artículo especial

Guidelines for indicating, obtaining, processing, and evaluating kidney transplant biopsies

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INTRODUCTIÓN

Despite the significant transplant activity in Spain,[1] no consensus has been reached yet in our country about the clinical situations in which a diagnostic biopsy would be recommended, or about how use of protocol or donor biopsies may be improved in clinical practice. Thus, an overall view detects a high heterogeneity between the different centres and even certain practices that may be considered inadequate. For instance, despite the significant evidence advising performance of biopsies in certain donor types or use of protocol biopsies,[2,3] a recent epidemiological study demonstrated that the former only account for 3% of all biopsies performed in Spain every year,[4] while only two Spanish centres perform serial protocol biopsies. An analysis of diagnostic biopsies also shows significant differences as regards activity in the different centres, with up to 10-fold differences being found in the reported biopsy/transplant ratios in certain cases.[4] In addition, no clear agreement appears to exist either about how renal tissue samples should be obtained and processed. There are relevant differences as regards use of different puncture needle gauges, as well as a quite limited use of more specific diagnostic procedures such as immunofluorescence, immunohistochemistry, or electron microscopy.

Because of this situation, a group of clinicians and nephropathologists experienced in kidney transplant jointly addressed, in a consensus conference held in Toledo in 2007, the various issues of interest related to the indication, processing, and evaluation of kidney biopsies in transplant patients. The main conclusions and recommendations on the subject of such consensus group are summarised in this document.

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CLINICAL VALUE OF BIOPSY IN KIDNEY TRANSPLANT

Donor biopsy

Multiple studies have shown that the presence of pre-existing lesions in the donor biopsy is associated to the occurrence of acute rejection, a poorer function, development of subsequent lesions, and a reduced graft survival.[5-20] Despite methodological differences between the different studies and certain disparate results,[21] there is currently a fair amount of agreement in that the finding of glomerular, tubulointerstitial, and vascular lesions in the biopsy at the time of implant represents one of the main donor-dependent factors that may condition kidney graft evolution. Indeed, it has been shown in several series that the finding in the donor biopsy of ~20% glomerulosclerosis is associated to the presence of delayed graft function (DGF) immediately after transplant, and also to a reduced kidney function or long-term graft loss.[8,13-15,18] The presence of tubulointerstitial damage inherited from the donor, such as interstitial fibrosis, tubular atrophy, or acute tubular necrosis, also has a relevant role and is correlated to subsequent development in the recipient of glomerulosclerosis and chronic interstitial damage. Such events significantly influence kidney function and survival of the implanted organ[18-20] and emphasise the importance of indication for performance of this biopsy in the donor using a good analytical methodology.[22]

As regards vascular damage, Nankivell et al found in 2001 that small vessel lesions in the donor predicted for detection of typical chronic nephropathy lesion in biopsies taken at 3 months of transplant,[17] whereas in the long term, detection of fibrous intimal thickening in the biopsy at the time of implant has been associated to the occurrence and severity of interstitial fibrosis, as well as to a reduced creatinine clearance.[12] Based on all the foregoing, it is understandable that interest in performance of donor biopsies has significantly increased in recent years, in parallel to the increasing use of marginal organs from elderly, diabetic, or hypertensive patients. The presence of these factors is obviously associated to development in the kidney of the abovementioned histological lesions,[23,24] which are consistently related to poorer graft evolution outcomes,[25,26] as already discussed.

Histological evaluation of the potential graft thus represents, together with clinical and laboratory data, a procedure that has been used to decide whether a single or double transplant should be performed or organs from that donor should be discarded.[27-29] This is the main current indication for donor biopsy in many centres, but it should not be forgotten that taking a sample at time zero, even in optimum donors, is of great help for adequate interpretation by pathologists of potential subsequent lesions in the recipient. Biopsy should therefore not be limited to the study of graft viability.

Finally, it should also be reminded that biopsy performance in donors is of particular interest in the setting of clinical trials in order to have paired morphological data before and after transplant.

