images patients that are symptomatic or in those in which a stricture is suspected.

It is difficult to predict the formation of biliary strictures in adult patients post liver transplant. MRCP is a non-invasive technique that enables detailed visualization of the biliary tree, however is a limited and expensive resource[19]. Through analyzing post-operative recipient blood work, total bilirubin was found to be elevated in those that developed biliary strictures, in particular, a total bilirubin level > 55 μmol/L on post-operative Day 7. These findings suggest that total bilirubin levels could be used as an inexpensive tool to identify those patients more at risk of biliary stricture formation and these patients could potentially benefit from a surveillance MRCP.

It is important to note that bilirubin has previously been recognized as a significant marker in the identification of liver graft dysfunction and graft survival. This is demonstrated in the currently accepted definition of early allograft dysfunction which includes one or more of the following variables: (1) total bilirubin ⩾ 171 μmol/L on post-operative day 7; (2) INR ⩾ 1.6 on post-operative Day 7; and (3) an aminotransferase level (ALT or AST) ⩾ 2000 IU/mL within the first seven post-operative days[20]. To support this, a study conducted by Wagener *et al*[21] concluded that elevated total bilirubin levels on post-operative Day 0-7 significantly correlated with graft dysfunction within the first 90 day post-operatively. This study went on further to report post-operative Days 1-2 bilirubin > 112 μmol/L should warn clinicians of potential EAD[21]. On the other hand, Olthoff *et al*[20] contradicts this statement suggesting that elevated total bilirubin levels on post-operative Days 1-3 should be excluded when predicting EAD as these values may reflect the pre-transplant status and not graft functionality.

The underlying mechanism of the association between elevated total bilirubin levels at Day 7 post-transplantation and biliary stricture formation remains to be determined. Previous studies have identified a more toxic bile composition in recipients developing non-anastomotic biliary strictures[22,23]. Furthermore, prolonged graft ischaemia was found to cause an unparalleled impairment of bile acid transporter expression in cholangiocytes leading to prolonged biliary transit time of bile acids inducing apoptosis[24,25]. Although not formally assessed in this study, increased total bilirubin levels at Day 7 post transplantation might be the results of impaired bile transporter function post transplantation and associated increased in bile toxicity resulting in stricture formation.

Currently, the literature reports biliary stricture formation presents as a later complication, between 5-8 mo post-transplant[18]. This is dependent on the type of stricture that has formed, with non-anastomotic stricture presenting between 3.3-5.9 mo[26,27]. Our study demonstrated AS formation as the most common complication (55.6%), with 33.3% of these forming within the first six-months post-transplant. Therefore, the proposed surveillance MRCP should be completed within the first three to six-months post-transplant.

Risk factors for biliary stricture formation have been well documented in the literature and are multifactorial including factors related to the recipient, donor and operative characteristics[5,11,28]. In line with previous findings, the results of our study identified stroke as donor cause of death, hepatic artery thrombosis (OR = 22.93) and Day 7 total bilirubin > 55 μmol/L as significant risk factors for biliary stricture formation on univariate regression. Upon multivariate analysis, PSC as primary indication for transplantation, HAT and Day 7 total bilirubin > 55 μmol/L were all significant risk factors. Specific donor characteristics, such as increased donor age (> 55 years) and DCD donor type were not found not to be a significant risk factor in the formation of biliary strictures in this study which could be due to our small cohort size and relative underrepresentation of DCD donor grafts.

In our cohort, we had a low percentage of ITBS compared to previous reports in the literature[29]. It was found that radiologically, it is difficult to distinguish between the development of PSC recurrence and ITBS formation[29]. For the purposes of this study, the investigator classified these questionable lesions as PSC recurrence, this in turn could have underestimated the presence of ITBS on our data set. Another point to note is that as DCD donation is infrequently used in our transplant center and therefore we were unable to assess this as a risk factor for stricture formation. Previously, the risk of biliary complications and ischemic cholangiopathy has been found to be significantly increased in DCD donors by Foley *et al*[19]. As immunological factors have been associated with biliary stricture formation, post-operative Days 1-7 serum Tacrolimus levels were measured by found not to be significantly associated with the development of biliary strictures.

