RISK FACTORS ASSOCIATED WITH THE DETERIORATION OF RENAL FUNCTION: THE ROLE OF PROTOCOL BIOPSIES

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Abstract: Protocol renal allograft biopsies allow the early detection of histological damage in the renal allograft even before renal function deterioration or proteinuria appears. Two different lesions have attracted the main interest in protocol biopsy studies: subclinical rejection, namely, the presence of tubulo-interstitial inflammation and chronic allograft nephropathy, nowadays termed interstitial fibrosis/tubular atrophy (IF/TA), and the presence of tubulo-interstitial chronic lesions. The incidence of subclinical rejection is maximal after transplantation and decreases during the first few months despite the fact that this condition persists in a proportion of cases even in late protocol biopsies done after the first year. In studies of serial protocol biopsies, the presence of subclinical rejection is associated with a higher probability of the progression of chronic tubulo-interstitial lesions and, recently, it has been shown the subclinical rejection in early protocol biopsies is associated with poorer allograft survival. The incidence of IF/TA rapidly progresses after renal transplantation, following an exponential curve. Its presence is associated with a decreased graft survival and its predictive value on outcome is independent from other predictors of survival such as serum creatinine or acute rejection. The association of IF/TA with transplant vasculopathy, subclinical rejection or transplant glomerulopathy implies a poorer outcome than IF/TA without other histological lesions. Taken together, these data suggest that protocol biopsies allow the early detection of acute and chronic lesions and the recognition of different patterns of damage that are associated with allograft survival.

Key words: protocol biopsy, subclinical rejection, chronic allograft nephropathy, interstitial fibrosis/tubular atrophy.
Introduction

Renal allograft biopsy is indicated in renal transplant patients with acute or chronic allograft dysfunction in order to establish the diagnosis, prognosis and to decide the most adequate treatment. In the case of chronic allograft dysfunction the criteria for indicating a renal allograft biopsy vary between centres. Despite the presence of proteinuria or renal function deterioration, the decision to perform a renal biopsy is often delayed by some empirical therapeutic manoeuvres such as the introduction of angiotensin-converting enzyme inhibitors in patients with proteinuria and the reduction of anti-calcineurin dose in patients with renal function deterioration. However, this is not a reasonable approach since both proteinuria and renal function deterioration can be due to many different causes such as subclinical rejection, chronic anti-donor specific mediated rejection, de novo glomerulonephritis, recurrence of the original disease, hypertension, polioma virus infection, hepatitis C virus related glomerulopathy or obstruction. Thus, when indication of renal allograft biopsy is delayed we deny the patient the possibility of receiving the best treatment for a specific cause.

Characterisation of the progression of histological lesions by means of protocol biopsies

At the end of the 70s and the beginning of the 80’s it was suggested that the histological lesion of the allograft could precede proteinuria or renal function deterioration. This hypothesis stimulated the first pioneering studies of protocol biopsies. The most striking observation of the first studies was the presence of a severe interstitial infiltrate in many stable grafts [1] and soon it was possible to establish a relationship between the severity of the interstitial infiltrate and the evolution of the renal function [2] suggesting that the presence of an interstitial infiltrate might represent a minor form of rejection and could be responsible for a smouldering process leading to graft function deterioration (Figure 1). In 1995, the term subclinical rejection was introduced to refer to the presence of histological lesions of acute rejection in stable grafts evaluated by means of protocol biopsies [3]. In 1992 a relationship between the presence of chronic lesions in protocol biopsies and a higher probability of renal function deterioration was established [4]. These observations stimulated the study of the potential utility of protocol biopsies for the early diagnosis of histological lesions.
Figure 1 – Subclinical rejection characterised by the presence of interstitial infiltrate (long arrow) and tubulitis (short arrow)

The predictive value of chronic allograft nephropathy diagnosed by means of protocol biopsies on outcome

In 1995 an association between the presence of chronic lesions in 6 month protocol biopsies and graft outcome was described for the first time [5]. Later on, in 1997, it was observed that the presence of chronic allograft nephropathy in 3 month protocol biopsies is not only associated with a poorer renal allograft survival, but it was also shown that its predictive value on graft survival was independent of other classical predictors of graft outcome such as acute rejection, serum creatinine or proteinuria [6]. This observation implied that protocol biopsies (Figure 2) contain valuable information for predicting the outcome that was not present in clinical data. This result was confirmed by other centres [7–9] and the question was raised about which parameter was a better predictor of outcome: renal histology evaluated in protocol biopsies or renal function. However, despite the fact that serum creatinine or estimated glomerular filtration rate are predictors of graft survival in epidemiological studies [10] its predictive value is not precise enough to be a trustworthy parameter for evaluating the risk in individual patients [11].
Correlation between chronic allograft nephropathy and renal function