Recipient biopsy

Histological examination of renal biopsy continues to be the test par excellence for diagnostic identification of graft pathology, and its value is shown by the fact that more than 90% of biopsies performed in kidney transplant patients in Spain are indicated for diagnostic reasons.[4] The main value of biopsy lies in the possibility of differentiating in detail the presence of lesions suggesting acute rejection, nephrotoxicity induced by calcineurin inhibitors, or chronic lesions, and also of diagnosing not clinically suspected conditions such as *de novo* glomerulopathies, disease recurrence, or nephropathy associated to polyomavirus type BK. In this regard, introduction in recent years of the Banff criteria and their respective updates[30-33] has allowed for having a reproducible tool[34] with a high clinical-pathological correlation[35] and internationally accepted for description of acute and chronic lesions of the different kidney compartments. In addition, recent incorporation of new histological and marker techniques, such as detection of C4d and donor-specific antibodies, has increased the diagnostic and prognostic capacity of renal biopsy, giving pathologists the possibility of performing increasingly accurate diagnoses. In this regard, the growing volume of evidence accumulated using these procedures recently allowed the Banff conference for proposing introduction of a new concept, late antibody-mediated rejection, as well as criteria required for its diagnosis.[33]

With regard to protocol biopsies, recent studies have been able to establish the natural history of lesions affecting the renal graft,[36] as well as the impact of each of them on transplant prognosis. Use of serial biopsies has revealed that prevalence of interstitial infiltrates and tubulitis in stable grafts, the so-called subclinical rejection (SCR), is maximum during the initial months after transplant, after which prevalence gradually decreases (though SCR may persist for longer than one year in some patients).[38] Presence of these acute lesion, even in cases with minimal inflammation, is consistently associated to the occurrence of chronic lesions such as interstitial fibrosis and tubular atrophy (IF/TA)[36,39-41] and to a decrease in long-term graft survival, as recently seen in patients with SCR lesions two weeks after transplant followed up for 10 years.[42] Once established, IF/TA progresses rapidly during the first months, and despite being clinically silent at the start,[36,43,44] it is also related to a poorer kidney function and to long-term graft loss[45,46] even when detected early at 6 months of transplant.[47] In fact, the predictive value of IF/TA is independent from other classical markers such as serum creatinine, proteinuria, or acute rejection.[48]

In this setting, there has been recently an increasing interest in detection of lesion patterns in biopsies that would help predict transplant evolution better and earlier than separate lesion assessment. The combination of IF/TA and vascular damage has been shown to be significantly associated to a poorer 10-year graft survival as compared to IF/AT alone,[49] and similar results were seen at 5 years in patients with IF/TA lesions and transplant glomerulopathy.[50] Moreover, the combination of acute and chronic lesions also predicts for poorer long-term results, so that IF/TA has a particularly poorer prognosis when it is concomitantly associated to the presence of SCR.[41,50,51] In fact, certain lesion patterns found in protocol biopsies within 6 months of transplant, such as the presence of IF/TA plus transplant vasculopathy or IF/TA plus SCR, have shown no less sensitivity and specificity for predicting 7-year graft viability than classical markers such as acute rejection or kidney function.[3]

It is also particular interesting to note that protocol biopsies could have a significant role for improving graft prognosis. There are two reports on this regard that, despite their methodological limitations, support this statement.

Table I. Guidelines for indicating, obtaining, processing, and evaluatin donor biopsies

Indication

- Expande criteria donors (advisable in all transplants to assess lesion progression).
- All clinical trials including protocol biopsies.

Sampling and size ob biopsy

- Using needle or wedge (5 x 5 x 5 mm).
- In representative areas of the renal morphology of both donor kidneys.
- Minimum sample size should be 50 glomeruli between both kidneys (no less than 15 in the sample with less glomeruli) with 2 or more arterial sections containing internal elastic lamina.

Processing

- Rapid processing in paraffin using microwave oven technology.
- Histological section thickness of 3-4 microns.
- HE and PAS staining (Masson's trichromic recommended).

Evaluation

- Joint evaluation (both kidneys from a same donor) following Banff 97 criteria.