Currently, there is no clear consensus as to which anastomotic reconstruction technique (duct-to-duct *vs* Roux-en-Y) of the biliary system is superior regarding biliary stricture formation. It is important to note that the surgical technique used if often dependent on the indication for transplant (*e.g.,* PSC with previous diseased bile duct) or split liver graft and is usually weighed up against the need to restore original anatomy[11]. In saying this, a running suture, without a bile tube has been proven to be of benefit in preventing early

biliary complications[30]. Although a greater percentage of patients that underwent Roux-en-Y anastomosis developed biliary complications compared to those in the end to end anastomosis group, Roux-en-Y was not found to be a significant risk factor for biliary stricture formation. Our study demonstrated a higher incidence of biliary stricture formation in the Roux-en-Y technique, with this being the less common method used at our center (26.4%). T-tube use was not associated with biliary stricture formation.

In addition, recipient WIT has been identified as a risk factor for non-anastomotic biliary stricture formation[31]. It has previously been found that there is a 2.64 ($P \leqslant 0.01$) relative risk of developing non-anastomotic biliary strictures with every hour increase of WIT[14]. Our study did not identify CIT or WIT as a risk factor for biliary stricture formation, with the average CIT and WIT being comparable. Again, the smaller cohort represented in our study may have accounted for this finding.

Our study was limited by the fact that it consisted of a relatively small cohort and because data was collected retrospectively collected and some cases were not documented appropriately or contained missing data. Overall 98.5% of parameters included in the total dataset were available for analysis. Furthermore, due to the limited number of patients in our cohort that received right split grafts (n = 37, 12.5%), we were unable to draw comparisons between whole and split liver grafts on risk of biliary stricture formation. Wan *et al*[32] demonstrated an OR = 0.64 favoring right split grafts compared to whole grafts in the formation of biliary strictures post liver transplant in adults. Similarly, only 11 patients received a DCD liver graft in out cohort. In conclusion, the incidence of biliary stricture formation post liver transplant in our center was 15%. Serum total bilirubin levels > 55 μmol/L at Day 7 post-operatively were associated with an increased risk of stricture formation, suggesting that bilirubin could be used to identify those that need closer surveillance following liver transplantation.

ARTICLE HIGHLIGHTS

Research background

Liver transplantation is a lifesaving surgical procedure available to those eligible with end-stage liver failure. Biliary strictures can cause a disruption in the flow of bile and formation post liver transplantation is a frequent cause for patient morbidity and mortality. Due to the significant burden of disease biliary strictures cause, those patients with biliary strictures often require either endoscopic intervention, surgical re-do of the anastomosis or even re-transplantation.

Research motivation

Biliary strictures post liver transplantation can be classified into two categories, non-anastomotic stricture and anastomotic stricture. Non-anastomotic strictures are often difficult to treat. They are associated with worse outcomes as they often present in numbers and are situated anatomically in a difficult to access location outside the biliary tree. Earlier identification and subsequent treatment of biliary strictures post liver transplant have been associated with improved patient outcomes and decrease the need for re-transplant. Current identified risk factors for biliary stricture formation post liver transplant include sub-optimal surgical technique, the presence of bile leak, hepatic artery thrombosis, primary sclerosing cholangitis, donation after circulatory death donors and prolonged cold or warm ischemic time. Identifying risk factors and clinical indicators for the development of biliary strictures would allow clinicians to identify at risk patients and potentially predict stricture formation. This would allow for earlier treatment of strictures, improving clinical patient care and allograft survival.

Research objectives

This study investigates the risk factors and clinical indicators associated with biliary stricture formation post liver transplantation. In order to translate these findings clinically, this study also aimed to describe potential surveillances method for biliary strictures formation post liver transplantation. These clinical tools would allow for the early identification and treatment of biliary strictures, with the aim of improving patient outcomes.

Research methods

Electronic data for this study was collected retrospectively on all liver donors and recipients in the state of Queensland between 2005 and 2014. Within this data set we analyzed demographic, intra-operative and post-operative characteristics of each procedure. In addition, post-operative liver function tests, serum bilirubin and Tacrolimus levels were collected from post-operative Days 0 to 7. Biliary stricture formation post-operatively was recorded, the interventions used to treat and their timing was also identified. This study was unique in that is used logistical regression to identify potential risk factors and clinical indicators for biliary stricture formation.