In studies of serial protocol biopsies, it has been shown that chronic renal lesions tend to progress. Tubulo-interstitial chronic lesions rapidly progress during the first few months and more slowly thereafter. This observation was important since it demonstrated that chronic lesions can be evaluated in early protocol biopsies. Other chronic lesions such as glomerulosclerosis, chronic vascular lesions, hyaline arteriolar changes also progress, but following a more linear mode [12]. It has been estimated that at 3 months 30–40% of patients already display chronic allograft nephropathy, 50% at 1 year, 60% at 2 years and 100% at 10 years. However, the correlation between structure and function in studies of protocol renal allograft biopsies is very poor. For example, in a study in which a 4 month and 1 year protocol biopsy was performed in 155 patients, chronic lesions significantly progressed in the glomerular, interstitial, tubular and vascular compartment while serum creatinine remained stable [13]. These data clearly show that serum creatinine is not a reliable marker of the progression of chronic lesions, reinforcing the notion that, in early stages, chronic allograft nephropathy can only be diagnosed by means of protocol biopsies. In a study in which a protocol biopsy was performed in renal transplants with a serum creatinine < 200 µmol/l and proteinuria < 1g/24h, glomerular filtration rate measured according to the inulin method, effective renal plasma flow, mea-
Risk factors associated with the deterioration of renal function ... 295

sured according to the paraamino-hipurate technique and renal functional re-
serve after the administration of intravenous aminoacids or aminoacids with
dopamine were determined. There were no differences in renal function be-
 tween patients with and without chronic allograft nephropathy [14], further
documenting the lack of correlation between structure and function in studies of
protocol biopsies.

Subclinical rejection

Subclinical rejection is defined as the presence of histological lesions of
acute rejection defined according to the Banff criteria [15] in patients with
stable renal function. The incidence of subclinical rejection is maximal just after
transplantation and rapidly decreases during the first months, following an expo-
nential curve [12]. Despite the fact that the incidence of subclinical rejection
varies between centres, approximately 10% of patients show subclinical rejec-
tion after the first year. The persistence of subclinical rejection in serial biopsies
is associated with progressive renal function deterioration. Furthermore, in
studies of serial protocol biopsies, it has been observed that patients with sub-
clinical rejection in the first biopsy have a higher probability of renal function
deterioration in the second protocol biopsy, suggesting that subclinical rejection
is a risk factor for the appearance or progression of chronic allograft nephro-
pathy [16].

In studies of protocol biopsies it has been observed that, after transplan-
tation, glomerular volume increases as an adaptation mechanism to the recipient
metabolic demand. It has also been observed that the larger the glomerular
volume in 4 month protocol biopsies, the better the renal function [17] sug-
gesting that glomerular enlargement after transplantation is a necessary con-
dition for achieving a better renal function. In a study of paired 4 month and 1
year protocol biopsies, not only was it observed that the presence of subclinical
rejection was associated with a higher probability for the progression of tubulo-
interstitial chronic lesions, but also that the presence of subclinical rejection in
the first biopsy was associated with impaired glomerular enlargement in the
second one [18]. These data suggest that early subclinical rejection impairs
glomerular adaptation to the recipient metabolic demand. Recently, Choi et al.
[19], in a large study including more than 300 recipients from living donors in
whom a protocol biopsy was performed 15 days after transplantation, showed for
the first time an association between the presence of subclinical rejection and
decreased renal allograft survival. Taken together, all these data point out that
the presence of subclinical rejection is associated with a poorer outcome.

The incidence of subclinical rejection is associated with timing of pro-
tocol biopsy, the presence of an episode of acute rejection before performing the
protocol biopsy and degree of sensitisation [16, 20]. Moreover, the incidence of
subclinical rejection is closely associated with the immunosuppressive treatment. The incidence of subclinical rejection is minimal in patients treated with tacrolimus, mycophenolate mofetil and prednisone, intermediate in patients treated with cyclosporine mycophenolate mofetil and prednisone, higher in patients receiving cyclosporine, azathioprine and prednisone and very high in patients receiving a calcineurin-free regimen consisting of thymoglobulin, mycophenolate mofetil and prednisone [12, 20, 21]. At present, there is little data on the incidence of subclinical rejection in patients treated with sirolimus or everolimus. However, the incidence of subclinical rejection with anti-calcineurin free regimens based on anti-proliferative signal inhibitors may be slightly higher than in regimes based on tacrolimus. However, in serial protocol biopsies it has been observed that despite a higher incidence of subclinical rejection in patient treated with sirolimus at 1 year, the severity of chronic lesions was significantly lower at 3 years [22].

