

REVIEW ARTICLE

BIOPSY OF THE TRANSPLANTED KIDNEY – ROLE OF PROTOCOL BIOPSIES

Masin-Spasovska J. and Spasovski G

University Department of Nephrology, Medical Faculty, Skopje, R. Macedonia

Abstract: Traditionally, renal allograft biopsies were performed mainly in the setting of acute graft dysfunction. Recently, there has been a change of paradigms. Several reports suggested that acute rejection of the graft and chronic allograft nephropathy are often subclinical without any deterioration in the graft function. This raises the issue of biopsies in functionally stable allografts (e.g. protocol biopsies) and the clinically useful information they provide. Namely, recent reports provide evidence in favour of treating biopsy-proven subclinical rejections. Moreover, by early identification of chronic histological lesions, protocol biopsies give an opportunity for individualized immunosuppressive regimen and use of targeted therapeutic strategies, in order to prevent chronic allograft dysfunction and improve long-term graft outcome. In this review, diagnostic, therapeutic and research benefit information on protocol biopsies performed in stable kidney recipients are described.

Key words: renal transplantation, acute rejection, allograft nephropathy, protocol biopsy.

Introduction

The gold standard for assessing structural abnormalities in the transplanted kidney is a graft biopsy sample for histopathology. However, the role of protocol biopsy seems to be still debatable. At the end of the 70's and the beginning of the 80's the first pioneer studies of protocol biopsies were performed [1, 2]. The most intriguing observation in these studies was the presence of interstitial infiltrate in grafts with stable function, suggesting that this histological finding might reflect a minor form of silent rejection that could be respon-

sible for further allograft histological and functional deterioration. In 1995, the term of subclinical acute rejection (SAR) was introduced as a finding of histological lesions of acute rejection (AR) in stable grafts by means of protocol biopsies [3]. In addition, the association between the presence of chronic lesions in protocol biopsies and the probability of graft functional deterioration was hypothesised [4], and later on confirmed as a many-fold time higher risk for graft loss in high scored chronic lesions in 6-month protocol biopsies [5]. These observations have stimulated further studies trying to establish the potential utility of the protocol biopsies in the early diagnosis of acute and chronic lesions and their impact on the graft histology and function later.

Technique of renal biopsy

Several techniques for transplant renal biopsies have been published. Iversen and Bran first introduced the percutaneous technique for renal biopsy over 50 years ago [6]. Historically, the first reports on superiority of real-time fine-needle ultrasound guidance for renal biopsy over "blinded" techniques were published in the 1990s [7, 8]. Today, it is adopted as a universal technique, with a needle mounted in an automatic spring-loaded device (gun-biopsy). The needle-size used is 14, 16 or 18 gauge. The 14-gauge needle is preferred for histopathological evaluation but has theoretically a higher risk of bleeding complications. However, when adequacy of biopsy specimens obtained by conventional 14 vs. 18 gauge automated biopsy device was compared the yield of glomeruli was even slightly higher with the 18-gauge needle in the presence of a lower complications rate [9]. Preparations for transplant biopsy are similar to those for biopsy of the native kidney. Informed consent is required from patients, who should be specifically warned of the potential risks (complications) and benefits from the procedure. In fact, there could be only a few contraindications of transplant renal biopsy (bleeding disorder, sepsis, enormously enlarged and distended graft, etc.), while the early recognition of factors contributing to graft failure may critically improve outcomes after kidney transplantation.

Although several methods have been used to diagnose renal allograft dysfunction (clinical evaluation and laboratory tests), the core biopsy remains "gold standard" for diagnosis of renal transplant abnormality. In this respect, percutaneous allograft biopsy represents an indispensable diagnostic tool, as a thorough histological analysis allows a subtle differentiation of factors contributing to allograft dysfunction and consequently, a different approach in the management.

The Banff schema of allograft pathology

Renal function is not always included in the definition of condition of stable grafts, despite the fact that it is well known that serum creatinine and proteinuria are powerful predictors of the graft outcome. Furthermore, both parameters correlate with the severity of acute and chronic renal lesions. The Banff schema has contributed to an international uniformity in the evaluation of renal allograft biopsies, but there is still scant information on the utility of this classification system in clinical practice, especially when it is applied to the evaluation of stable grafts.