Research results

This study demonstrated the incidence of biliary strictures post liver transplantation at our center at 15%. Significant risk factors for the formation of biliary strictures post-transplant included primary sclerosing cholangitis as the primary indication for transplant and the presence of hepatic artery thrombosis. As a clinical indicator, Day 7 total serum bilirubin > 55 μmol/L was found to be associated with an increased risk of stricture formation. Investigation into potential mechanisms explaining this rise in bilirubin in patients with strictures would be beneficial.

Research conclusions

As well as known risk factors for biliary stricture formation, this study identified Day 7 total serum bilirubin > 55 μmol/L as a significant clinical indicator for the development of biliary strictures post liver transplant. As biliary strictures pose a significant burden of morbidity and mortality on patients post liver transplantation, identifying clinical indicators such as elevated total serum bilirubin for stricture formation is a useful tool to enable clinicians to provide early and more successful care to those transplant recipients more at risk. This study identified previously known risk factors for biliary stricture formation post transplantation including primary sclerosing cholangitis are the primary indication for transplant and the presence of hepatic artery thrombosis. Previous studies have identified elevated bilirubin in the post-operative period as a risk factor for biliary stricture formation. This study adds to this body of evidence as it proposes a specific measure of total serum bilirubin (> 55 μmol/L) that is associated with biliary stricture formation post liver transplant. The results of this study can be translated into clinical practice by applying a clinical algorithm to patients that are considered at higher risk of biliary stricture formation post-transplant. The authors suggest focused surveillance of these patients for biliary stricture formation within the immediate three to six-month post-operative period with a magnetic resonance cholangiopancreatography scan.

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CASE REPORT

Mucocele mimicking a gallbladder in a transplanted liver: A case report and review of the literature

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Abstract

Biliary mucoceles after deceased donor liver transplantation are a rarity, and mucoceles mimicking a gallbladder from the recipient remnant cystic duct have not been described until this case. We describe a 48-year-old male who presented with right upper quadrant pain and was found to have a recipient cystic duct mucocele 3 mo after receiving a deceased donor liver transplant. We describe the clinical presentation, laboratory and imaging findings (including the appearance of a gallbladder), multidisciplinary approach and surgical resolution of this mucocele originating from the recipient cystic duct, and a review of the literature.

Key words: Liver; Transplantation; Mucocele; Complications post-transplant

Core tip: Biliary mucoceles after deceased donor liver transplantation are a rarity, and mucoceles mimicking a gallbladder from the recipient remnant cystic duct have not been described until this case. We describe a 48-year-old male who presented with right upper quadrant pain and was found to have a recipient cystic

duct mucocele 3 mo after receiving a deceased donor liver transplant. We describe the clinical presentation, laboratory and imaging findings (including the appearance of a gallbladder), multidisciplinary approach and surgical resolution of this mucocele originating from the recipient cystic duct, and a review of the literature.

Chaly T, Campsen J, O'Hara R, Hardman R, Gallegos-Orozco JF, Thiesset H, Kim RD. Mucocele mimicking a gallbladder in a transplanted liver: A Case report and review of the literature. *World J Transplant* 2017; 7(6): 359-363 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/359.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.359

INTRODUCTION

Orthotopic deceased donor liver transplantation has become the standard of care for patients with end stage liver disease secondary to hepatitis C virus (HCV) and hepatocellular cancer (HCC). The standard biliary anastomosis is a duct-to-duct anastomosis performed in either a running or interrupted fashion using absorbable suture. In addition, a donor cholecystectomy is routinely performed after reperfusion and in instances of a long recipient cystic duct, this duct is either opened or over sewn. We present a case of a 48-year-old male who underwent an uncomplicated deceased donor liver transplant and was found to have a mucocele which mimicked a gallbladder 3 mo post-operatively. A biliary mucocele is a complication after deceased donor liver transplantation that has not been well described in the literature. Furthermore, mucoceles from the recipient *vs* donor remnant cystic duct have not yet been described. Such a biliary abnormality can be difficult for the surgeon to both diagnose and treat. It can be extremely difficult to make the diagnosis of a recipient duct *vs* a donor duct mucocele preoperatively even with excellent imaging.