It has been observed that patients treated with tacrolimus, in comparison to patients treated with cyclosporine, not only show a lower incidence of subclinical rejection soon after transplantation, but also a lower incidence of chronic allograft nephropathy at 12 months [12] suggesting a link between prevention of subclinical rejection and prevention of chronic allograft nephropathy. However, other studies have not observed this benefit [23, 24].

It has also been studied whether treatment of subclinical rejection with steroid boluses could be associated with a better outcome. A prospective randomised study was performed in one centre in Canada in order to address this question. Patients were randomised into two groups. In the study group, patients were biopsied at 1, 2 and 3 months and treated with boluses if they showed acute rejection in the protocol biopsy. In the control group, patients were not biopsied and, accordingly, were not treated for subclinical rejection [25]. The severity of chronic tubulo-interstitial lesions at 6 months and serum creatinine at 2 years were lower in the study group. At present, no multicentre trial has been done to confirm this observation.

Are there different types of chronic allograft nephropathy?

At the 8th Banff Conference, the elimination of the term chronic allograft nephropathy was proposed. This term had been introduced to describe biopsies with interstitial fibrosis and tubular atrophy (IF/TA)) as an alternative to the term chronic rejection. However, despite the success of the new term, in many papers it is now assumed that the term chronic allograft nephropathy refers to an entity and not to chronic tubulo-interstitial lesions. Thus, in order to avoid any over-interpretation, it was proposed that it should refer to biopsies with tubulo-interstitial chronic lesions that cannot be ascribed to any specific cause as IF/TA. Furthermore, at this conference, criteria were defined for recog-
nising chronic antibody mediate rejection, and this represents an important step forward, since a proportion of patients with IF/TA can be now diagnosed with a specific disease and, accordingly, offered a more rational treatment. The criteria for defining chronic antibody mediated rejection are: a) morphological lesions including transplant glomerulopathy, multilamination of the basal membrane of the peritubular capillaries, fibrous intimal thickening and IF/TA; b) C4d in peri-tubular capillaries and c) donor-specific antibodies. At this conference the necessity for further recognition of specific entities leading to tubulo-interstitial chronic damage was stressed [26].

In recent years, epidemiological studies based on protocol biopsies have suggested that prognosis of tubulo-interstitial chronic lesions partly depends on the accompanying lesions in other renal compartment. At present, there is some evidence to suggest that the association of transplant vasculopathy, subclinical rejection or even transplant glomerulopathy with tubulo-interstitial chronic lesions implies a poorer prognosis than the presence of tubulo-interstitial chronic damage alone.

**IF/TA associated with transplant vasculopathy**

When protocol biopsies are classified into two groups: presence or absence of tubulo-interstitial chronic lesions, graft survival is lower in the first group [5, 6]. However, if we classify protocol biopsies in three groups: no tubulo-interstitial chronic lesions, tubulo-interstitial chronic lesions without transplant vasculopathy and tubulo-interstitial lesions with transplant vasculopathy, only the last group shows a poor allograft survival, while the outcome in patients with tubulo-interstitial chronic lesions without transplant vasculopathy is just slightly inferior to that in patients with a normal biopsy [7, 27]. These results point to the importance of chronic vascular lesions of the renal allograft, the so-called transplant vasculopathy (Figure 3), which is characterised by intimal thickening. In heart transplants, the presence of transplant vasculopathy can be precisely evaluated by means of intravascular ultrasound and it is associated with a reduced graft survival. Transplant vasculopathy in the heart can be prevented by the immediate introduction of statins just after transplantation [28] or by the use of everolimus [29]. In the kidney it has not been determined whether transplant vasculopathy could be prevented with statins or proliferative signal inhibitors. In the ALERT study, statins were introduced at the 5th year of follow-up and graft survival was not different in patients receiving fluvastatin or a placebo [30]. In a Spanish epidemiological study, the use of statins during the first year was associated with improved graft survival [31]. Recently, a protective effect of sirolimus on chronic vascular lesions has been described [32]. All these data suggest the possibility that renal transplant patients with trans-
plant vasculopathy in an early protocol biopsy represent a group with an especially poor prognosis that might benefit from specific treatments.