HE: Hematoxylin-eosin; PAS: Periodic acid-Schiff.

The first report refers to a study where protocol biopsies were performed 1, 2, 3, 6, and 12 months after transplant. In this study, Rush et al found that detection and early treatment of SCR with corticosteroid boluses resulted in a decreased progression of lesions at 6 months and a better kidney function at 2 years as compared to the control group.[52] In the second study, Buehrig et al reported that early detection of nephropathy by the polyomavirus using protocol biopsies within one year of transplant allowed for modifying immunosuppression in early disease stages. At 6 months, graft survival and function in these patients were significantly superior to patients in whom the disease was detected late when a diagnostic biopsy was performed for kidney function impairment.[53]

In conclusion, the biopsy not only represents the best alternative for diagnostic evaluation of renal graft lesions, but is also a good tool for prognostic and viability assessment of the graft. Based on the available evidence, it has been stated that protocol biopsies could be considered as a surrogate marker for graft survival,[54] and several groups of researchers are currently focusing their efforts on the search for different lesion patterns, as well as new quantitative parameters for assessing these biopsies, that would improve their predictive value.[3] Finally, the significant value of protocol biopsies as secondary endpoint in the setting of clinical trials should not be forgotten. A remarkable experience in this field has already been obtained in several studies intended to describe potential differences in the impact of the different immunosuppressive treatments on renal histology.[55-58]

RECOMMENDATIONS FOR PERFORMING DONOR BIOPSIES (table I)

Indications

Definite indication

In recent years, the growing disparity between the number of patients in a waiting list for receiving a kidney transplant and the number of grafts available has prompted the need for expanding the criteria for considering a kidney adequate to be transplanted (59). In 2001, the UNOS (United Network for Organ Sharing) defined expanded criteria donors (ECD) as cadaveric donors aged ≥ 60 years or with ages ranging from 50 and 59 years and at least 2 of the following 3 risk factors: death from stroke, hypertension, or end-stage serum creatinine levels < 1.5 mg/dL (60). Several series have reported that implantation of grafts from ECDs is associated to an increased risk of primary dysfunction and DGF, and also to a long-term reduction of graft and recipient survival.[61-63] Thus, performance of a biopsy in these suboptimal donors may provide significant prognostic information[2,29] to help make clinical decisions about organ viability, and significant improvements

in transplant evolution have recently been found using this strategy.[27] In view of the foregoing, a biopsy is advised in all grafts from ECDs in order to assess viability of organs and to help take an adequate decision about in what recipients must they be implanted, as well as to ascertain the baseline histological status to allow for subsequent evaluation of changes in graft lesions.

It is also considered appropriate to have a biopsy from the donor (standard or expanded) in all clinical trials including protocol biopsies to assess new drugs or changes in the immunosuppression regimen existing at transplant time zero in order to more adequately interpret lesion changes over time.

Indication recommended

In standard donors not enrolled into any clinical trial, biopsy is recommended (but not mandatory) to be able to monitor progression of graft lesion after transplant. However, biopsy advantages should be weighed against the potential risks of the procedure for the patient, mainly the bleeding risk. The financial impact of this strategy should also be considered. The decision must therefore be taken after assessing the risk-benefit ratio in each individual patient.

Sampling and size of biopsy

By way of introduction, it should be emphasised that the whole biopsy procedure (sampling, processing, and evaluation) should not significantly lengthen the cold ischaemia time.

In donors, needle or wedge biopsies may be interchangeably taken (according to the standard practice at the centre) from a representative area of renal morphology. It is recommended to take a sample from both donor kidneys. To try and ensure an adequate sample size, wedge biopsies should be no less than 5 x 5 x 5 mm in size. Care should be taken not to exceed 1 cm in depth to avoid compromising arcuate arteries, which would increase the risk of bleeding after implant surgery. In fact, prognostic implications derived from the potential complications associated to donor biopsy, such as the presence of perirenal haematoma, have not been adequately analysed yet. Hence, in the absence of clinical evidence, close laboratory and ultrasound monitoring of patients receiving a biopsied renal graft is recommended.

