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NEFROLOGIA

SPANISH CONSENSUS DOCUMENT ON CHRONIC ALLOGRAFT NEPHROPATHY (CAN)

Editors

D. Hernández, A. Sánchez Fructuoso and D. Serón

Authors

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ADDENDUM NCT

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NORMAS

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ABBREVIATIONS

ACE	Angiotensin-converting enzyme
AHT	Arterial hypertension
AR	Acute rejection
ARA	Angiotensin receptor antagonist
BD	Brain death
CA	Calcium antagonist
CDC	Complement-dependent cytotoxicity
CMV	Cytomegalovirus
CNI	Calcineurin inhibitor
CR	Chronic rejection
CsA	Cyclosporine
CT	Clinical trial
CAN	Chronic allograft nephropathy
DA	Donor age
DBP	Diastolic blood pressure
DGF	Delayed graft function
DNA	Deoxyribonucleic acid
EBM	Evidence-based medicine
ELISA	Enzyme linked immunoabsorbent assay
HCV	Hepatitis C virus
MBP	Mean blood pressure
MHC	Major histocompatibility complex
MMF	Mycophenolate mofetil
PCR	Polymerase chain reaction
RCT	Randomized clinical trial
RGF	Renal glomerular filtrate
SBP	Systolic blood pressure
SRL	Sirolimus
TAC	Tacrolimus
UNOS	United Network Organ Sharing
USRDS	United States Renal Data System

PREFACE

There is an enormous amount of information in the medical literature, from experimental studies to controlled clinical trials (CT), which would be impossible to examine due to limitations of time and resources in daily clinical practice. However, only a small part of this information responds immediately and accurately to the questions we often ask. From a clinical viewpoint, evidence-based medicine (EBM) could represent the best approach. Conceptually, EBM enables us to use the best scientific contributions to improve prevention, diagnosis and treatment of diseases. In other words, it helps us to optimize clinical practice by applying the best evidence from the most conclusive clinical trials[1]. EBM does not claim that randomized trials and meta-analyses are the only way forward in daily clinical practice. Rather it merely attempts to avoid inefficient therapeutic approaches based on contributions or procedures not supported by sufficient scientific evidence. Before we can attain clinical excellence, a suitable seam between professional experience and the most important scientific contributions is necessary. In diagnosis and prognosis, cross-sectional or cohort studies (prospective or retrospective) can also contribute a suitable level of evidence as long as they provide reliable, audited data and are analyzed using powerful regression models (Cox proportional analysis, logistic regression, propensity analysis, etc). In these terms, the information from registers and large databases such as the Spanish Register for the Study of Chronic Allograft Nephropathy is a valuable clinical tool to improve the prognosis of this condition[2-5].

From this viewpoint, the level of evidence of the published studies has been classified according to its scientific quality. In particular, meta-analyses and controlled CT with large numbers of patients are at the top of the classification, whereas studies from registers or databases occupy lower positions. Thus, Clinical Practice Guides or therapeutic, prognostic and diagnostic guides have been developed, expressed as levels of evidence according to the relative scientific qualtity of these studies (table I). Basically, a level-A recommendation for therapy would be supported by a systematic review of controlled clinical trials (Level 1a) or a controlled clinical trial with a narrow margin in the confidence interval (Level 1b), and would represent suitable evidence for recommending a specific approach. With regard to prognosis and diagnosis, systematic reviews of cohort studies with validated clinical decision guides in different centres and populations would be the corresponding options. A level-B recommendation (Evidence level 2a, 2b or 3) in diagnosis would be supported by the systematic review of cohort studies (Level 2a) or by an individual cohort study, including those with an incomplete follow-up and low-quality clinical trials (Level 2b) or a systematic review of case-control studies (Level 3a). In terms of diagnosis and prognosis, this corresponds to retrospective cohort studies (historical cohorts), non-consecutive exploratory cohort studies or follow-up of clinical trial control groups. Thus, level B would provide evidence to support a recommendation. Finally, levels C and D do not provide a sufficient level of evidence to support a recommendation.

Few areas of medicine need the integration of clinical practice with the best evidence possible as much as kidney transplantation. The wide variability in the clinical management of these patients may possibly be due to an imbalance between the abundant level of evidence in some areas, such as acute immunological dysfunction, compared with others, such as chronic allograft nephropathy (CAN), which lacks this scientific backing. In fact, despite the potent current therapeutic arsenal and significant decrease in the rates of acute rejection, CAN is still the main cause of long-term graft loss. Immunological and non-immunological factors contribute to the development of this entity, but it is still not clear which are the best therapeutic options in order to avoid this complication.

In 2002, this concern led to the publication of the European Best Practice Guidelines for Renal Transplantation[6] in an attempt to improve the results of different aspects of kidney transplant, including CAN. Obviously, the large number of studies published since then means that our knowledge in this area must be updated. Parting from this premise, a group of experts in the field of kidney transplantation has reviewed in-depth each of the clinical aspects of CAN to draw up a consensus document that would optimize the management of this condition. Essentially, it tries to provide a global vision of the magnitude of the problem, of the risk factors involved in its pathogenesis, of the most sensitive diagnostic methods and of the most promising therapeutic strategies in CAN. As a result, some recommendations are made in the area of diagnosis, prognosis or therapy beginning with the maximum level of evidence from

Table I. Modified levels of evidence from the Oxford Center for Evidence-Based Medicine[7]

Recommendation	Level of evidence	Treatment-Prevention	Prognosis	Diagnosis
A	1a	Systematic review (with homogeneity*) of RCT	Systematic reviews of prospective cohort studies (with homogeneity*); Clinical decision rules§ validated in different populations	Systematic reviews (with homogeneity*) of prospective cohort studies; Clinical decision rules§ validated in different centers
A	1b	RCT (with narrow confidence interval)	Study of a prospective cohort with ≥80% follow-up; Clinical decision guidelines§ validated in a single population	Cohort study by validating specific diagnostic tests based on previous experience with good** reference standards; Validated clinical decision guidelines § in a single center
B	2a	Systematic review (with homogeneity*) of cohort studies	Systematic review (with homogeneity*) of either retrospective cohort studies (historical cohorts) or untreated control groups in RCTs	Systematic review (with homogeneity*) of > level 2 diagnostic studies
B	2b	Individual cohort study (including low-quality RTC; e.g., <80% follow-up)	Retrospective cohort studies or follow-up of untreated control patients in an RCT group	Exploratory cohort study with good** reference standards; Validated clinical decision rules on split samples or databases
B	3a	Systematic review (with homogeneity) of case-control studies		Systematic review of 3b and better studies
B	3b	Individual case-control study		Non-consecutive case series or without consistently applied reference standards
C	4	Case series (and poor-quality cohort or case-control studies §§)	Case series (and poor-quality prognostic cohort studies***)	Case-control studies with poor or non-independent reference standards
D	5	Expert opinion without explicit critical appraisal, based on physiology or preclinical research	Expert opinion without explicit critical evaluation, based on physiology or preclinical research	Expert opinion without explicit critical appraisal, based on physiology, or bench research

*: Does not have worrisome variations (heterogeneity) between the results between individual studies.

RCT, randomized clinical trial

§: Clinical decision rules: scoring systems or algorithms which lead to a prognostic estimation or diagnostic category

**: The good reference standards are independent of the diagnostic test and can be applied blindly

§§: By poor-quality cohort study we mean one which failed to clearly define comparison groups and/or failed to measure exposures or outcomes in the same way in the exposed and non-exposed individuals and/or failed to identify or appropriately control known confounders and/or failed to carry out a sufficiently long and complete follow-up of patients. By poor-quality case-control study we mean one which failed to clearly define comparison groups and/or failed to measure the exposures and outcomes in the same way in both cases and controls and/or failed to identify or appropriately control known confounders.

***: By poor-quality cohort study we one in which sampling is biased in favor of patients who already had the target outcome or the measurement of target outcomes was determined in at least 80% of the study patients, or outcomes were determined blind or non-objectively **CWOor** there was no correction of confounding factors.

the different studies in each of these areas. As is to be expected, much of the medical information on CAN is based on studies of registers or large databases that lack the scientific rigor of CT and, therefore, only reach evidence levels B or C. Bearing this in mind, some recommendations are not always backed up by high-quality scientific studies, and sometimes this recommendation reflects the particular vision of the experts based on their experience and an exhaustive review of the field in question.

In any case, the recommendations made in this consensus document should not be considered as definitive, but should remain open to future modifications that will come about as a result of therapeutic or diagnostic innovations in the field. Therefore, they should be reviewed and adapted regularly as relevant scientific research appears.

Here, we would like to acknowledge the collaboration of the experts involved in the preparation of these guidelines, as well as Wyeth laboratories for their constant logistic support. Lastly, we hope this effort is another small step on the road to prolonging graft survival and that of the kidney transplant recipient.

1. Impact of CAN on transplant outcome

INTRODUCTION

Kidney transplant is the best therapeutic option for patients with end-stage renal insufficiency[8]. Kidney transplant outcome has improved significantly during the last twenty years thanks to improved surgical techniques, better medical attention, prevention and treatment of infections, but above all thanks to advances in immunosuppression[9]. In this sense, the contribution of the new immunosuppressive agents has been very important, since the incidence of acute rejection during the first few months after transplant has fallen dramatically by as much as 15-20%. Even though outcome has obviously improved during the first year (90-95% graft survival), long-term survival has not been as good, for two basic reasons: the continuous loss of grafts after the first year, and the death of patients with a functioning graft, as a result of cardiovascular involvement[10].

Chronic allograft nephropathy is the first cause of graft loss after the first year post-transplant, and is followed by death of the patient with a functioning graft[2,11]. It is currently the main problem for kidney transplant recipients. The importance of this entity can also be seen in the fact that chronic graft failure is one of the main causes of chronic renal insufficiency. In fact, almost 20% of all transplants carried out in the USA are for patients who have already had one or two previous transplants[10,11].

CLINICAL AND HISTOLOGICAL DEFINITION OF CAN

Chronic allograft nephropathy is a clinical-pathological entity with a multifactorial origin characterized by vascular and tubulo-interstitial involvement accompanied by a progressive deterioration of renal function, hypertension and proteinuria[10,12].

The term «chronic alograft nephropathy» is more widely accepted today than the traditional term of «chronic rejection», since it has been clearly shown that not only immunological factors but also non-immunological factors contribute to the development of chronic graft lesion (see below). In fact, in line with the results of Nankivell et al., CAN represents the damage accumulated and increased by immunological and non-immunological causes during its development[13].