CASE REPROT

A 48-year-old man with end stage liver disease secondary to HCV and HCC received a liver transplant from a deceased donor, and per routine, a graft cholecystectomy was performed and confirmed by pathology. The donor cystic duct was ligated with a 2-0 silk tie, and the recipient cystic duct was over sewn with a 4-0 proline suture. The patient's post-operative course was unremarkable, and the patient was subsequently discharged on post-operative day 8. The patient did well in the weeks after the transplant with his liver functions tests (LFTs) indicating excellent graft function.

Three months after the transplant, the patient complained of right upper quadrant pain. He was found to have abnormal LFTs, specifically an elevated gamma-glutamyltransferase (GGT), alkaline phosphatase (alk phos), and total bilirubin (T.bili). A liver biopsy showed no signs of rejection or recurrent HCV. A magnetic resonance cholangiopancreatography (MRCP) showed an anastomotic biliary stricture. An endoscopic retrograde cholangiopancreatography (ERCP) demonstrated an anastomotic stricture, a covered stent was placed, and the biliary duct then displayed free flowing contrast through the biliary system (Figure 1). The alk phos, GGT and T.bili normalized.

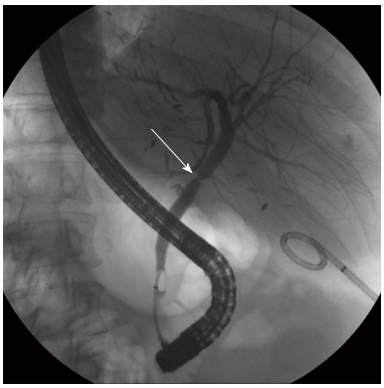

Figure 1 Endoscopic retrograde cholangiopancreatography which showed biliary stricture.

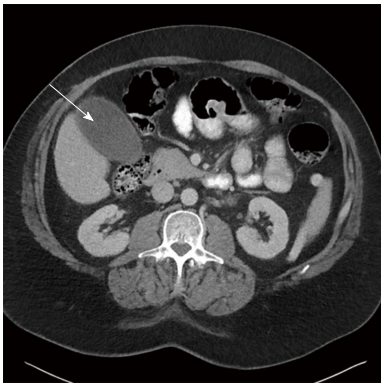

Figure 2 Collection inferior to segment 5 in the donor gall bladder fossa mimicking a gallbladder.

In the 4 wk following the procedures, the patient complained of persistent right upper quadrant pain with associated nausea, vomiting, and intermittent elevated temperatures. A CT scan showed what appeared to be a fluid collection mimicking a gallbladder (Figure 2). The suspected collection was presumed to be either a hematoma or biloma. Given the patients clinical symptoms of right upper quadrant pain, nausea, and vomiting, it was decided to place a drain in the collection *via* interventional radiology.

Following drain placement, bile tinged fluid was extracted, and a biloma was presumed. The patient was asymptomatic following drain placement, and his liver graft function was normal. The patient was seen for a follow up visit in clinic, during which the drain exhibited signs of minimal output and was subsequently removed.

Three weeks following drain removal the patient had a recurrence of his symptoms. His right upper quadrant pain was now increased in caliber, which forced in-