As regards sample size, and since one of the most interesting aspects to be studied in donor biopsies is glomerulosclerosis, it is extremely important that samples include a minimum number of glomeruli that allows for estimating the percent sclerosis of the graft with an acceptable coefficient of variation. Remuzzi et al suggested some years ago that at least 25 glomeruli in each kidney were required to adequately calculate the percentage of glomerulosclerosis in the donor.[28] In view of the good results subsequently seen using the proposed model,[27,28] 50 glomeruli (adding samples from both kidneys) are recommended as the minimum sample size that should always be attempted to be taken. Below this number, the value of biopsies for estimating glomerulosclerosis has a low reliability, as shown in the simulation in Figure 1. It is also advised that the kidney sample with less glomeruli has no less than 15. It is additionally recommended that the donor biopsy includes at least 2 arterial sections containing internal elastic lamina to be able to adequately assess the chronic vascular damage in the donor (it is generally advised that the biopsy report describes the number of glomeruli and arterial sections obtained).

Finally, a good practice in relation to this procedure would be to implement a biopsy registry for each centre to record data from its donor biopsies, in order to be able to retrospectively assess after a time whether its methodology allow for consistently obtaining an adequate amount of material.

Processing

Taking into account the importance of clinical decisions depending on donor biopsy, it is recommended that, to the extent possible, both the technician handling tissues and the pathologist are experts in renal pathology.

Most transplant centres in Spain still use today freezing procedures for processing donor samples. The study of frozen tissue allows for an adequate assessment of the percentage of glomerulosclerosis and arterial intima thickening, provided vessels are cross-sectioned. Sample viability usually depends to a great extent on the quality of freezing and the section made. Adequate evaluation of interstitial fibrosis and tubular atrophy is difficult if tissue processing has not been adequate. Moreover, assessment of arteriolar hyalinosis, which is a key morphological datum for some authors,[9] is highly complex even in good quality samples. By contrast, it is well established that paraffin embedding of tissues allows for a detailed evaluation of the different renal compartments,[22] though it is true that there is currently no comparative study in renal biopsies from donors assessing the performance of this procedure as compared to frozen sections. Most these approximations have occurred in the setting of histological study of breast cancer, where significantly poorer diagnostic results have been noted with frozen material, so that its use is currently advised against, even for intraoperative biopsies.[64] Based on the foregoing, in cases where the purpose of the renal donor

biopsy is to assess organ viability for transplant, use of rapid processing in paraffin using microwave oven technology (~ 3 hours) is recommended.[65] In this regard, it would be convenient that such rapid processing could be centrally performed in a healthcare area, so that a permanent 24-hour service would be provided to give logistic support to centres that required it.

The recommended thickness of histological preparations is 3-4 microns for samples processed in paraffin, and 6-10 microns for frozen samples. In general, all samples should always be stained with hematoxylin-eosin (HE) and with the PAS (periodic acid-Schiff) stain. Use of Masson`s trichromic stain for adequate assessment of interstitial fibrosis is also advised.

Table II. Model proposed for the study of donor biopsies in the autonomous community of Andalusia (under review)[66]

1. Glomeruli sclerosed or with total atrophy due to cyst formation

0: Absence.
1: 0-10%.
2: 11-20.
3: More than 20%.

Note: Subcapsular involvement alone shoud not be considered as a condition excluding the organ unless it is associated to relevant tubular vascular disease of the underlying parenchyma.

2. Hyaline arteriolar disease

0: no Pas (+) hyaline thickening of ateriolar walls.
1: Mild to moderate PAS (+) hyaline thickening in at least one arteriole.
2: Moderate to severe PAS (+) hyaline thickening in more than one arteriole.
3: severe Pas (+) hyaline thickening in most arterioles.

3. Fibrous thickening of the vascular intima

0: Absence of chronic vascular lesions.
1: Less than 25% narrowing of vascular lumen from myontimal thickening.
2: Increase in lesions reported in 1 involving from 26%-50% of vascular lumen.
3: Increase in lesions reported in 2 involving more than 50% of vascular lumen.