From a histological viewpoint, it is defined according to the Banff criteria as the presence of interstitial fibrosis and tubular atrophy that may or may not be associated with the presence of transplant vascular disease[14] (see below).

PREVALENCE AND INCIDENCE

The prevalence of CAN taken as the number of patients to be diagnosed in a specific time is measured using histological criteria after renal transplant protocol biopsies. In the different published studies of protocol biopsies, the prevalence of CAN affects approximately 35% of grafts at 3 months, 50% at 12 months and 66% at 24 months[15,16]. In the Nankiwell 10-year series of kidney and pancreas transplants with protocol biopsies, histological data for CAN grade I were observed in 94.5% of cases during the first year after transplant. At ten years, severe CAN was evident in 54.5% of patients, whereas nephrotoxicity induced by anti-calcineurin drugs was almost universal[13].

The incidence of new cases of CAN could be around 60% in protocols with CsA and sirolimus, and 30% in regimens with sirolimus after suspending CsA[17].

NATURAL HISTORY IN CLINICAL TERMS

CAN normally appears in patients who have had several episodes of acute rejection, especially when these are multiple and late-onset, or single episodes with incomplete recovery of the renal function, understood as the return to baseline serum creatinine before rejection. Sometimes it presents in patients who have never had a rejection episode, and is occasionally related to non-adherence to therapy. It is characterized clinically by a progressive, asymptomatic deterioration of renal function, accompanied

by variable-grade proteinuria and arterial hypertension (AHT)[10,18].

Proteinuria is usually moderate, between 1 and 3 grams per day, but it may be in the nephrotic range. Very often, when nephrotic-range protenuria is present (more than 3.5 g/day), it is not accompanied by hypoproteinemia or hypoalbuminemia. Nevertheless, on other occasions, there may be complete nephrotic syndrome, which is sometimes related to transplant glomerulopathy. In fact, CAN is the most frequent cause of nephrotic syndrome in kidney transplant recipients[18].

The progress of CAN to graft failure is variable, and may range from months to years. In most cases the deterioration is slow but inexorable, although in some cases there may be a spontaneous decrease in the rate of kidney function loss. As is the case with chronic renal insufficiency in native kidneys, poor monitoring of AHT and massive proteinuria are poor prognostic factors and favor kidney impairment. In this sense, the introduction of hypotensive drugs and anti-proteinuria drugs can slow up the progression to end-stage renal failure[10].

The differential diagnosis must be AHT, including stenosis of the graft renal artery, chronic nephrotoxicity due to CsA or tacrolimus and recurring or de novo glomerulonephritis. A correct diagnosis of CAN needs a graft biopsy, to provide prognostic histologic data about graft survival. It must be stressed that chronic nephropathy lesions can sometimes coexist with nephrotoxicity or glomerulonephritis lesions[10,13,18].

CAN, HALF-LIFE AND GRAFT URVIVAL

The graft half-life is defined as the time 50% of patients who survive after the first year post-transplant remain alive and with the graft functioning[19]. Therefore, half-life is determined by the death rate and the return to dialysis. In fact, half-life is a measure of graft survival, in other words, of the late loss of the graft. Half-life is based on a survival forecast and is calculated using the following formula:

$$\text{Estimated half-life} = T \log(2) / n \text{ of events}$$

«T» being the accumulated survival after the first year and *«n of events»* the number of patients dead or the number of grafts which stopped functioning after the first year.

CAN, HALF-LIFE AND PATIENT AND GRAFT SURVIVAL

Recently, an increase in the half-life of kidney transplants in the USA have been: for patients who received a cadaveric transplant in 1988, the forecasted half-life without censoring death was 7.9 years, whereas in 1995 it was 13.8 years[20]. The increase in half-life was clear in patients who did not experience acute rejection. In live donor transplants during the same years, half-life was 12.7 and 21.6 years, respectively. However, this improvement was not corroborated when the real half-life analysis was carried out[21]. In patients who received a cadaveric transplant in 1988, the half-life was 6 years and in 1995 it was 8 years. In fact, there was only a slight improvement of two years in graft survival. In this analysis, the patients with the best improvement were clearly the high-immunological risk patients: retransplant recipients and African-Americans. Given the bias of the estimated half-life (incomplete follow-up and variability of progression), Meier-Kriesche proposes calculating graft survival by comparing the area under the Kaplan-Meier survival curve between two groups and thus calculating the cumulative half-life gained throughout the follow-up.

During the 1990s in Spain, the forecasted half-life of patients was similar in transplant recipients in 1990 and in 1998: 21.8 and 21.5 years, respectively. However, the half-life of the graft by censoring death was 15.4 years in 1990 and 17.7 years in 1998. This improvement was observed despite the increased age of donors and lower HLA compatibility[2].

The most recent data from the American register (OPTN/UNOS) reported from the period 1999-2003 show the results of 66 843 renal transplants. Estimated graft survival at 10 years was 67% for a live donor transplant, 51% for a cadaveric donor transplant with standard criteria and 33% for a cadaveric kidney transplant with expanded criteria (donor aged > 60 years or between 51 and 59 years who died from cerebro-vascular disease, with a history of hypertension or serum creatinine above 1.5 mg/dl). Estimated survival of the patient was 82%, 71% and 59%, respectively. The half-life of the grafts was 17.8, 10.8 and 6.8 years and the half-life of the patients was 35.5, 21.3 and 11.9 years, respectively[22].

Indeed, these data show that the results of cadaveric kidney transplants have improved only modestly in the long term. Therefore, the most impor-

tant causes of late graft loss —CAN and death of cardiovascular origin— should receive special attention so that results can improve. New therapeutic strategies are essential if we are to modify the natural history of CAN and control cardiovascular risk factors.

2. Risk factors associated with CAN

NON-IMMUNOLOGICAL RISK FACTORS

Pre-transplant risk factors

Genetic factors

Both immunological and non-immunological factors are involved in the development of CAN. Nevertheless, when faced with similar risk factors, not all patients develop this complication, which suggests the existence of a genetic susceptibility based on polymorphisms in the DNA sequence of the genes involved in pathogenesis. Therefore, the interaction between environmental factors and these genetic variations may be decisive in the onset of CAN. Previous studies have analyzed the influence of some genetic polymorphisms of the molecules involved in the development of CAN, and the results have been contradictory (table II).

Cytokines are mediators of the immune response and their production may be genetically influenced. Specifically, polymorphisms of TNF-a (-308 G/A), IL-10 (-1082 G/A) and TGF-b1 (aa10 L/P) have aroused the most interest due to the action of these molecules on the early immune response and prognosis of the graft in the long term[23-29]**.** Similarly, genetic variants of chemokine receptors, which regulate leukocyte traffic, have been associated with longer graft survival[30].

Different polymorphisms of adhesion molecules (ICAM-1), endothelial growth factors and factors of the coagulation and fibrinolysis system (PAI-1, factor V Leiden and glycoprotein IIIa/IIb), which take part in endothelial damage and repair, have also been associated with acute rejection and worse graft survival[31-33] (Table I).

Given the wide similarity between the histological lesions of CAN and atherosclerosis, several genetic polymorphisms related to vascular risk factors (nitric oxide, G protein, renin-angiotensin system, homocysteine and apolipoproteins) are firm candidates for the development of CAN[23,34-37]. On the contrary, other authors have not found an association between the polymorphisms of the renin-angiotensin system and renal graft outcome[38]. In this sense, the unfavorable genotype (DD) of the polymorphism of the ACE gene has been related to worse graft survival in patients with CAN[39]. Similarly, an interesting finding observed in different studies is the association between the polymorphism of apolipoprotein E (E3/E4) and the development of atheromatous disease and CAN[33,40-43], which highlights the importance of lipid alterations in renal transplant outcome.

If these findings are confirmed in large-scale population studies, knowledge of the genetic risk profile will help to tailor the most suitable immunosuppressive therapy in the hope of minimizing this complication of kidney transplant.

Donor characteristics

Donor age (DA) is an important predictor of poor graft survival in the long term, with a high correlation between the elderly donor and CAN lesions[2,44-46]. UNOS data show that kidneys from elderly donors have a higher initial incidence of delayed graft function (DGF), poorer renal function and a higher frequency of acute rejection[45,47]. Moreover, it has been suggested that the greater percentage of acute rejection in the kidneys of elderly donors is due to an increase in immunogenicity[48,49]. It is conceivable

Table II. Genetic polymorphisms of molecules associated with the immune response and renal transplant outcome

Risk factors and system	Molecule	Polymorphism
Cytokines and receptors	TNF-α	-308 G/A
	IL-10	-1082 G/A
	IL-4	-590 G/T
	IL-6	-174 G/C
	IFN-γ	(CA)n
	TGF-β1	aa10 L/P
Chemokines and receptors	MCP-1	2,518 G/A
	CCR5	59,029 A7G
Adhesion molecules	ICAM-1	+241 G/R
		+469 E/K
Coagulation-fibrinolysis	PAI-1	-675 (4G/5G)
	Factor V	-1,691 G/A
	GP IIIA	PIA2
Cardiovascular risk	Proteína G	-825 C/T
	ECA	I/D
	AGT	-235 M/T
	MTHFR	-667 C/T
	Apo E	E2/E3/E4
Co-estimulation and growth factors	CTLA4	Exón 3 (8AT)n
	VEGF	-1,154 G/A
		-2,578 C/A

that donor arteriosclerosis returns to the most immunogenic vessels and parenchyma, indeed arteriosclerosis has findings of chronic inflammation, with an increase in T cells and monocytes in the vascular intima, and increased expression of MHC antigens, adhesion molecules and cytokines.

The impact of DA can be seen clearly in an analysis of the course of 220 pairs of grafts in which it is observed that graft function and survival is similar in kidneys from the same donor. The older the donor, the greater the glomerulosclerosis, tubular atrophy and interstitial fibrosis and, therefore, the greater the decrease in long-term graft function[50]. Furthermore, elderly donors who die of a vascular illness usually have a previous history of AHT that may be the cause of nephroangiosclerosis, with the result that graft quality is even lower. In any case, it seems that the reduction in acute rejection and improvements in transplant management have reduced the impact of DA[51,52].