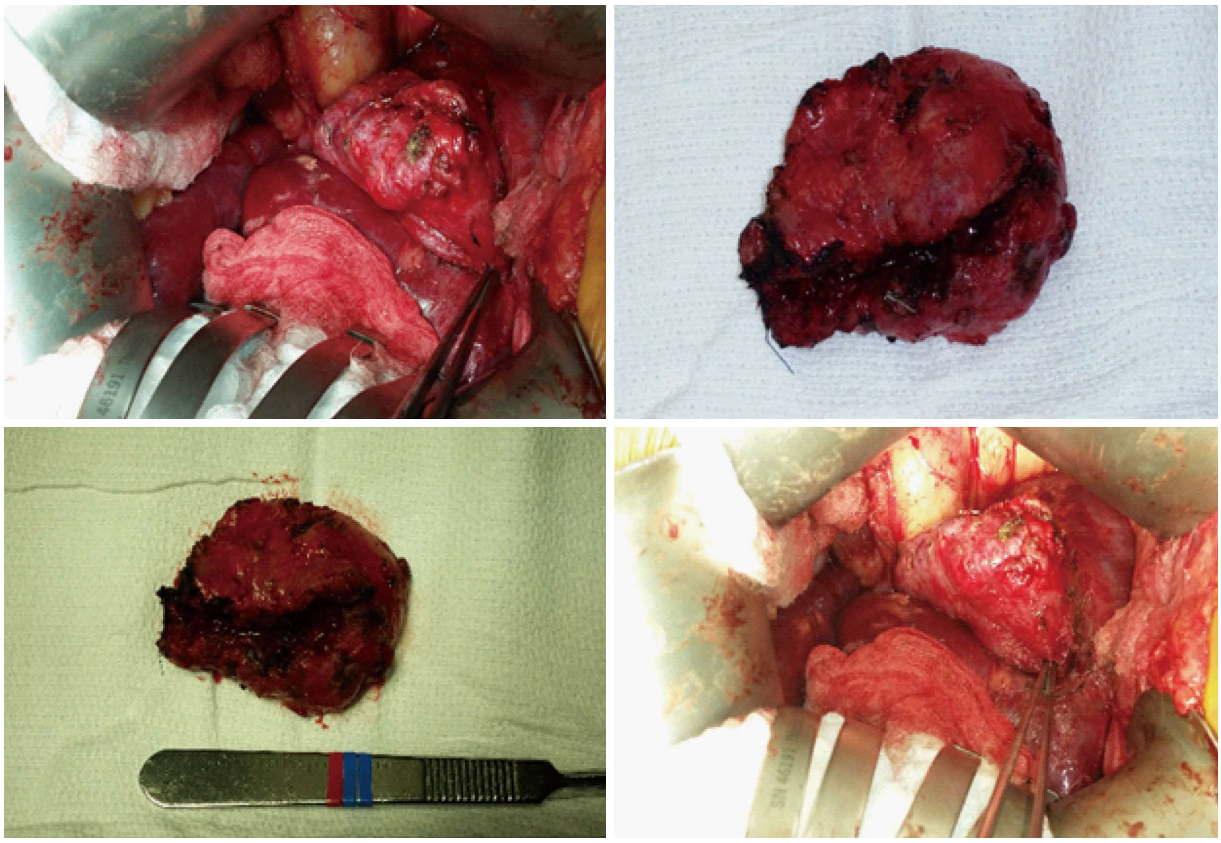

Figure 3 Intraoperative views and gross images of cystic duct mucocele.

patient admission. The patient clinically appeared to have cholangitis. On repeat imaging, the collection had now re-appeared inferior to the liver. A drain was once again placed and had evidence of purulence and bile tinged fluid. In addition to the interventional radiology procedure, the patient had an ERCP for stent removal, which showed resolution of his previous biliary stricture.

A diagnosis of cystic duct remnant mucocele was made given its appearance and recurrence, and ablation with sclerosing agents (ETOH) was attempted by interventional radiology on three separate occasions but was of little benefit. The patient continued to have recurrent symptoms with drain removal and a re-appearance of the mucocele.

After repeated attempts at minimally invasive techniques, 12 mo after transplant, the patient underwent an exploratory laparotomy. Abdominal exploration revealed a mucocele originating from the remnant of the recipient's cystic duct (Figure 3). This was subsequently surgically excised, and the defect was closed with proline suture. The patient did well after the operation with complete resolution of his symptoms. Furthermore, the patient's liver graft function remained excellent, with normalization of his alk phos, GGT and T.bili. The final pathology report revealed a cystic duct mucocele with signs of cholangitis and scarring. The mucosal wall had biliary mucosa (Figure 4).