4. Tubular atrophy

0: Absence of cortical tubular atrophy.
1: Less than 25% of atrophic cortical tubules.
2: 26%-50% of atrophic cortical tubules.
3: More than 50% of atrophic cortical tubules.

5. Interstitial fibrosis

0: 5% or less of cortical area involved.
1: 6%-25% of cortical area involved.
2: 26%-50% of cortical area involved.
3: More than 50% of cortical area involved.

Total score assessment:

Renal sample with favourable histology if score is ≤ 7.

Evaluation

There is currently no agreement about how should donor renal biopsies be assessed, and there are hardly any validated scales for that purpose. To date, some centres and institutions have proposed their own local protocols for the study of donor biopsies, such as the one several hospitals from Andalusia are now completing for such region (table 2).[66] In 2006, Remuzzi et al followed the scheme previously developed by an expert panel[28] to evaluate the viability of kidneys from donors over 60 years of age. The empirically propose system consists of evaluation of chronic damage in all four renal compartments using a semiquantitative scale graded from 0 to 3. Results are added, and grafts with a score ranging from 0 and 3 are used for single transplant, those with a score ranging from 4 and 6 are used for double transplant, and grafts with a score ≥ 7 are discarded. Authors of this study noted that long-term survival of grafts selected using this scale was significantly longer as compared to a cohort of patients who had also been implanted grafts from donors older than 60 years but with no prior histological assessment.[27] Despite the good results reported, the model in question is not free from certain limitations such as, for instance, the small number of patients included in the study, the fact that single and double transplants were included, and the need for finding a very severe glomerulosclerosis (> 50%) for this item to be given the maximum score in the proposed scale.

In the last Banff meeting, held in La Coruña in 2007, evaluation of donor biopsies following the criteria established by the Conference itself was advised,[67] but most transplant groups have been indeed using for some time the Banff scheme[31] for testing organ viability before transplant. While such protocol was not specifically developed for such purpose, it is clear that it adequately describes the presence of chronic lesions, and the value of its application to donor biopsy has recently been shown. Indeed, Lopes et al found in 2005 in paraffin-processed samples that evaluation according to Banff of the donor biopsy has a predictive power of kidney function and graft survival similar to morphometric evaluation. Based on a detailed statistical analysis, authors

Table III. Guidelines for indicating, obtaining, processing, and evaluating diagnostic biopsies in kidney transplant recipients

Indication

- Patients with DGF if worsening is seen in the renogram or DGF lasts longer than 2-3 weeks.
- Patients with kidney function lower than expected based on donor characteristics after the first post-transplant months.
- Patients with sudden graft function impairment attributable to kidney disease.
- Patients with a progressive increase in creatinine levels (≥ 20% from creatinine nadir) over a period of 3-6 months.
- Patients with proteinuria > 1 g.
- Patients with sediment changes without apparent urological causes.
- Advisable before changes in immunosuppressive treatment.

Sampling

- Sampling of 2 cores using a 16 G needle.
- Use of automatic guns and ultrasound guidance.

Processing

- Tissue embedding in paraffin according to Banff methodology and staining with HE, PAS, and Masson's trichromic.
- Processing of frozen tissue for measuring immunoglobulins and complement using immunofluorescence.
- C4d staining of all diagnosctic biopsies.
- Use of immunohistochemistry or electron microscopy procedures (at the pathologist's discretion).

Reporting

- Report the presence and severity of lesions in the different renal compartments using Banff criteria.
- Report the number of glomeruli present in the sample and the percentage of glomerulosclerosis.
- Describe the biopsy diagnoses.
- Discuss lesion changes if a donor biopsy is available.