These poor results can be ascribed to reduced nephron mass leading to glomerular hypertension, or as recently been suggested, accelerated senescence[53]. Whereas DA explains approximately 30% of the variance in graft survival after the first year[44], other factors such as donor size or sex, which also determine the quantity of nephron mass transplanted, contribute to no more than 1-2% of the variance. This has led us to believe that intrinsic changes in the older kidney (cellular aging) play a greater role in reducing survival than nephron mass per se[53]. By "senescence" we understand the process observed in cultured cells by which, after a certain number of stages, the cells stop dividing and show characteristics different from those of normal cells, such as resistance to growth factors. Recent studies have examined this process in CAN, and have found a greater presence of cellular senescence markers such as b-galactosidase[54], or a greater expression of cycline-dependent kinase inhibitor genes p16 and p27[55]. Senescent cells in the graft do not respond normally to stimuli, which could lead to an anomalous repair, while they continue to produce fibrogen factors, leading to an increase in interstitial fibrosis[56].

Donor type

Brain death (BD) is not a static process and the events occurring during this process can affect the future of the transplant. Our knowledge of the systemic damage that follows massive central damage is limited. Cerebral ischemia and herniation of the brain stem during BD are associated with complex hemodynamic, neurohumoral and immunological alterations. After an intital increase in the parasympathetic tone, the organs are exposed to intense sympathetic stimulation and release of cathecolamines. The resulting vasoconstriction leads to tissue ischemia that alters the production of ATP, generates free radicals of oxygen, an increase in the cytosolic calcium concentration, and activates different enzymes such as nitric oxide synthases or endonucleases. This is followed by a hypotension phase with low sympathetic activity that reduces even more the supply of tissue oxygen. This can all damage the graft. Additionally, experiments with rats have shown that an explosive increase in intracranial pressure followed by systemic hypotension stimulates different cytokines from lymphocytes and macrophages in different somatic organs in rats. One interesting study shows that animals that receive a kidney from a BD donor die due to acute renal insufficiency after rejection more quickly than those who receive a graft from an anesthetised live animal. In the receptors of a BD donor we can observe a marked infiltrate of neutrophils, followed by a marked infiltrate of macrophages and T cells, together with an increase in pro-inflammatory mediators (E-selectin, ICAM-1, TNFα and IFNγ) and factors which are chemically attractive for macrophages (MCP-1, MIP-1, RANTES) and for leucocytes (IL-8)[57]. This line of research was promoted by UNOS data that showed not only that the survival of unrelated live donors was similar to that of related live donors who shared a haplotype, but also that the organs from live donors, regardless of differences in the genetic relationships, had better results than those of the cadaveric donors[58]. Other authors have reported that grafts from donors who die of cerebral trauma or cerebrovascular accident have a poorer survival than those who die of other causes[59], and that the duration of brain death has an effect on the survival of the graft in donors who die of a cerebrovascular accident. There have been reports in humans of higher levels of expression of pro-inflammatory adhesion molecules such as ICAM-1, VCAM-1, E-selectin, IL-1b and MIP-1 and HLA-DR antigens in kidneys from BD donors compared with kidneys from live donors.

Comparing the results of BD donors with those of live donors may not be appropriate, as the latter are not subject to the lesions produced by ischemia and reperfusion. Grafts from non-heart-beating donors share these latter stages with BD donors and therefore the in vivo model is the most appropriate for inferring which are the effects of brain death. A Cox analysis comparing 197 kidney transplants from BD donors compared with 175 kidney transplants from non-heart-beating donors shows that the recipients of BD donors have a greater risk of vascular rejec-

tion, and that this risk is even higher if the donor dies of a cerebrovascular accident[60]. In this study, the receptors of the BD donors who developed DGF had a greater incidence of vascular rejection, and it was speculated that the presence of DGF could be an expression of a more catastrophic BD, with greater injuries and therefore greater inflammatory stimulation. The Spanish study on CAN also found that the presence of DGF is a poor prognostic marker for graft survival in BD donors, and this is not the case in non-heart-beating donors[61].

Ischemia-reperfusion

Ischemia-reperfusion injury is associated with DGF and epidemiological data point to a relationship between cold ischemia and the incidence of DGF, acute and chronic rejection, and survival in the long term[62,63]. This injury is the result of cellular energy deprivation after ischemia and the inflammatory process generated after reperfusion. Although the term «acute tubular necrosis» has been considered synonymous with ischemic renal damage, apoptosis has recently begun to emerge as a potential mechanism leading to death of the tubular cell after ischemia. Apoptosis has been reported in cultures of tubular cells after ischemia in animal models and in the biopsies of kidneys transplanted from cadaveric donors, with a significant correlation being found between apoptosis and time of cold ischemia in experimental models[64].

Post-transplant risk factors

Hyperfiltration

Transplantation of reduced nephron mass carries a risk of hyperfiltration and graft loss. In multivariate analyses, large body mass and male sex have lower graft survival[65,66], whereas low body mass carries a lower risk of graft loss due to chronic rejection[67]. In an analysis made using data from USRDS, Kasiske et al[51]) found that kidneys from donors with a small body surface index have a 43% higher risk of graft loss if they are implanted in recipients with a large body mass and by 16% more if they are implanted in medium-sized recipients.

Nevertheless, despite the abundant literature in the clinical field supporting the role of hyperfiltration in the development of CAN, some authors are doubtful and argue that focal glomerulosclerosis —the basic histopathological marker of glomerular hyperfiltration and hypertension— is not a prominent finding in CAN[53]. Nevertheless, it has been shown that double kidney transplants from pediatric donors, and therefore with double nephron mass, have an overload of aminoacids by increasing glomerular filtrate and renal plasma flow, which is indicative of a renal functional reserve and thus a lower risk of hyperfiltration, a finding not observed in single adult kidney transplants[68]. Furthermore, chronic kidney disease of the transplant is unusual in patients that receive double nephronal mass, as is the case of *en bloc* pediatric transplant recipients[49].

Hyperfiltration after transplant of a small nephron mass can cause early expression of adhesion molecules in the endothelial cells and stimulate production of cytokines by endothelial and mesangial cells and, consequently, lead to adherence of inflammatory cells. In fact, an interesting experimental study published by Azuma et al.[69] showed that changes in transplanted nephron mass cause important changes in the expression of cell surface molecules, cellular infiltrate and expression of products derived from T cells and macrophages. Thus, these authors observe that expression of ICAM-1, class II MHC antigens, VLA-4, TNF-α, TGF-β, PDGF, RANTES, MCP-1, endothelin and different interleukins appeared more quickly in transplants with reduced nephron mass. In clinical practice, a study comparing three groups of kidney transplants with different nephron mass (*en bloc* pediatric transplants considered as near normal nephron mass, single transplants from a young donor interpreted as nephron mass reduced to 50% and an elderly donor representing lower nephron mass) observed that one of the factors associated with cortico-resistant acute rejection is receiving grafts with a low nephron mass[49]. A greater incidence of rejection has also been reported in transplants from elderly or pediatric donors if only a single graft is transplanted or female donors are used for male recipients, in other words, the transplanted nephron mass may not only be a factor that affects the development of CAN, but it may also be a factor for acute and chronic rejection.

Infection

Kidneys from CMV-seropositive donors have a slightly lower graft survival[67]. It is not clear whether this reflects an increased risk of CAN, since the relationship between CMV disease, acute rejection and chronic kidney disease is complex to analyze, as there are close links between the three factors. It is difficult to know whether CMV infection can be a consequence of more intense immunosuppressive

therapy as a consequence of more severe acute rejection and whether the latter explains the greater incidence of CAN. Humar et al., in a retrospective study, reported that CMV disease did not seem to be a risk factor for CAN in the absence of acute rejection[70]. However, a more recent prospective study found a relationship between CMV and CAN, regardless of acute rejection, although the number of cases is small[71].

In experimental studies, CMV infection significantly increases the development of CAN, since it increases the intensity of interstitial inflammation, enlargement of the capillary base membrane and vascular intimal proliferation[72]. Nevertheless, in kidney transplants with fibrosis of the intima, studies with in situ hybridization, immunohistochemistry and PCR have not found viral DNA or proteins in the arteries[73].

Infection by other viruses such as polyoma BK virus and herpes 6 virus can also contribute to graft loss in the long term[71]. A recent prospective study of BK virus infection in 104 transplants detected viruria in 57% and viremia in 29% of patients[74]. The presence of BK virus-associated kidney disease is calculated to be more than 8% in kidney transplants, and graft loss after this isolated infection or after rejection is significant.

With regard to the hepatitis C virus, even though the first published studies show similar graft survival in infected and non-infected patients, the most recent studies, including the Spanish CAN study[2,75-77] support the thesis that outcome is poorer. A recent meta-analysis including only observational studies found a higher risk of graft loss in HCV-positive kidney recipients than in HCV-negative kidney recipients[76]. These discrepancies can probably be explained by the different methods used to diagnose HCV infection and the activity and severity of the liver disease. Most studies use the presence of antibodies to diagnose HCV infection, and this has proven to be an incorrect estimator of real incidence. Studies analyzing viral RNA observed that patients with positive viremia and abnormal ALT have poorer graft survival[78]. One hypothesis that might explain the greater incidence of CAN is the reduction in immunosuppression usually observed in these patients. Other hypotheses could include alteration of the carbohydrate mechanism[79], or induction of fibrogenetic mechanisms.

Renal function and proteinuria

Retrospective studies have shown that high levels of serum creatinine at different months after transplant are associated with an increased risk of graft loss[2]. The reverse curve of creatinine is also a prognostic marker, as is the time it takes for renal function to deteriorate[80]. Post-transplant proteinuria is an important risk for CAN[2,81,82] and affects the progressive impairment of renal function in such a way that patients with persistent proteinuria of more than 2 g/day have a higher risk of later deterioration of renal function[20]. In the Spanish CAN study, the persistence of proteinuria ≥ 0.5 g/day during the first year post-transplant is an independent marker of risk of graft loss and mortality[2,83]. Reabsorption of excessive quantities of proteins by proximal tubular cells can lead to the release of inflammatory mediators from tubular cells and interstitial damage, and contribute to the progression of renal insufficiency.

Arterial hypertension (AHT)

AHT, both in the donor and in the recipient, is a risk for CAN that has been proven in the clinical practice and in experimental research. However, retrospective clinical studies have shown that there may be confusion between the variables hypertension and renal function[84,85]. Animal studies have shown that hypertensive recipients present accelerated impairment both in renal function and in renal histology if they are compared with normotensive recipients[86]. The prevalence of post-transplant hypertension, defined as the need for hypotensive therapy, is approximately 75%. Both high SBP and high DBP one year from transplant are predictors of graft survival in the long term[87] and they are related to progressive impairment of renal function[88,89]. AHT can lead to arteriosclerosis in renal vessels and glomerular hypertension, which can increase glomerular permeability and thus protein loss. There are no prospective studies designed to ascertain whether a strict control of BP can prevent the development of CAN, although, given that renal insuficiency has shown that good control of blood pressure reduces progression of the disease, it seems reasonable to believe that these data can be extrapolated to kidney transplant recipients.