The use of the Banff scheme to classify allograft pathology provides for a more rational allocation of immunosuppressive therapy than does descriptive terminology and may decrease the incidence of therapeutic complications [10]. The Banff scheme on allograft pathology defines three types of acute or active rejection [11].

Type I rejection is tubulointerstitial rejection without arteritis. Grade IA rejection represents cases with significant interstitial infiltration (> 25% of parenchyma affected), and foci of moderate tubulitis (> 4 mononuclear cells per tubular cross-section or group of 10 tubular cells) whereas grade IB rejection shows significant interstitial infiltration (> 25% of parenchyma affected), and foci of severe tubulitis (> 10 mononuclear cells per tubular cross-section or group of 10 tubular cells).

Type II rejections are vascular rejections where type IIA represents cases with mild to moderate intimal arteritis and type IIB rejections are those with severe intimal arteritis comprising > 25% of the luminal area.

Type III rejections show transmural arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells. Biopsies with only mild inflammation are graded "borderline" or suspicious for rejection.

Rejection due to anti-donor antibodies is categorized as a separate group in the Banff scheme with finding of C4d deposition as a diagnostic marker of AR caused by humoral immune components, both in early and late post-transplant periods [12]. In addition, C4d is a significant predictor of graft survival and might be of great help when treating acute rejection [13].

On the other hand, by the Banff scheme chronic allograft nephropathy (CAN) is recognized and semi-quantitatively scored according to the presence of interstitial fibrosis (IF), tubular atrophy (TA), and transplant chronic vasculopathy (CV) [10, 11].

Use of protocol kidney allograft biopsy – when and why?

Transplant renal biopsy is generally performed upon indication, i.e. when an acute or chronic renal allograft rejection is suspected. However, sur-

veillance (protocol) biopsies performed in stable kidney transplants allow timely recognition of pathological conditions causing later deterioration of the graft function. There is accumulating evidence that the performance of protocol biopsies could be a worthwhile strategy to improve long-term outcomes in kidney transplantation.

The histological changes in protocol biopsies at different time-points have been well described in studies with sequential protocol biopsies [14, 15]. Unfortunately, at present, there is no consensus yet on when to take protocol biopsies.

Protocol biopsies are invaluable for research purposes, not only to help understand the pathophysiology and progression of chronic changes in the graft, but also as surrogate markers for clinical immunosuppressives and other drug studies. At the recent consensus meeting the usefulness and valuable role of protocol biopsies in the follow-up of kidney transplant recipients was pointed out, while the principal barrier to their wider use is the knowledge of benefits of such intervention [16].

Perhaps the strongest argument in favour of protocol biopsies is the possibility of detecting subclinical rejection, usually defined with histological signs of acute or borderline cellular rejection in protocol biopsy specimens without concomitant clinical presentation of graft dysfunction [17]. In addition, an early occurrence of features of SAR or CAN may help establishing an individually targeted immunosuppressive regimen (anti-rejection treatment, use of novel less toxic immunosuppressant or even reduction or withdrawal of some immunosuppressive drugs).

The main clinical indicator of renal allograft dysfunction is a trend towards increasing serum creatinine levels above a baseline value. There is no targeted level of creatinine and surely a single abnormal laboratory value does not warrant renal biopsy, while other early clinical indications of allograft rejection include unexplained fever, oedema, hypertension, graft tenderness, eosinophilia, oliguria, anuria, and proteinuria unrelated to glomerulonephritis. Clinical criteria alone cannot predict graft dysfunction in 50% to 70% of the patients; therefore, histological confirmation of the diagnosis is often required [18, 19].

In the last decades there was a consensus on international standardisation of histological criteria of renal allograft pathology – Banff classification [10, 11, 20]. Thus, renal transplant biopsy findings can sometimes alter patient management in ~ 40% of cases where a presumptive diagnosis was made on the basis of clinical and laboratory findings, while in 19% of cases unnecessarily immunosuppression could be avoided [21]. Concerning the debate on protocol biopsy at scheduled intervals to diagnose subclinical allograft dysfunction, it was concluded in another study of 228 biopsies on 108 patients that, when

regularly performed, clinically silent acute rejection in 13% and chronic rejection in 52% of their patients could have been diagnosed and treated [22].