DGF: Delayed graft function; HE: Hematoxylin-eosin; PAS: Periodic acid-Schiff.

proposed a result adding system taking into account glomerulosclerosis (assessed as absent or present depending on whether the rate of sclerosed glomeruli was more or less than 10%), the grade of interstitial fibrosis (ci-score), and chronic vascular damage (cv-score) evaluated according to Banff criteria. In this study, grafts with a result from 0 to 2 were seen to be useful for single transplant, and grafts with a result of 3, because of their poor evolution, could be considered for double transplant.[29]

As a conclusion, until the various local protocols are adequately validated, this consensus group thinks that the most recommendable approach today is to perform histological evaluations of donor biopsies following Banff criteria[67] in order to meet international guidelines. It is also advised that biopsies from both kidneys of a same donor are jointly evaluated whenever no gross differences suggesting the existence of a specific pathology in any of them are seen. The main general advantage of using the Banff criteria for donor biopsies is that this approach allows for an easier study of lesion progression when recipient biopsies are assessed. However, information currently available is still limited as to advise an algorithm based on the sum of results for accepting or discarding a graft.[27,29] On the other hand, if only frozen tissue is available, the percentage of glomerulosclerosis will only be assessed because of the difficulty for studying interstitial fibrosis and tubular atrophy in these samples. In this regard, a consensus document of the Spanish Society of Nephrology has proposed that if more than 20% glomerulosclerosis is found, a double transplant is considered.[59]

RECOMMENDATIONS FOR PERFORMING BIOPSIES IN KIDNEY GRAFT RECIPIENTS (table III)

Indications for diagnostic biopsy

As a general recommendation, transplant groups are urged to maintain an active attitude to diagnostic biopsy, that should always be indicated early when any problem is suspected, and not be delayed until the clinical condition is already very advanced.

Table IV. Kidney function (glomerular filtration rate estimated by the abbreviated MDRD formula) seen in kidney transplant patients three months after transplant by donor age (data from Hospital Universitario Ramón y Cajal, Madrid)

Donor age (years)	N	Glomerular filtration rate (mL/min) at 3 months (mean ± SD)	Median
< 30	359	55.4 ± 21.2	53.7
30-40	131	50.6 ± 20.1	47.8
40-50	179	50.9 ± 17.7	48.8
50-60	142	42.0 ± 15.9	41.5
60-70	114	36.5 ± 14.5	34.8
> 70	26	33.7 ± 9.0	31.9

SD: Stándar deviation.

Delayed graft function (DGF)

DGF represents a form of acute renal failure that is associated after transplant with an increase risk of acute rejection and long-term graft loss.[68] In large multicentre studies, DGF has been clinically defined as the need for at least one dialysis session during the first week after organ implantation.[69] As discussed, several donor-dependent factors are associated to the occurrence of DGF, but there are also multiple factors present during transplant and in recipients themselves that promote delayed function.[68] In fact, one of the most important factors is the degree of sensitisation of recipients, that may lead to the development of antibody-mediated early rejection.

Based on the foregoing, when DGF occurs in kidney transplant patients with a standard risk (not hyperimmunised), indication of a diagnostic biopsy after the first post-transplant week (between days 7 and 12) is recommended in order to find and treat the underlying cause early. In hyperimmunised patients, this biopsy must be performed at an earlier time, after considering the results of supplemental tests (renogram, ultrasound, and Doppler ultrasound).