Few studies evaluate the effect of donor AHT on the development of CAN. Experimental studies have shown that recipients from hypertensive donors suffer from accelerated morphological and functional damage if they are compared with recipients from normotensive donors. Inflammatory activity is observed in the hypertensive organ before transplant (with an important increase in TNFa and MIP-1a that continues to increase progressively after the transplant),

which can trigger an allo-specific response and development of fibrosis, and CAN[86].

Hyperlipidemia

Hypercholesterolemia appears in 70-80% of kidney transplants and hypertriglyceridemia in 30-40%. Both hypertriglyceridemia and hypercholesterolemia have been associated with graft dysfunction[90-93]. In an interesting study with protocol biopsies, Moreso et al found that post-transplant hypercholesterolemia plays a role in the development of vascular disease in the graft[94]. It is speculated that hyperlipidemia can lead to an accumulation of oxidated low-density lipoproteins in the renal interstitium and fibrosis. In any case, it is not completely clear if there is a relationship between hyperlipidemia and the development of CAN, as, in most studies it is difficult to separate the role of lipid alterations from other predictors of CAN.

Smoking

Different studies have reported smoking as a risk factor for graft loss[95-97]. Kasiske et al reported that smoking more than 250 packets per year at the time of transplant increases the risk of graft loss by 30%[96], although they found no differences in renal function among functioning transplants regardless of whether the patients were smokers or not. There is evidence in different renal diseases that smoking speeds up progression to renal insufficiency[95]. It reduces renal plasma flow and filtration fraction, probably because it increases vasoconstrictive endothelin synthesis by reducing generation of vasodilating nitric oxide and leads to accelerated atheromatosis in several vascular territories[98].

Nephrotoxicity of calcineurin inhibitors

Chronic kidney disease due to tacrolimus or cyclosporine leads to bands of interstitial fibrosis associated with degenerative changes in arteriole walls not easily distinguishable from CAN lesions. Furthermore, neither process is exclusive and they often coexist. Some studies show that long-term therapy with calcineurin inhibitors can play a role in the development of CAN, although others have shown that insufficient immunosuppression as a result of low doses of these drugs can increase the risk of CAN by triggering an immune response[12]. A case-control study in patients treated with cyclosporine found that young patients and those with highly variable exposure to cyclosporine (ie, high doses of drug per kg of body weight and high coefficient of variation in doses by trough level) are more predisposed to develop CAN[99]. This greater variability may be a reflection of poor adherence to therapy, occasional drug-drug interactions, interference with absorption, etc. A recent prospective study with 119 consecutive kidney-pancreas transplants showed that data on nephrotoxicity due to calcineurin inhibitors at 10 years post-transplant appeared in 100% of patients and that this histological damage was the most obvious finding at this point in follow-up, and thus marked impairment of renal function[13]. The high relevance of this nephrotoxicity led the authors to suggest two phases of therapy in our patients, with low doses of calcineurin inhibitors in the second phase in order to improve the results. Even though nephrotoxicity is very likely to be one of the determining factors in the development of CAN, its role may have been overestimated in this study, given that there were few other factors contributing to graft loss in the long term. Thus, these kidney-pancreas transplants had special characteristics (young donors, short ischemia time, and low incidence of DGF, high doses of cycolsporine, high rate of acute rejection and excellent adherence to therapy) that are not common in all kidney transplants. Recently, Gallagher et al[100] published the results of a study involving 489 patients followed up for 15 years and randomized to three treatment groups: 1) bitherapy with azathioprine and prednisone, 2) monotherapy with long-term cyclosporine 3) initial therapy with cyclosporine and substitution with azathioprine and prednisone at three months. Better function and graft survival were observed in those patients who used cyclosporine for a short period, with more significant results when the functioning transplants were analyzed after the first year. Even though the authors do not provide data on the levels of cyclosporine, the doses used are high as the patients receive monotherapy. An older Spanish study with a longer follow-up also confirms that the initial improvement in graft survival after therapy with CsA compared with combined therapy with azathioprine and prednisone is not maintained in the long term[101]. In summary, these articles confirm the deleterious effect of high-dose CsA.

In the last few years, mycophenolate and sirolimus —more potent immunosuppressive agents than azathioprine— have allowed CNI-free or lower-dose regimens or early suspension of these drugs with favorable results in some studies[102-104]. Thus, a prospective study with a small number of cases comparing sirolimus/MMF/prednisone with cyclos-

porine/MMF/prednisone has shown better renal function and lower histological prevalence of CAN in the CsA-free group[105]. Despite the fact that these data point to the deleterious effect of CNI, we still need studies with a greater number of cases, longer follow-up and protocol biopsies, in order to state with evidence level A the possible inferiority of regimens with low doses of CNI, mainly tacrolimus, combined with MMF or sirolimus, compared with CNI-free regimens.

IMMUNOLOGICAL RISK FACTORS

The importance of the human histocompatibility system (HLA) for patient and graft survival was fundamental in the early days of kidney transplant. However, the introduction of immunosuppressive agents and, in particular, improved efficacy has led some authors to minimize the importance of HLA compatibility in kidney transplants. This debate has intensified due to problems of recipient disposition. There are likely to be other, more important factors for the favorable outcome of kidney transplant in the short term (first year), although it is well known that long-term immunosuppression does not only avoid, but also leads to a series of harmful effects in the recipient and in the graft. This is known as chronic allograft nephropathy[106]. Immunological factors could be considered risk factors for chronic rejection (CR), which is the initial cause of CAN, whereas non-immunological factors contribute to the progression of CAN once CR takes place. There are clinical and experimental data on the contribution of the humoral, direct and indirect cellular immune response in CAN[107-109]. Nevertheless, no studies have simultaneously evaluated all the components of the immune alloresponse and, therefore, we do not know to what extent each of them contributes to the development of CAN. All this is further complicated by the high polymorphism of the HLA system.

The ideal objective when trying to avoid CAN is to develop immune tolerance, but there is absolutely no way to achieve this at present, or laboratory method to measure this state in clinical practice. The only way to demonstrate it is by eliminating immunosuppression, which carries the risk of acute rejection. At present, the only established methods in clinical practice are for detecting and quantifying the humoral response[110].

Below we describe existing evidence and provide recommendations to follow with regard to immunological risk factors, especially those that refer to the HLA system and the humoral immune response, to avoid the development of CAN.

Anti-HLA antibodies

The absence of anti-HLA antibodies, whether donor-specific or not, is accompanied by a lower frequency of chronic rejection and greater graft survival[111,112]. Curiously, anti-HLA antibodies determined by ELISA, both those related to the donor and those that are not, have been associated with a greater frequency of rejection. Nevertheless, if the antibodies are detected by CDC, only those related to the donor are associated with acute and chronic rejection[112]. Currently, there is no clear level of scientific evidence on the role of anti-HLA antibodies determined by ELISA or flow cytometry in the development of CR and CAN. A recent study shows a low relative risk (RR 1.8) of CAN in the case of high PRA by ELISA and microlymphocytoxicity[113]. The RR of a PRA >15% obtained by the Spanish Group for Chronic Kidney Transplant Nephropathy was similar, although it was not statistically significant (RR = 1.476, p = 0.0739).

Although the study of anti-HLA antibodies before kidney transplant is an established practice, monitoring of anti-HLA antibody production after transplant is not routine practice, mainly because of technical problems. The development of methods such as ELISA or flow cytometry to quantify these antibodies has led to several studies on the association between post-kidney transplant production of anti-HLA antibodies and development of rejection. There is sufficient evidence (level A) to justify the quantification of anti-HLA antibodies after a kidney transplant using either of the three techniques available for the diagnosis of humoral acute rejection[114-120].

On the contrary, there are no prospective studies with wide series that establish an association between post-transplant production of anti-HLA antibodies and CAN. One study, from the University of Tainan (Taiwan), with the collaboration of Terasaki, uses biopsy to show the formation of anti-HLA antibodies before the development of chronic rejection in 100% of cases[121]. On the other hand, only 27% of patients who maintained a stable renal function produced these antibodies after the transplant. Although this was not clearly established, we can conclude from this study that the association established between the production of antibodies post-transplant and chronic rejection was independent of other immunological factors (eg, previous acute rejections) and non-immunological factors involved in CAN. The importance of this study lies in the fact that it is the first to show an association between the production of anti-HLA antibodies after transplant and the development of CR. Furthermore, it shows the chronology of detection of

positive anti-HLA antibodies in 14 patients with no pre-transplant anti-HLA antibodies. Given that most antibodies appear at least one year before CR, Lee et al. defend the idea of using detection of anti-HLA antibodies annually to monitor kidney transplant recipients and thus predict chronic rejection[121]. The previous finding seems to correspond with the data obtained by Worthington et al. who studied the production of anti-HLA antibodies periodically and correlated it with the outcome of the graft at five years. In this study, the recipients who developed anti-HLA-I antibodies experienced graft failure long before those who only produced class II anti-HLA antibodies[120]. Nevertheless, this study did not correlate these findings with the histopathology of the rejections.

After these studies at the 13th International Histocompatibility Workshop, a prospective study was started to evaluate the capacity of anti-HLA antibodies to predict kidney graft failure. The first data published at one year of follow-up showed that 8% of the patients who developed anti-HLA antibodies de novo lost the graft, compared with 3% of those who did not produce them[122]. These findings are confirmed by more recent data at two years of follow-up[123]. Although serial determination of the presence of anti-HLA antibodies is not recommended, it will be made in those recipients with impairment of renal function, serum creatinine levels of > 2 mg/dl and when the degree of immunosuppression is going to be modified[122-124].

A recent analysis by the CTS states that the prevalence of antibodies not targeting the HLA system in recipients of kidneys from twin donors is associated with graft loss in the long term[125].