Complications of transplant renal biopsy

Transplant renal biopsy carries a lower complication rate than the native renal biopsy of about 0.06 to 13% [7, 23, 24], although there are isolated reports even up to 19.5% in grafts compared to 28.9% in native kidneys [25]. The transplanted kidney is much more superficial than the native kidney, separated from the skin only by a thin layer of muscle, fascia, and subcutaneous tissue of the anterior abdominal wall, and is therefore much more visualized and disposable for the biopsy. The variation in the frequency of the reported complications depends on multiple factors including the experience of the operator, utilization of imaging guidance, gauge of the biopsy needle and a proactive effort on the part of the operator to pursue subclinical complications by follow-up imaging. All major complications after needle biopsy manifest as perinephric or urinary bleeding. Haemorrhage is the predominant complication related to transplant biopsy and may occur acutely as microscopic or gross haematuria or subcapsular haematoma. Transient macroscopic haematuria follows about 3% of biopsies and is of little clinical significance. Urethral obstruction occasionally occurs, requiring placement of a percutaneous nephrostomy. A massive haemorrhage necessitating surgical exploration, angiographic embolization, or postbiopsy arteriovenous fistulas is rare [7, 23, 24].

Differences in the clinical practice based on the protocol biopsy

Biopsy proven rejection without deterioration of graft function (SAR)

Recent reports provide evidence in favour of treating biopsy-proven SAR. Rush et al. showed that corticosteroid treatment of SAR found in the first 3 months after transplantation is associated with a better outcome [26]. A decrease in the frequency of early (month 2 and 3) and late (months 7) acute rejection episodes was noted. This was accompanied by a decrease in chronic tubulointerstitial score at 6 months post-transplant, and by a lower serum creatinine concentration at 24 months post-transplant, when compared to untreated controls. Importantly, when SAR episodes occurring later than 6 months after transplantation were treated with corticosteroids, the treatment failed to prevent subsequent deterioration of renal function. These findings indicate that a reversible rejection process may be present early after renal transplantation (< 6 months). Beyond 6 months after transplantation, however, such rejections can no longer be fully reversed by standard immunosuppressive therapy [26].

On the other hand, the controversy about the management of Banff-borderline rejection (BR) continues. While the benefit of treating borderline rejection in the setting of graft dysfunction has been documented beyond any doubt, it is still not certain whether the patients with stable graft function diagnosed by protocol biopsy derive benefit from the treatment [27].

Acute rejection (AR) in patients with delayed graft function (DGF)

Both DGF and AR are strongly associated with poor long-term graft outcome. In patients with DGF, it is difficult or impossible to diagnose graft rejection clinically. Early treatment of AR is crucial to prevent an adverse effect on the long-term graft outcome. Consequently, there is a need for a method to promptly diagnose AR in patients with DGF which could be surely provided by the protocol biopsy. Many studies reported high rates of acute rejection biopsies performed in patients with DGF. Qureshi et al. reported a prevalence of SAR in 50% of recipients with DGF [28]. Similarly, in two other studies it was reported for 18 and 21% of patients with DGF having AR, and without protocol biopsies, these AR episodes would have been surely missed [29, 30].

Thus, it seems prudent to perform protocol biopsies in patients with DGF in whom diagnosis of AR cannot be made on clinical grounds. The data justifying routine biopsies to detect SAR is, as yet, only suggestive and requires confirmation.