In a recent study of 1 year protocol biopsies performed on children, it has been observed that, when biopsies were classified as normal, chronic allograft nephropathy and chronic allograft nephropathy associated with subclinical rejection, graft survival was shorter in the last group and not different in the first two subgroups [33], suggesting two important messages. The first is that the association of chronic tubulo-interstitial lesions with subclinical rejection implies an ominous prognosis, and the second is that the presence of chronic tubulo-interstitial damage without other lesions is associated with a rather good outcome. This observation was later confirmed in a study including more than 300 adult recipients [34]. Recently in a large study including more than 400 patients, we were not only able to confirm these data, but we also observed that patients with subclinical rejection without chronic tubulo-interstitial chronic lesions, fare as well as patients with a normal biopsy [20]. These observation raise the question why patients displaying both conditions in the protocol biopsy, subclinical rejection and chronic tubulo-interstitial damage show such a bad prognosis. There are two theoretical explanations for this observation. The first is that the activity of the infiltrate may be increased in patients displaying both conditions in comparison to patients showing only subclinical rejection. The alternative explanation is that, even if the severity and activity of infiltrating cells is similar in patients with and without tubulo-interstitial chronic
lesions, the capacity for tissue repair is decreased in patients already displaying renal scarring.

**Conclusions**

Protocol biopsies allow the early detection of subclinical rejection and tubulo-interstitial chronic lesions before renal function deterioration. Both subclinical rejection and chronic allograft nephropathy are associated with a poorer renal allograft survival. The predictive value of chronic lesions evaluated in protocol biopsies is independent of clinical and analytical parameters. Recently, evidence has accumulated suggesting that the association of tubulo-interstitial chronic lesions with transplant vasculopathy, subclinical rejection or transplant glomerulopathy is associated with a poorer outcome than the presence of tubulo-interstitial chronic damage alone, which suggests that different patterns of chronic allograft nephropathy can be recognised by means of protocol biopsies (35).

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Резиме

РИЗИК ФАКТОРИ АСОЦИРАНИ СО ВЛОШУВАЊЕ НА РЕНАЛНАТА ФУНКЦИЈА: УЛОГАТА НА ПРОТОКОЛ БИОПСИТЕ

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Протокол реналните аллографт биопси дозволуваат рана детекција на хистолошкото оштетување дури и пред појавувањето на влошувањето на реналната
функција и протеинуријата. Најголем интерес во студиите со протокол биопсии предизвика две различни лезии: субклиничко отфланање, кое всушност претставува присуство на тубуло-интерстицијално воспаление и хронична алографт нефропатија, сега прифатена под терминот интерстицијална фиброза/тубуларна атрофија (ИФ/ТА), односно, присуство на хронични тубуло-интерстицијални лезии. Инциденцата на субклиничкото отфланање е најголема непосредно по трансплантацијата и се намалува постепено во текот на првите неколку месеци и покрај тоа што оваа состојба перзиства во одреден процент и во протокол биопсните по првата година. Во овие студии на сериите на протокол биопсии, присуството на субклиничкото отфланање е асоциирано со повисока веројатност за прогресија на хроничните тубуло-интерстицијални лезии, а во поново време, беше соопштено дека субклиничкото отфланање кај раните протокол биопсии е асоциирано со постоио преживување на графтот. Инциденцата на ИФ/ТА е со брза прогресија по реналната трансплантација презентирано во вид на експоненцијална крива. Нејзиното присуство е асоциирано со намалено преживување на графтот и нејзината предиктивна вредност за исходот е независна од другите предиктивни фактори на преживување како што се серумскиот креатинин или акутното отфланање. Асоцираноста на ИФ/ТА со трансплантационата васкулопатија, субклиничкото отфланање или трансплантационата гломерулопатија имплицира постоио преживување на графтот отколку во случај на ИФ/ТА без присуство на другите хистолошки лезии. Земено во целост, овие податоци укажуваат на тоа дека протокол биопсните дозволуваат рана детекција на акутните и хронични лезии, како и препознавање на различните типови на оштетување кои се асоциирани со преживувањето на графтот.

Ключни зборови: бубрежна трансплантација, протокол биопсии, субклиничко отфланање, хронична алографт нефропатија, интерстицијална фиброза/тубуларна атрофија.

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Legends for figures

Figure 1. Subclinical rejection characterised by the presence of interstitial infiltrate (long arrow) and tubulitis (short arrow)

Figura 1. Subklini–ko otfrGaњe со карактеристично присуство на интерстициелен инфилтрат (долга стрелка) и тубулит (кратка стрелка).

Figure 2. Chronic allograft nephropathy characterised by the presence of interstitial fibrosis and tubular atrophy (arrow).

Фигура 2. Хронична алографт нефропатија со карактеристично присуство на интерстициелна фиброза и тубуларна атрофија (стрелка).

Figure 3. Transplant vasculopathy characterized by fibrous intimal thickening

Фигура 3. Трансплантациска васкулопатија со карактеристично присуство на фиброзно интимално задебелување.