Specific antibodies against the donor

The Taiwan group study[121] only shows the production of anti-HLA antibodies by ELISA after a kidney transplant but it does not define whether these antibodies are donor-specific or not. On the contrary, the study by Worthington, with 112 recipients determines the specificity of anti-HLA antibodies by ELISA and shows that, for prognosis of the transplant, it is more important for antibodies to be donor-specific than the level of sensitization (120). This study, however, lacks histological correlation with clinical and serological data. Using flow cytometry, other authors managed to show a 91% sensitivity and 83% specificity for the production of anti-HLA antibodies to detect CR and graft loss[118]. This study is based on samples collected prospectively from 120 recipients over one year. Other groups have recently presented at congresses prospective studies[126] which, together with those quoted above, seem to show level-B evidence in favor of monitoring anti-HLA donor-specific antibodies during the post-transplant period. A recent study of 1229 kidney recipients with a graft functioning for at least one year and who were followed up for 5 years found that the presence of anti-HLA antibodies, both donor-specific and not, is associated with lower graft survival, poorer function and proteinuria[127].

Therefore, the abovementioned international, multicenter, prospective study by Terasaki is under way and aims to recruit 5,000 kidney transplant recipients and analyze the presence of donor-specific anti-HLA antibodies with graft outcome at one year post-transplant, and to confirm the preliminary findings. This is also one of the objectives of the Transplant Centres Interactive Thematic Network. Furthermore, it is necessary to establish the ability to diagnose CAN using the three available techniques for determination of anti-HLA antibodies, and their accessibility in clinical practice. At present, the techniques are not standardized and, while cytotoxicity is subjective, there is no consensus on the recently adapted cut-off points which must be established in ELISA and flow cytometry for the detection of these antibodies.

Degree of histocompatibility

The recipients of kidneys from live donors with poor HLA histocompatibility present long-term results equivalent to those of recipients of a graft from a haploidentical live donor[128,129]. That is, even though HLA identity is still an essential factor in the medium-long term[130], the quality of the allograft and peri-operative management are considered more important. These data are corroborated by those obtained by the Spanish CAN Group Register, where the degree of A, B or DR incompatibility did not affect graft survival.

Acute rejection

The immunological factor most closely related to CAN and graft loss in the long term is considered to be a high rate of acute rejection[131,132]. Even subclinical rejection, the form of acute rejection with no functional impairment, contributes to the development of CAN[133]. The effect of the acute rejection rate on CAN and long-term graft survival is so obvious that it is very difficult to isolate the effect of the possible CAN risk factors.

The greatest risk of developing CAN is found at three months after an episode of acute rejection (RR = 14.5; p = 0.0001)[113].

The Spanish CAN Register also shows an increased risk of suffering from CAN, the greater the number of acute rejections the recipient has suffered. Thus, the relative risks of suffering from CAN at one year after transplant are 1.497, 2.310 and 3.215 in the case of having suffered 1, 2 or 3 episodes of acute rejection.

3. Diagnosis of CAN

DIAGNOSIS AND SEVERITY OF CAN ACCORDING TO THE BANFF CRITERIA

CAN is defined as the presence of interstitial fibrosis (ci) and tubular atrophy (ct) and can be accompanied by vascular disease of the transplant (cv). The severity of these lesions is described according to their extension as absent (grade 0), mild (grade 1), moderate (grade 2) and severe (grade 3). There is considered to be no interstitial fibrosis (ci=0) when this lesion affects less than 5% of the cortex, ci=1 between 6 and 25%, ci=2 between 26 and 50%, and ci=3 more than 50%. Similarly, ct=0 is defined as the absence of tubular atrophy in the cortex, ci=1 as involvement of up to 25%, ci=2 between 26 and 50% and ci=3 more than 50%. With regard to chronic vascular lesion, cv=0 is defined as the absence of vascular lesion, cv=1 as the reduction of the area of the arterial lumen <25%, cv=2 between 26 and 50% and cv=3 more than 50%. The severity of CAN is described using Roman numerals followed by «a» or «b», to mention the absence or presence of transplant vascular disease. For example, CAN II (a) means moderate grade chronic nephropathy with no vascular disease. Other lesions, such as the percentage of sclerosed or ischemic glomerules, mesangial enlargement and glomerular disease of the transplant are associated with CAN but do not constitute diagnostic criteria[14]. There is some variability among centers when evaluating biopsies using the Banff criteria[134]. Biopsies taken from patients with suspected CAN must be processed using optical microscopy, immunofluorescence / immunohistochemistry and electronic microscopy to carry out the differential diagnosis with relapse of the primary disease, de novo glomerulonephritis (including that associated with HCV), transplant glomerular disease and BK virus nephropathy.

INDICATION FOR BIOPSY

The presence of proteinuria, slow and progressive deterioration of renal function, or elevated creatinine suggests the presence of CAN. However, the clinical criteria for biopsy vary widely from center to center, therefore the study of the incidence and prevalence of this entity is only known thanks to studies carried out using protocol biopsy.

PROTOCOL BIOPSIES AND CAN

CAN is diagnosed in 40% of protocol biopsies at 3 months, 50% at one year, 66% at two years and 100% at 10 years[13,15,135]. In order to calculate the incidence of CAN, we must remember that, in approximately 15% of donor biopsies, there are chronic lesions which cannot be distinguished from CAN[136]. The incidence and severity of CAN increases rapidly during the first months and more slowly after the first year[13,137].

The presence of CAN in protocol biospsies is associated with poorer graft survival, especially if it is accompanied by transplant vascular disease[135,136,138-140]. The predictive value of CAN for graft survival is independent of other clinical variables such as creatinine, acute rejection or proteinuria[136,141].

The risk factors associated with CAN in protocol biopsies[13,15,136-143] are summarized in Table 3. The large epidemiological studies agree on these factors and those associated with graft survival after one year[2,144]. Nevertheless, risk factors associated with chronic tubulo-interstitial injury are not the same as the risk factors for transplant vascular disease. For example, thickening of the intima in the donor biopsy, vascular rejection before the protocol biopsy, the degree of HLA incompatibility between the donor and recipient, or lipid alterations are all associated with transplant vascular disease, whereas donor age, interstitial rejection, cold ischemia time, or delayed graft function are associated with CAN without vascular involvement[94,136,142,143].

The close relationship between CAN in protocol biopsies and graft survival has led to protocol biopsies being proposed as an efficacy variable in clinical trials[94,136].

CAN AND SUBCLINICAL REJECTION

The diagnosis of subclinical failure, that is, the presence of histologic lesions of acute rejection in patients with stable renal function, is a frequent finding, whose highest incidence is observed during the first few months[133,145,146]. In studies using serial biopsies, subclinical rejection is associated with an increase in the severity of chronic lesions in later biopsies[133,145,146]. One prospective study has reported that therapy for subclinical rejection using steroid bolu-

ses prevents the development of fibrosis and preserves renal function in the medium term[147]. This observation has stimulated the study of a possible relationship between immunosuppressive therapy and the prevalence of subclinical rejection or fibrosis[148]. Therapy with mycophenolate mophetil compared with azathioprine[13], and tacrolimus compared with CsA[13,16,133] is associated with a lower incidence of subclinical rejection. To date, there are no conclusive histological studies that confirm that the rejection of subclinical rejection in patients on tacrolimus and mycophenolate is associated with less CAN[15,149], although some epidemiological data do suggest this[150,151]. In protocol biopsies taken from patients treated with CsA and sirolimus, early withdrawal of CsA has been associated with a lower incidence of CAN at three years[17,103]. Similarly, the incidence of CAN at three months in patients treated with sirolimus and mycophenolate mofetil is lower than in patients treated with tacrolimus and sirolimus[152].

It has recently been reported that the association of subclinical rejection and CAN in the same biopsy implies a worse outcome of the graft than the presence of CAN without rejection[153,154]. One study of 435 transplants which underwent protocol biopsy at approximately 3-6 months in our center revealed that patients with subclinical rejection and CAN in the biopsy had a lower survival rate at 15 years than patients with CAN and no subclinical rejection or in patients with subclinical rejection and no CAN.

Table III. Risk factors associated with the presence of chronic kidney disease diagnosed using protocol biopsies

Donor age
Thickening of the intima in the donor biopsy
Number of incompatibilities in the HLA system
Cold ischemia time
Delayed graft function
Acute rejection
Subclinical rejection
Cytomegalovirus infection
Exposure to cyclosporine
Number of episodes of acute kidney disease by cyclosporine or tacrolimus
Total cholesterol
HDL-cholesterol
Creatinine
Proteinuria
Blood pressure

CAN AND HUMORAL RESPONSE

The determination of recipient-specific antibodies against the donor and staining of the stable fraction of the C4d complement in the biopsy has facilitated the diagnosis of acute humoral rejection (155, 156). These techniques have recently been used to evaluate the contribution of the humoral response to CAN. An association has been reported between transplant glomerular disease and the presence of donor-specific antibodies and/or deposition of C4d. These data suggest that transplant glomerular disease (cg) is a disease mediated by the humoral response[113,157,158].

Transplant glomerular disease is characterized by the detection of double contours in optic microscopy with negative immunofluorescence and usually positive glomerular C4d deposits[113]. Electronic microscopy shows thickening of the subendothelial space and abnormal accumulation of a similar material to the dense lamina of the basal membrane. From a clinical viewpoint it is characterized by the presence of proteinuria and the progressive deterioration of renal function. A recent study of protocol biopsies revealed that graft survival is lower in patients with CAN associated with transplant glomerular disease than in patients with CAN and no glomerular disease[153].

Transplant capillary disease or duplication of the dense lamina of the peritubular capillaries observed using electronic microscopy is associated with transplant glomerular disease and it has been suggested that this could be a marker of damage mediated by the alloimmune repsonse[159-161].

4. Treatment and prevention of CAN

MODIFICATION OF NON-IMMUNOLOGICAL FACTORS

Treatment of arterial hypertension (AHT)

AHT (>140/90 mmHg) is very prevalent after kidney transplant (60-90%). It is a clinical marker of CAN and contributes to graft loss and the morbidity and mortality of these patients[11,85,162,163]. On the contrary, reducing blood pressure can curb progression of kidney disease in non-diabetic patients with moderate and severe impairment of glomerular filtrate[164]. Regardless of the causal factors, reducing blood pressure is a clinical priority in this group[165]. Nevertheless, there is no suitable level of evidence to show that strict control of AHT minimized the cardiovascular disease and CAN, but it seems prudent to adopt the therapeutic measures of the general population.

Given that AHT and proteinuria are often associated with the course of CAN[53], a joint therapeutic approach seems more rational when both occur simultaneously. In this sense, a blood pressure of ≤ 130/80 is recommended in kidney transplant recipients without proteinuria and ≤ 125/75 in those with proteinuria[166-168].