The "late" protocol biopsy – presence of chronic allograft nephropathy (CAN)

CAN is the most common cause of late renal allograft failure and loss. Unfortunately, when biopsies are performed after the deterioration of renal function, the degree of histological scarring is usually advanced and often beyond the point of no return. This consideration prompted some groups to perform protocol biopsies in an attempt to diagnose CAN at an earlier stage. Seron et al. reported CAN in about 42% of the biopsies performed at 3 months after transplantation in patients with stable graft function [31]. Expectedly, patients with CAN had experienced more AR episodes, had a higher mean cyclosporine concentration, and had a worse graft survival. In contrast, if only borderline changes were found, late allograft outcome was the same as that of the patients with a normal histology. In addition, several groups have examined the prognostic value of 6- or 12-month biopsies on the 5-years outcome and found that chronic histological changes correlate with long-term outcomes [5, 32–34]. At this stage, it is unclear whether interventions prompted by biopsy findings will improve the intermediate- or ultimate long-term outcome.

Conversely, it is well documented in previous studies that subclinical inflammation, scored under the Banff schema as SAR and BR, leads to increased interstitial fibrosis and CAN within the first year [35–39]. There is also evidence that protocol biopsies of a stable renal allograft may be of value to diagnose CAN before deterioration of the graft function [31, 40].

Our data also confirmed that CAN is frequently found in clinically normal renal transplants, when assessed by protocol biopsies. We reported incidence of CAN in 45% and 87% of protocol biopsies performed at 1 and 6 months after transplantation, respectively. Furthermore, in our study at a 1-month biopsy no histological evidence of CAN was found in 63%, CAN grade I (according to the Banff classification) was found in 37%, while no patient had CAN grade II or III. At a 6-month biopsy the proportion of these findings was 14.3%, 37% and 46%, respectively, and CAN grade III in 2.9%. In addition, a progression of CAN was observed in 71% of patients while regression was observed in only 28.6%, confirming that the probability that progression was higher than the probability of regression [41–43].

Another argument in favour of protocol biopsies is the clinical evidence from several studies showing analysis of chronic changes, such as the semi-quantitative chronic allograft damage index (CADI), as a strong predictor of graft outcome long before clinical signs of graft deterioration are evident. Using the CADI score, Isoniemi et al. reported a significant correlation of the 2-year score with transplant function at 6 years. Of the patients with a low CADI score (< 2) 7% were in clinical chronic rejection at 6 years when compared to 42% of patients with a CADI score > 2 and stable graft function at 2 years [44].

Finally, early CAN detected in protocol biopsies may ultimately result in deterioration of graft function. Therefore, chronic graft damage detectable in well-functioning kidney allografts may be a valuable predictor of late graft loss, and protocol biopsies a valuable tool for future studies evaluating strategies aimed to treat CAN.

Humoral rejection in kidney allograft protocol biopsies

Recent reports reinforce an important role of humoral immunity as a mediator of allograft rejection [39, 45]. Deposition of the C4 complement split product C4d along the endothelium of peritubular capillaritis (PTC) represents a specific marker of antibody-mediated rejection (AMR). The incidence of C4d deposition was reported to be 25–50% in biopsies performed because of an acute allograft dysfunction. However, several studies have suggested that C4d may represent a valuable tool to uncover subclinical humoral rejection in protocol biopsies as well [46, 47]. In addition, patients with chronic AMR had worse graft function and survival [48, 49], requiring development of new treatment protocols for those found to be at risk.

Protocol biopsy and cyclosporine nephrotoxicity

The reported frequency of cyclosporin A nephrotoxicity following kidney transplantation varies between 10 and 54% [50]. Large inter-individual differences in susceptibility to cyclosporine nephrotoxicity, or insufficient precision of the pre-dosing blood cyclosporin A measurements, are strong arguments for more frequent allograft biopsies or even protocol biopsies. The diagnosis of calcineurin inhibitor (CNI) nephrotoxicity is extremely important, since this condition is reversible if immunosuppression is modified in time, by reduction of the cyclosporine dose and increased dosing with other immunosuppressants [51]. Only a few studies have reported the results of protocol biopsies to exclude CNI nephrotoxicity in patients with stable renal allografts. Takeda et al. found cyclosporin A nephrotoxicity (histologically expressed as the presence of arterial hyalinosis) in up to 42% of protocol biopsies performed later than 12 months after transplantation [52].