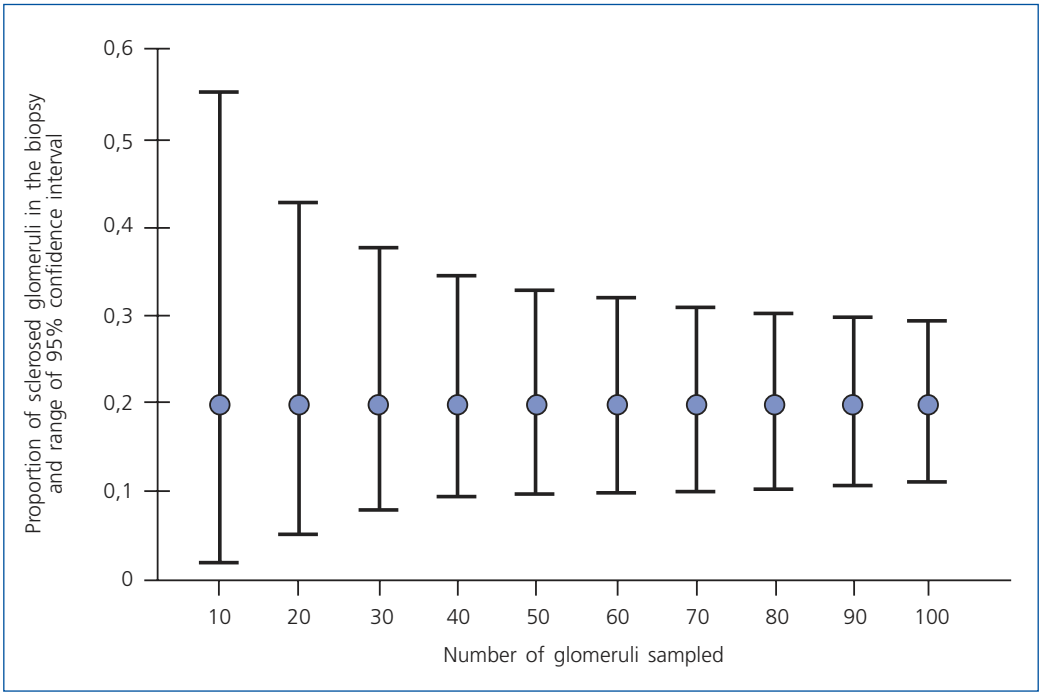

Figure 1. In this figure it is represented how the 95% confidence interval decreases in a biopsy with 20% sclerosed glomeruli as the number of glomeruli increases.

Suboptimal function

Organ function after transplant depends on both donor and recipient characteristics, and it is really difficult to precisely establish in each case what the expected kidney function of a graft should be based on such characteristics. Despite this, and though this is not an universally extended practice yet, there has been an increasing interest in recent years in biopsying patients who stabilise in serum creatinine values that are not adequate or are higher than could be expected. Such suboptimal levels could suggest the presence in the graft of early lesions that would already be affecting its function. Table 4 shows the mean glomerular filtration rates seen in kidney graft recipients three months after transplant, classified by donor age. Based on data shown in this table, performance of a diagnostic biopsy is advised in patients who have after the first few months since transplant a glomerular filtration rate more than two standard deviations below the expected mean based on donor characteristics.

Acute impairment

A diagnostic biopsy should always be performed in transplant patients who experience a sudden kidney function impairment, defined as a rapid increase (1-2 days) in serum creatinine levels greater than 10%-25% as compared to the previous measurement.[70] Such increase has to be attributable to parenchymal kidney disease, and possible prerenal or obstructive causes should therefore previously excluded.

Chronic impairment

Chronic graft dysfunction, characterised by a progressive impairment in kidney function associated to the occurrence or worsening of arterial hypertension and proteinuria, is the most significant determinant of long-term organ survival.[71] In these patients, gradual increase in creatinine levels over time, a phenomenon known as «creeping creatinine», is considered a characteristic suggesting such dysfunction,[72] and it has been suggested that its definition requires demonstration of the existence of a negative slope in the creatinine inverse using a minimum of 6 measurements performed during the last months of follow-up (from 3 to 18 months).[73] Thus, and in order to diagnose graft lesions in their initial stages, early biopsies are recommended in patients showing in a minimum approximate period of 3-6 months a ≥ 20% increase in serum creatinine levels as compared to creatinine nadir, whether or not associated to proteinuria.

Proteinuria

A biopsy is recommended in patients with proteinuria greater than 1 g, particularly if they are treated with angiotensin converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs), because the risk of diseases such as chronic nephropathy or recurrence of the primary disease is greater in these cases.

On the other hand, there is still few experience today with performance of biopsies in patients with proteinuria values ranging from 0.5-1 g (with or without treatment with ACEIs or ARBs) to determine whether histological diagnosis may help modify the natural history of lesions. In this regard, centres are advised to record data from biopsies performed in patients with this profile to be able to make recommendations on this matter later.

Sediment changes

When sediment changes are found in kidney transplant patients (persistent microhaematuria or casts in urine).