Any antihypertensive agent can be useful for controlling post-kidney-transplant AHT, and they have their advantages and disadvantages (Table IV). Nevertheless, in these patients the renin-angiotensin system (RAS) is generally activated. Therefore, it is advisable to begin with drugs which reduce intrarenal vasoconstriction and/or intraglomerular pressure, initially by avoiding substances that stimulate the RAS (eg, diuretics), except when the clinical situation requires it (congestive heart failure, edematous processes, etc.). Thanks to their pharmacological properties, these effects are achieved by calcium antagonists (CA) and drugs that block the RAS, namely angiotensin II converting enzyme inhibitors (ACE inhibitors) or antagonists of its AT1 receptor (ARA). This is seen as an improvment in renal function and in the outcome of CAN[169,170]. In the non-transplanted population, ACE inhibitors and ARA have clearly proven to be better at reducing proteinuria and progression of renal insufficiency than other antihypertensive therapies[171-174]. However, no clear benefit of CA over ACE inhibitors or ARA has been observed with regard to renal function or graft survival in patients who have undergone a kidney transplant[175]. Therefore, the initial choice of antihypertensive drug will depend basically on the clinical characteristics of the patient (age, diabetes, severity of AHT, clinical data of atheromatosis, ventricular hypertrophy, etc.) and the presence of proteinuria, which can be modified by antihypertensive therapy.

Figure 1 shows is a simplified representation of the recommendations for the treatment of AHT depending on whether or not proteinuria is present. Obviously, the first measures involve restricting salt intake, controlling weight, avoiding alcohol and other drugs which can induce hypertension (non-

Table IV. Advantages and disadvantages of some antihypertensive drugs

Drug	Advantages	Disadvantages
Low-dose thiazides	Control of edema Reduction of kalemia Low cost	Renal insufficiency Not very effective with GFR <30 ml/min Hyperlipidemia, hyperglycemia
Beta-blockers	Indicated in ischemic heart disease Low cost	Hyperlipidemia
ACE inhibitors	Fall in proteinuria Control of erythrocytosis Cardioprotection Delay in the progression of CAN?	Cough, anemia Increased creatinine Hyperkalemia
ARA	Fall in proteinuria Control of erythrocytosis Cardioprotection Delay in the progression of CAN?	Anemia, increase in creatinine, hyperkalemia
Calcium--antagonists	Increase in the levels of CsA and sirolimus (mainly non-dihydropyridine) and of renal plasma flow	Edema
Vasodilators	Fall in cardiac postload	Tachycardia

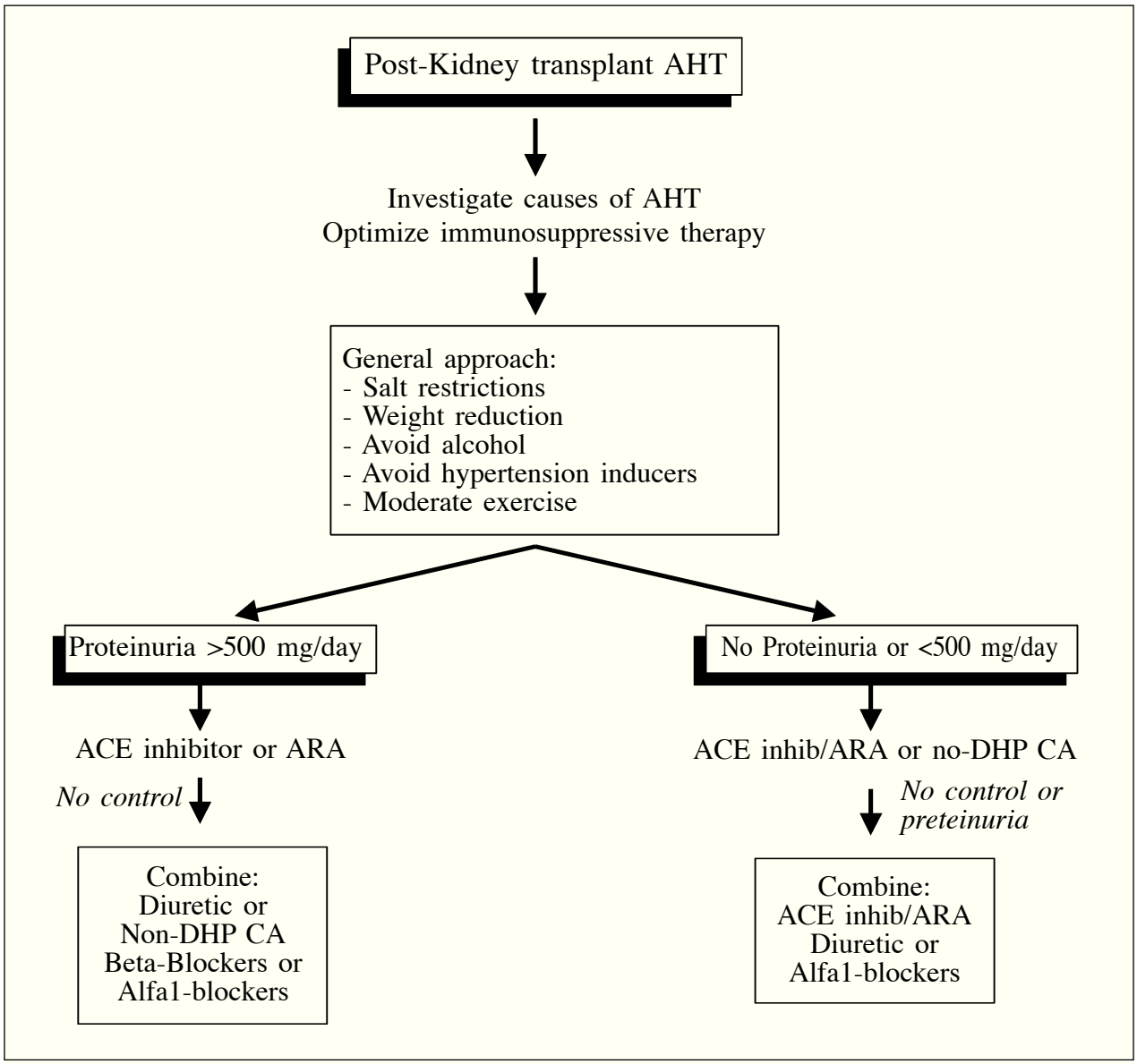

Fig 1.—Initial pharmacological management in patients with arterial hypertension and chronic allograft nephropathy.
Abbreviations: AHT, arterial hypertension. ACE, angiotensin-converting enzyme. ARA, angiotensin II AT1 receptor antagonist. Non-DHP-CA, non-dihydropyridine antagonist.
**Consider the combination of both drugs in the case of severe proteinuria.*
^ If a DHP-CA is necessary, monitor proteinuria and/or combine with an ACE inhibitor

steroid anti-inflammatory drugs, anabolic drugs etc.), as well as the optimization of immunosuppressive therapy[12]. The change from CsA to tacrolimus may be beneficial in cases of severe AHT[176]. In patients with proteinuria (≥500 mg/day), therapy should begin with an ACE inhibitor or an ARA II antagonist whose doses will gradually be increased as required by the monitoring of blood pressure. Additionally, these drugs provide cardio and renoprotection, inlcuding reduction of ventricular mass, inhibition of TGF-b, and preservation of renal function[177-180]. Similarly, the combination of an ACE inhibitor and an ARA II antagonist may be beneficial in cases of intense proteinuria[181]. Close monitoring

of the renal function, plasma potassium and hemoglobin should follow the use of these drugs. In patients without proteinuria or with <500 mg/day, treatment with an ACE inhibitor/ARA II antagonist can begin, or if this is not possible, a non-dihydropyridine CA can be used (diltiazem or verapamil), since dihydropyridines (nifedipine, amlodipine, nitrendipine, etc.) can lead to proteinuria and greater impairment of renal function[181,182]. Nevertheless, a randomized trial comparing lacidipine with placebo found better renal function in the group treated with the dihydropyridine CA[169]. The non-dihydropyridine CA can interact with the plasma levels of anti-calcineurin drugs and sirolimus[183,184]. If dihydropyridine CA are necessary, proteinuria should be monitored and/or combination with an ACE inhibitor or ARA should be considered in order to mitigate these effects.

If AHT cannot be controlled using these measures, other antihypertensives such as diuretics, b-blockers or α1-blockers can gradually be introduced (Figure 1), by tailoring the best therapeutic option in each specific clinical situation. Table V shows the ranges and intervals of the doses of some antihypertensives that could be used in kidney transplant patients. Obviously, the doses will be adjusted to the degree of renal insufficiency for those drugs that are mainly eliminated in urine.

Finally, in patients with uncontrolled AHT and/or renal insufficiency, arterial stenosis of the renal graft should be suspected (2-6%)[185]. In these cases, an angiographic study should be made and therapeutic measures should be adopted.

Table V. Antihypertensive drugs used in renal transplant

Drug	Dose range (mg/day)	Dose interval (hours)
Diuretics		
Thiazide diuretics		
Chlortalidone	12.5-50	24-48
Hydroclorothiazide	12.5-50	24
Indapamide	1.25-2.5	24
Loop diuretics		
Furosemide	40-240	8-12
Torasemide	2.5-20	12-24
Betablockers		
Atenolol	25-100	12-24
Bisoprolol	2.5-10	24
Metoprolol	50-200	24
Propanolol	40-320	8-12
Alfa-Betablockers		
Carvedilol	12.5-50	12
Labetalol	200-1.200	8-12
Calcium antagonists		
Dihydropyridine		
Amlodipine	2.5-10	24
Felodipine	2.5-20	24
Lacidipine	2-6	24
Nifedipine	30-90	12-24
Nitrendipine	10-40	12-24
Non-dihydropyridine		
Diltiazem	120-360	8-24
Verapamil	120-480	12-24
ACE inhibitors		
Captopril	25-150	8-12
Enalapril	5-40	12-24
Fosinopril	10-40	12-24
Lisinopril	5-40	24
Ramipril	1.25-10	24
Trandolapril	0.5-4	24
ARA		
Losartan	25-100	12-24
Candesartan	8-32	24
Irbesartan	75-300	24
Telmisartan	40-80	24
Valsartan	80-320	24
Alfablockers		
Doxazocine	1-16	12-24
Prazocine	1-15	12
Central action drugs		
Clonidine	0.3-1.2	12
Moxonidine	0.2-0.6	24

Management of proteinuria

Proteinuria is a well known marker of renal damage and contributes to the progression of renal insufficiency, by generating a pro-inflammatory response and interstitial fibrosis[181,186,187]. Proteinuria is frequent after kidney transplant (25% during the first 6 months) and determines the survival of the graft and of the patient. In fact, the persistence of proteinuria ≥ 0.5 g/day during the first year after the transplant is an independent marker of risk of graft loss and mortality[2,83]. Given that a kidney graft is particularly vulnerable to the adverse effects of proteinuria (expression of MHC2 antigens, endothelin and fibrosis), starting therapy during the early stages is essential to avoid impairment of glomerular filtration and to reduce morbidity and mortality in these patients. Therefore, reducing proteinuria to < 0.5 g/day is a crucial therapeutic objective to achieve these benefits.