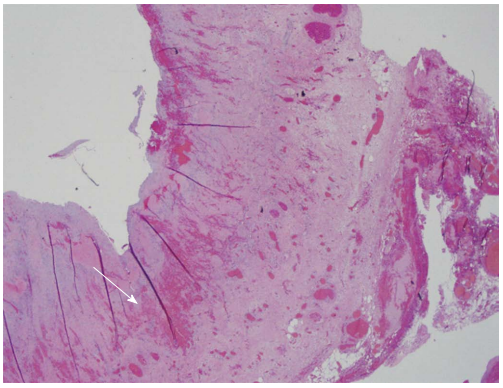

Figure 4 Section shows chronic cholangitis with prominent fibrosis, granulation tissue formation through mucosa, muscularis, and adventitia.

DISCUSSION

Orthotopic liver transplant is an accepted therapy for patients with end stage liver disease. Technical complications remain a significant cause of morbidity and mortality with biliary complications accounting for approximately 13% to 19% of the morbidities. Anastomotic biliary strictures and bile leaks account for most of these[1,2].

It is well known that the junction of the cystic and common hepatic ducts can be variable. In approximately 20% of cases, the cystic duct descends for a considerable distance, either along the right side of, or posterior to, the common duct before joining the common duct[2-4].

The presence of a recipient cystic duct mucocele after deceased donor liver transplant is a very rare anatomical complication. The reported cases in the literature are

few, and they all describe instances in which the donor cystic duct remained long to avoid damage to the donor common bile duct with resultant cystic duct mucocele. Chaterjee *et al*[5] describe a case in which there was high ligation of the donor cystic duct and incorporation of the cystic duct into the anastomosis to facilitate drainage but resulted in a donor duct mucocele nonetheless. Caputo *et al*[2] describe 13 patients in a 13-year history of 283 liver transplants, who developed donor duct mucoceles without encountering a recipient duct mucocele. Most of the reported cases demonstrate a mucocele derived from the donor anatomy as opposed to the recipient anatomy as described here[6-8]. In our case, it is believed that the recipient cystic duct was not adequately oversewn or ligated at the time of the initial liver transplantation. It is also possible that the ERCP stent had inappropriately obstructed the recipient cystic duct causing this mucocele; however, once removed, the mucocele persisted, making this unlikely.

The diagnosis of a mucocele, whether recipient or donor in origin, can be difficult but a rigorous diagnostic evaluation can aid in the diagnosis. With elevated liver enzymes after liver transplantation one must first exclude a vascular insult such as hepatic artery thrombosis or portal vein thrombosis, and an ultrasound with doppler of the liver transplant is necessary. In addition, an ultrasound may provide information on the biliary tree and whether there is intrahepatic biliary dilation indicating a possible biliary stricture. With a normal ultrasound examination, a liver biopsy is an appropriate next step to rule out rejection. With vascular compromise and rejection ruled out, the pathway may proceed in a variety of ways. A cholescintigraphy (HIDA) scan will provide the clinician with information in regard to the uptake and excretion of bile through the intrahepatic and extrahepatic biliary system. In rare instances, it may also reveal a possible mucocele. With a normal HIDA scan, a CT scan of the abdomen will give a better understanding of the hilar anatomy and assist in diagnosing any fluid collections, bilomas, or potential mucoceles. In addition, an MRCP can also aid in this diagnosis and exclude any biliary strictures. Given the condensed area of the hepatic hilum, it is difficult to distinguish the actual origin of the mucocele. If the mucocele were small and/or compressing the common duct, similar to Mirizzi's syndrome, this would point to the diagnosis of a donor duct mucocele. If there was no compression on the common duct and scans gave the impression of a remnant gall bladder, this may point to a recipient duct origin. From a clinical standpoint, however, the constellation of symptoms between a recipient duct and donor duct mucocele are very similar. Therefore, while imaging criteria may suggest a certain origin, the final diagnosis may not be achieved until operative intervention.

Treatment of cystic duct mucoceles, whether donor or recipient in nature, can be achieved by either interventional radiology (IR) or surgery. The IR approach is safe, effective, and can be achieved by percutaneous drain placement and ethanol ablation. Surgically, if the mucocele is donor duct in origin, excision of the mucocele followed by roux-en-y hepaticojejunostomy has been described as an accepted treatment method[2,5]. In the instance of a recipient duct mucocele, one would expect, depending on the recipient variation of cystic duct anatomy, to have a well-drained cystic duct either into the recipient common duct or directly into the duodenum. Regardless of the anatomy, our patient developed a mucocele from the recipient remnant cystic duct, and only required simple excision.