Protocol biopsy proven histological disorders and therapeutical management

A protocol biopsy provides new information not evident from any symptoms or laboratory values that had direct impact on the treatment of transplant recipients. SARs were mostly reacted to, but chronic changes mostly give information about the long-term prognosis, as currently no evidence-based therapy for CAN exists. Reduction of calcineurin inhibitor exposure and the use of sirolimus in the treatment of CAN may be of some benefit [53, 54]. Antifibrogenic and renoprotective therapy with ACEi or ARBs may also prevent the progression of CAN [55]. Currently, no clear data exist about the usefulness and impact of protocol biopsies to the actual treatment of the patients, except in the case of SARs.

Expectedly, the management of immunosuppression could be more appropriate if follow-up protocol biopsies are performed reacting to observed histological changes. Thus, late protocol biopsy seems to be a promising method of evaluating the safety of steroid withdrawal and a possible subsequent increase in the incidence of late AR's [56]. However, the true safety and benefit of steroid withdrawal can only be assessed in a study with a longer follow-up.

Recent reports provide evidence in favour of treating biopsy-proven SAR episodes with high-dose steroids lowering the rate of early and late acute rejections, SAR, and decreasing chronic histological changes with an improvement of allograft function later on when compared to untreated control patients [34, 57, 58].

Concerning the Banff "borderline" and subclinical rejections (grade I or II) it seems that withholding treatment would allow progression of the rejection process [59, 60]. In addition, it was hypothesized that the beneficial effect of

corticosteroids may be due to the interruption of early immune and nonimmune processes of tissue injury [42, 43, 59–61].

Conclusions

Protocol biopsies could help clinical decisions for treatment which might preserve graft function and its long-term prognosis. The presence of CAN and the associated risk of accelerated graft deterioration may be a useful surrogate marker for chronic rejection and subsequent graft failure and, in clinical trials evaluating the effect of different immunosuppressive drugs and their long-term graft outcome. Thus, protocol biopsy could help in maintaining immunosuppressive therapy, and better monitoring of the effectiveness and safety of novel immunosuppressive protocols. Timely treatment of allograft rejection that cannot be diagnosed on clinical ground is definitely of benefit for long-term graft function. Finally, the relative safety of the biopsy procedure is well documented and ethically justified as an investigative strategy.

Conflict of interest: *none declared*.

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Резиме

БИОПСИЈА НА ТРАНСПЛАНТИРАН БУБРЕГ – ЗНАЧЕЊЕ НА ПРОТОКОЛ БИОПСИИТЕ

Масин-Спасовска Ј. и Спасовски Г.

*Универзитетска клиника за нефрологија, Медицински факултет,
Скопје, Р. Македонија*

Традиционално, бубрежните алогографт биопсии биле правени најмногу при состојби на акутна алогографт дисфункција. Во последно време, доаѓа до промена на парадигмите. Неколку соопштенија, сугерираат дека акутните отфрлања на графотот и хроничната алогографт нефропатија најчесто се супклинички, без постоење на било какво влошување на функцијата на графотот. Оттука, произлегува улогата и значењето на примената на биопсиите кај функционално стабилните графтови (т.е. протокол биопсии) и клиничките корисни информации што ги даваат. Имено, најновите соопштенија ја потврдуваат очигледната корист на третманот на хистолошки потврдените супклинички отфрлања. Уште повеќе, преку раната идентифи-

кација на хроничните хистолошки оштетувања, протокол биопсиите даваат можност за индивидуализација на имunosупресивниот режим, употреба на насочено-целени терапевтски стратегии, а сè со цел да се превенира хроничната алографт дисфункција и да се подобри преживувањето на графотот. Во овој ревијален труд, ги претставуваме дијагностичките, терапевтските и истражувачките корисни информации при примената на протокол биопсиите кај пациентите со стабилна ренална функција.

Клучни зборови: бубрежна трансплантација, акутно отфрлање на графотот, алографт нефропатија, протокол биопсија.

Corresponding Author:

J. Masin-Spasovska
University Department of Nephrology
Medical Faculty
Vodnjanska 17,
1000 Skopje
R. Macedonia

E-mail: emasin@sonet.com.mk

Short title: Protocol biopsy in kidney transplantation

Manuscript type: review

Word count: 5031