Changes in immunosuppression

A kidney biopsy is recommended before changes are made in immunosuppressive treatment in order to be able to subsequently assess the potential evolution of graft lesions with the new drug regimen.

Technical aspects of diagnostic biopsy

Needle size and number of cores

Sampling of adequate material representative of renal cortex, together with minimisation of the potential morbidities associated to the process (such as gross haematuria, arteriovenous fistula, or perirenal haematoma),[4,74] represent the main objectives of diagnostic biopsy in transplant patients. There are currently available 3 needle gauges for performing renal biopsies: 14, 16, and 18 G. The larger gauge needle (14 G) obviously allows for obtaining a greater amount of tissue, but its use is usually associated to greater patient discomfort (75), as well as a greater incidence and extension of post-biopsy haematomas and a significant decrease in haematocrit.[76] In fact, use of this type of cores is currently quite neglected. Use of 16G needles is preferred because they offer a better relationship between sampling of an adequate amount of tissue and development of complications. It is generally recommended to always take 2 cores to ensure an adequate sample size.

In addition, it is advised that, to the extent possible, biopsies are performed by experienced staff using ultrasound guidance. Use of automatic guns is recommended over manual biopsies.

Processing

With regard to processing, it is first recommended to watch the cores obtained at biopsy with a stereoscopic loupe in order to be able to select the most adequate material to be processed for imunofluorescence and electron microscopy. Tissue selected must then be embedded in paraffin following the methodology described in the Banff protocol.

All samples should be studied with light microscopy using HE, PAS, and Masson's trichronic stains. It is also recommended to take a sample of frozen tissue for measuring immunoglobulins and complement using immunofluorescence techniques, particularly in biopsies performed from 6 months after transplant, though such measurements are also recommended in the first 6 months. In this regard, C4d deposit in peritubular capillaries has been associated both to acute rejection and progressive graft function impairment,[77-79] and all biopsies performed for diagnostic reasons must therefore be generally stained with C4d (preferably with immunofluorescence, because it provides for a greater sensitivity and easier observation). On the other hand, in situations requiring special immunohistochemistry procedures (such as polyioma, CMV, or lymphomas), these will be performed when indicated by the pathologist because clinical or pathological suspicion exists about the paraffin-embedded material, for which new histological sections of the material should be done. Similarly, and while use of procedures for characterizing leukocyte populations infiltrating the kidney is not standardized, there are data suggesting that persistence of certain cells, such as macrophages or B cells, is associated to a poorer prognosis.[81-84] Pathologists are therefore encouraged to perform these measurements and to report more data to increase understanding in this area.

Finally, it is also advisable to process a sample of biopsy tissue for electron microscopy, particularly in biopsies performed from the sixth month, though such processing could also be diagnostic immediately after transplant (such as in the event of early relapse of segmental and focal glomerulosclerosis). Such samples will be studied in situations where a diagnosis is not achieved with the prior procedures or when the pathologist deems it appropriate. In this regard, electron microscopy is of particular value in cases where differential diagnosis should be made between similar patterns of glomerular lesion (mesangiocapillary glomerulonephritis, chronic thrombotic microangiopathy, or transplant glomerulopathy) and to show «capillaropathy» in the peritubular capillary.[85,86]

Biopsy report

Pathologists are recommended to give in the biopsy report their evaluation of lesion severity at each renal compartment according to Banff criteria.[31] The number of glomeruli present in the sample and the percentage of glomeruli sclerosed, biopsy diagnoses, and any other comment considered of interest by the pathologist should also be included. Moreover, in cases where a donor biopsy is available, it is advisable that the pathologist's report includes a comment about progression of the lesion seen in the current biopsy.

Protocol biopsies

There is current evidence that protocol biopsies represent a surrogate measure of graft survival,[3] hence the increasing interest in including them as secondary endpoints in transplant clinical trials.[87] However, no adequate information allowing for recommending use of protocol biopsies in order to modify immunosuppression in transplant patients based on histological findings is still available. This consensus group therefore recommends that protocol biopsies are included in all clinical trials comparing the different immunosuppressant drugs.

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