When a transected cystic duct is encountered during preparation for liver transplant, it should be excised completely, even if it is close to the common duct. If this is not possible, the cystic duct should be excised as much as possible, rather than creating a blind mucosa-lined sac with the potential of enlarging and creating obstruction. However, this should be done under the discretion of the surgeon, as injury to the right hepatic artery, the common bile duct and its branches, and the biliary tract blood supply are possibilities. If excision of the cystic duct is thought too dangerous, its distal end can be incorporated in the anastomotic suture line to allow drainage of the cystic duct stump. However, wherever the valves of Heister are left to drain a blind biliary pouch that produces mucous, the risk of mucocele remains.

The diagnosis of cystic duct mucocele in the appropriate setting is usually made by radiologic modalities. Demonstration of fluid collection at the porta hepatis is a nonspecific finding. However, a combination of a well-defined, round, fluid collection adjacent to the common hepatic duct would confirm evidence. In some cases, the mucocele appears as a gallbladder and immediately calls into question whether a graft cholecystectomy was performed.

A recipient cystic duct mucocele is a rarity after deceased donor liver transplantation. It is a recognized complication to observe a donor duct mucocele that may compress the common bile duct. However, we describe for the first time a patient with a more ambiguous clinical picture found to have a recipient duct mucocele, showing the importance of the consideration of this complication in the post-transplant patient.

ARTICLE HIGHLIGHTS

Case characteristics

A 48-year-old male who presented with right upper quadrant pain and was found to have a recipient cystic duct mucocele 3 mo after receiving a deceased donor liver transplant.

Clinical diagnosis

A diagnosis of cystic duct remnant mucocele was made given its appearance and recurrence, and ablation with sclerosing agents (ETOH) was attempted by interventional radiology on three separate occasions but was of little benefit.

Differential diagnosis

Biloma, anastomotic bile leak, pancreatic cyst, pancreatic pseudo cysts, and cystic duct mucocele after transplantation.

Laboratory diagnosis

Liver function test revealed elevated canallicullar enzymes, complete blood count

showed elevated white blood cells, and blood cultures were unremarkable.

Imaging diagnosis

Abdominal X-ray (nonspecific), abdominal CT (mass or fluid collection at porta hepatis), abdominal MRI (mass or fluid collection at porta hepatis), MRCP (showed filling of the fluid collection), and ERCP (showed filling of the fluid collection).

Pathological diagnosis

Resection and histologic analysis of the fluid filled mucocele revealed chronic cholangitis with prominent fibrosis, granulation tissue formation through mucosa, muscularis, and adventitia.

Treatment

Surgical resection of the mass, and perioperative antibiotics.

Related reports

See the reference list: Ref. [2,4,7].

Term explanation

Mucocele: A swelling like a sac that is due to distension of a hollow organ or cavity with mucus; MRCP: Magnetic resonance cholangiopancreatography: A special type of magnetic resonance imaging (MRI) exam that produces detailed images of the hepatobiliary and pancreatic systems, including the liver, gallbladder, bile ducts, pancreas and pancreatic duct; ERCP: Endoscopic retrograde cholangiopancreatography: A technique that combines the use of endoscopy and fluoroscopy to diagnose and treat certain problems of the biliary or pancreatic ductal systems; Gamma-glutamyltransferase: A transferase (a type of enzyme) that catalyzes the transfer of gamma-glutamyl functional groups from molecules such as glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate); Mirizzi's Syndrome: A rare complication in which a gallstone becomes impacted in the cystic duct or neck of the gallbladder causing compression of the common bile duct or common hepatic duct, resulting in obstruction and jaundice; Hepaticojejunostomy: Biliary-enteric anastomosis is usually to smaller ducts, which can be multiple if the injury or stricture is above the bifurcation of the right and left ducts.

Experiences and lessons

Cystic duct mucoceles after transplantation require a high index of suspicion requiring h and p, lab tests (liver function test, complete blood count, blood cultures), and imaging studies (abdominal X-ray, abdominal CT, abdominal MRI, ERCP) with resolution through surgical resection.

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