Table VI shows antiproteinuria strategies according to the level of recommendation or evidence.

Regardless of optimal control of blood pressure, the choice of the most suitable therapy depends on the role of angiotensin II in the pathogenesis of proteinuria after kidney transplant.

ACE inhibitors are the drugs of choice for curbing proteinuria. They are safe and efficacious when treating this condition after kidney transplant[179,188], and have a beneficial effect on the cardiovascular profile including left ventricular hypertrophy[177,189].

ARA II antagonists have an antiproteinuric effect similar to that of ACE inhibitors, but there is less experience in kidney transplantation[190-192]. Current evidence indicates that combining ACE inhibitors and ARA II antagonists at the maximum dose tolerable in both drugs is more powerful than when each one is administered in monotherapy[181]. This may be extended to kidney transplantation. Other measures such as avoiding dihydropyridine calcium antagonists[193] and statins[194] could be additional methods of minimizing post-kidney transplant proteinuria.

Table VI. Antiproteinuria therapeutic strategies according to level of recommendation

Intervention	Objective/Comment
1. General non-pharmacological measures	
Control of protein intake	0,7-0,8 g/day
Reduce salt intake	Na 2-3 g/day
Reduce obesity	< 30 kg/m^2
2. Pharmacological measures	
ACE inhibitors	< 0,5 g/g/day. First choice, even in normotensive patients, due to its cardioprotective and renoprotective effect
ARA II antagonists	<0.5 g/day. Using the maximum dose tolerable
ACE inhibitor + ARA II antagonists	Add ARA II antagonists to the maximum dose of ACE inhibitor, if the latter fails
Control of BP: B-Blockers Calcium antagonist	Systolic <120 mmHg
Avoid dihydropyridine calcium antagonists (unless they are necessary to control BP)	Control BP well but can generate proteinuria
Statins	Lipid-lowering and antiproteinuria effect
Other therapies: allopurinol, pentoxyphylline, mycophenolate	Few studies, or studies based on animal experiments

Treatment of hyperlipidemia

Hyperlipidemia is common after kidney transplant (40-50%) and some factors inherent to the transplant itself, such as immunosuppressive medication (mainly steroids, CsA and sirolimus), may contribute to its development[195]. In addition to the negative impact on cardiovascular disease, hyperlipidemia has been associated with CAN and early loss of kidney graft (196-198). Similarly, therapy with statins can prolong kidney graft survival[199]. It is therefore reasonable to treat hyperlipidemia early with the aim of reducing these complications, especially in patients with a high risk of cardiovascular disease. In this sense, transplant recipients have recently been included in the clinical practice guidelines on the management of chronic kidney disease in patients with a high cardiovascular risk[200].

Therefore, kidney transplant recipients must undergo routine evaluation in order to detect dyslipidemia. This includes a quarterly fasting lipid profile (cholesterol, LDL, HDL and triglycerides). Similarly, if hyperlipidemia is detected, secondary causes should be investigated.

Table VII shows the therapeutic recommendations for dyslipidemia in kidney transplant recipients. In general, the first stage should be lifestyle changes: reducing weight and calorie intake, avoiding alcohol and doing moderate exercise (Table VII). If these measures do not resolve the dyslipidemia, lipid-lowering therapy should be started according to the underlying lipid disorder (Table VIII). Except in the case of pure hypertriglyceridemia, statins are the drug of choice. In addition to their beneficial effect on lipid and cardiovascular profile, these drugs have certain immunomodulator effects (reduction in inflammatory response and inhibition of cytokine expression) that give them a potentially protective role in CAN[197,201], a fact which has not been confirmed in humans. Table IX shows the recommended statin doses by degree of renal function. We must remember that CsA increases statin levels in plasma, mainly simvastatin, atorvastatin and lovastatin. In situations where dyslipidemia is persistent, gradual reduction of immunosuppression (steroids, CsA or sirolimus) may be efficacious in improving the lipid profile. In severe cases, the switch from

Table VII. Therapeutic recommendatiosn for a lifestyle change in kidney transplant recipients

- Diet (supervision by an endocrinologist / dietician):
- Reduction of saturated fats (<7% of total calorie intake)
- Polyunsaturated fats: up to 10% of total calories
- Monosaturated fats: up to 20% of total calories
- Total diet fats: 25-35% of total calories
- Intake of carbohydrates: 50-60% of total calories
- Fiber: 20-30 gr/day
- Weight maintenance: BMI 25-27 kg/m2
- Physical activity: Sporting activity for 20 minutes, 3-4 times per week
- Habits
- Moderate alcohol consumption
- Avoid smoking

Table IX. Statins and ranges of recommended daily doses for the treatment of dyslipidemia according to level of renal function

	GFR ≥ 30	GFR < 30 or dialysis	With CsA
Atorvastatin	10-80 mg	10-80 mg	10-40 mg
Fluvastatin	20-80 mg	10-40 mg	10-40 mg
Lovastatin	20-80 mg	10-40 mg	10-40 mg
Pravastatin	20-40 mg	20-40 mg	20-40 mg
Simvastatin	20-80 mg	10-40 mg	10-40 mg

GFR, glomerular filtration rate (mL/min/1.73 m^2); CsA, cyclosporine

CsA to tacrolimus should be considered, clinical conditions permitting[195,202]. In patients with CAN, this measure can be a useful strategy for optimizing the lipid profile[203]. Recently, there have been reports on the use of ezetimibe, a cholesterol absorption inhibitor, in the treatment of hypercholesterolemia in transplant recipients, although experience is limited and there is a potential interaction with CsA[204,205].

Given the deleterious synergic effect of hyperglycemia and hyperlipidemia on cardiovascular disease and maybe on CAN, in patients who develop diabetes post-transplant, lipid-lowering therapy should be intensified following the American recommendations for the control of hyperlipidemia in type 2 diabetes mellitus[206]. Finally, lipid-lowering therapy can slow down the progression to chronic renal insufficiency[194], which might be extrapolated to recipients of a renal graft with impaired glomerular filtrate.

Hyperglycemia

According to the Experts' Committee[207,208], the diagnosis of post-kidney transplant diabetes mellitus is relatively frequent (5-20%) and is an independent risk factor for patient and graft survival[209-212]. In general, post-transplant diabetes mellitus is a consequence of the insulin resistance syndrome, which is affected by several factors, including immunosuppression[213-215]. Similarly, hepatitis C virus has been related to this metabolic alteration, especially in patients receiving tacrolimus[216,217]. Therefore, early identification of this metabolic syndrome, adherence to non-pharmacological methods and correct treatment of glucose disorders are the best therapeutic measures against this devastating complication.

Table X shows the pre-transplant risk factors for the development of post-transplant diabetes. Figure

Table VIII. Recommendations for the treatment of post-trasplant dyslipidemia

Dyslipidemic disorder	Objective	Initial treatment	Further treatment	Alternative
TG ≥500 mg/dl	TG <500 mg/dl	LSC	LSC + fibrates or niacin	Fibrates, fatty acids omega-3 or nicotinic acid
LDL 100-129 mg/dl	LDL <100 mg/dl	LSC	LSC + low-dose statins	Cholestyramine or nicotinic acid
LDL≥ 130 mg/dl	LDL <100 mg/dl	LSC + statins (low doses)	LSC + statins (maximum dose)	Cholestyramine or nicotinic acid
TG ≥200 mg/dl y Non-HDL ≥ 130mg/dl	Non-HDL < 130 mg/dl	LSC + statins (low doses)	LSC + statins (maximum dose)	Fibrates or nicotinic acid

TG, triglycerides; LSC, lifestyle changes; LDL, low-density lipoproteins; HDL, high-density lipoproteins; Non-HDL colesterol includes LDL plus VLDL (very low-density lipoproteins).

2 shows the recommendations for avoiding this complication and detecting it during the early stages of follow-up.

At present, diagnosis of post-transplant diabetes is based on the ADA/WHO (American Diabetes Association/World Health Organization) criteria (Table XI). The glucose tolerance test or glycosylated hemoglobin plasma levels are not recommended as screening determinations for diabetes due to their high cost and low sensitivity[218]. There is evidence that strict control of blood glucose confers a lower risk of meta-diabetic complications[219]. Therefore, in patients in whom diabetes is detected (table XI), or who fulfill the criteria for impaired fasting glucose (100-125 mg/dl) or impaired tolerance test at any of the scheduled visits (fig. 1), general measures should be reinforced (diet, control of weight, physical exercise, etc.) and replacing tacrolimus with CsA should be considered, mainly in those patients with more than 6 months post-transplant and no immunological risk. Optionally, reducing or eliminating steroids can help avoid post-kidney transplant hyperglycemia. If these measures do not control glucose levels (<100 mg/dl) in 2-4 months, a gradual therapeutic schema should be followed, similar to the one used

Table X. Pre-transplant risk factors for the development of diabetes after kidney transplant

- First-degree family history of type 2 diabetes mellitus.
- History of glucose intolerance before the development of uremia
- Gestational diabetes or glucose intolerance
- Advanced age
- African American or Hispanic race
- Obesity (BMI >30 kg/m^2)
- Hepatitis C
- Dyslipidemia (specially hypertriglyceridemia)

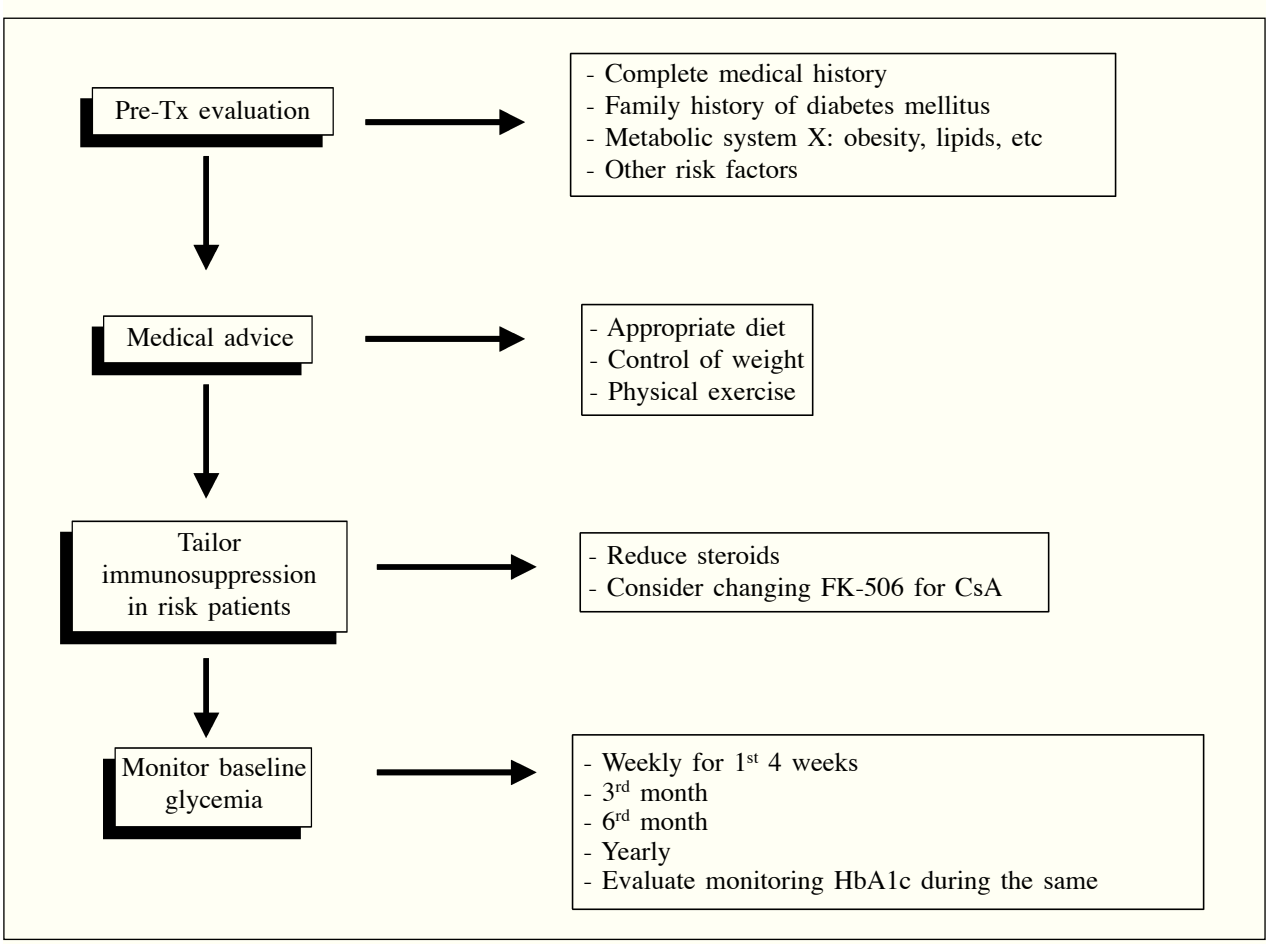

Fig. 2.—Detection and management of post-transplant diabetes.

in type 2 diabetes in the general population (fig. 3). Finally, treatment for diabetes should be accompanied by other recommendations including: a) Self-monitoring of glucose levels; b) Monitoring of hyperlipidemia and blood pressure (≤ 130/80 mmHg); c) Periodic determination of HbA1c (maintain levels <7%), and d) Follow-up and detection of diabetic complications, including retinopathy and neuropathy. ACE inhibitors or ARA II antagonists can be recommended in these patients, as long as renal function and potassium in plasma are closely monitored.

Table XI. Criteria for the diagnosis of post-kidney transplant diabetes according to the ADA (American Diabetes Society)[207, 208]

- Normal:

Fasting glucose <100 mg/dl (<5.6 mmol/L) or <140 mg/dl (<7.8 mmol/L) after 2 hours of oral overload of 75 g of glucose.

- Diabetes:

Fasting sugar ≥126 mg/dl (≥ 7 mmol/L) or
Symptoms of diabetes + glycemia at any time of the day ≥ 200 mg/dl (≥ 11.1 mmol/L) or
Glycemia ≥200 mg/dl after two hours with an oral overdose of 75 g of glucose

- Impaired fasting glucose:

Fasting glucose of 100-125 mg/dl (5.6-6.9 mmol/L)

- Impaired tolerance test:

Glycemia 140-199 mg/dl (7.8-11.0 mmol/L) after two hours of oral glucose overload

Smoking

The prevalence of smoking after transplant ranges between 25 and 40%[175,220], and it is known to be

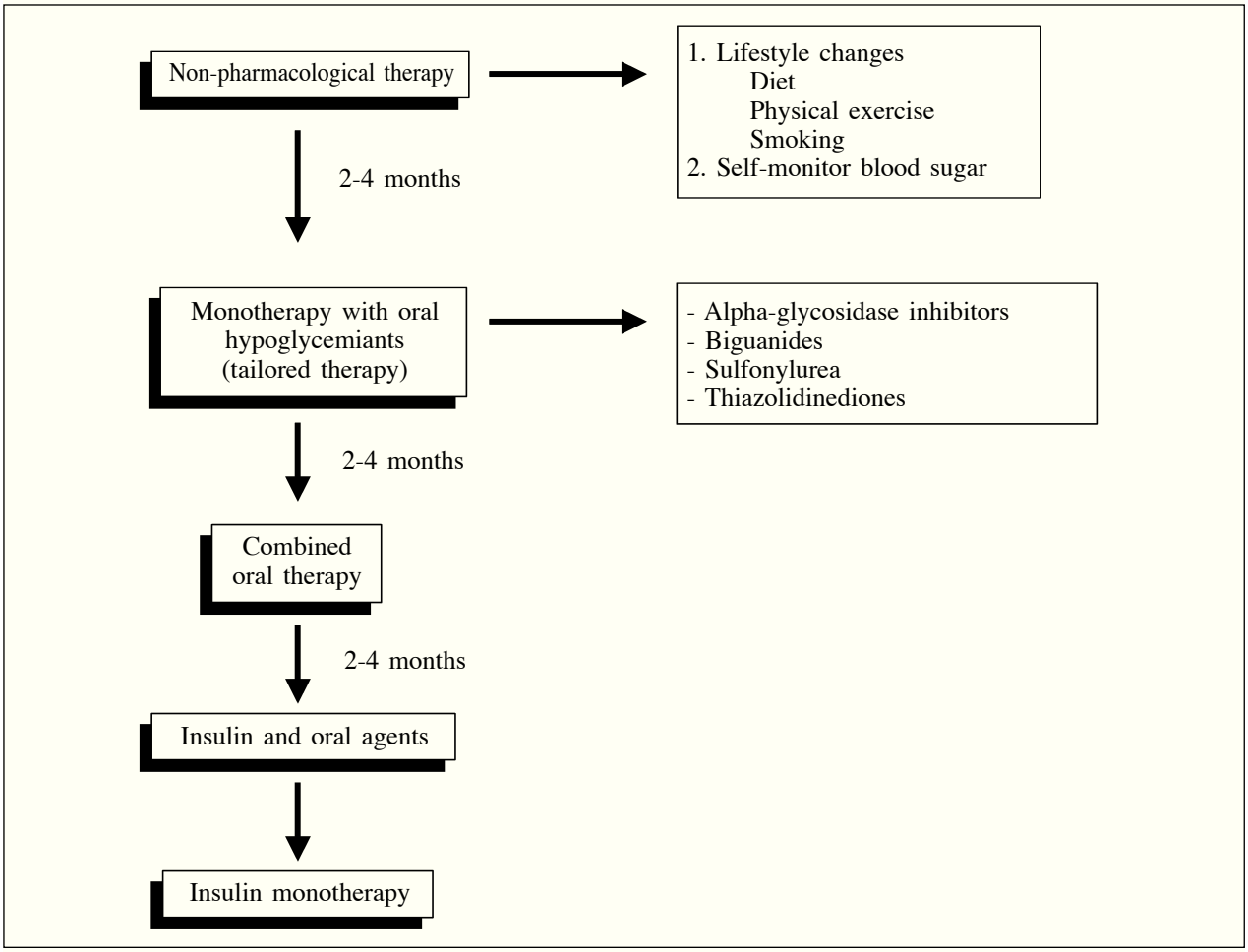

Fig. 3.—Treatment of post-kidney transplant diabetes mellitus.

associated with poorer graft and patient survival post-transplant[96,97], as well as with accelerated atheromasclerosis in different vascular territories[98]. Moreover, giving up smoking after transplant has been associated with an improvement in graft and patient survival rates[96,97]. Therefore, giving up smoking from the pre-transplant stage is strongly recommended in order to avoid these complications.

Overweight

Obesity (BMI > 30 kg/m^2) and overweight are associated with a higher rate of delayed renal function after a kidney transplant, but there is some controversy as to the prognosis of these patients in the longer term[175,221-223]. An imbalance between the metabolic demands of the recipient and nephron mass of the graft may explain, at least partly, the appearance of CAN. At the same time, obesity contributes to a poorer cardiovascular profile in kidney graft recipients, including hyperglycemic disorders. Although controlled studies are necessary to demonstrate it, patients with a BMI > 35 kg/m^2 should reduce weight in order to improve their long-term prognosis. Moderate exercise and a balanced low-calorie diet are the best measures for reaching this objective. Psychological support can be of great help and the withdrawal of steroids could help reduce weight. In some cases, it may be necessary to fit a temporary intragastric balloon to create a feeling of satiety, or resort to bariatric surgery[224]. All in all, obese patients (BMI > 30 kg/m^2) who receive a kidney transplant have a significantly better survival than those with similar overweight who remain on dialysis[225].

Antiproliferative drugs and other measures

There is evidence that some cytokines, mainly TGF-β1, take part in the development of the interstitial fibrosis that accompanies CAN. Similarly, some immunosuppressive agents activate the RAS, and angiotensin II is a powerful stimulus for the formation of TGF-β1. Therefore, blocking this system could be an alternative to avoid or minimize CAN. Recent studies have shown that administering losartan to transplant recipients with CAN significantly reduces levels of TGF-β1[180,226,227]. If the aforementioned effects are confirmed by future studies, these substances could be an attractive option for mitigating this complication.

Omega-3 fatty acids have antiproteinuric and anti-inflammatory properties by modulating the production of vasoconstrictive prostaglandins and reduce the generation of TNF-α and IL-1[228]. In kidney transplants, a modest fall in the levels of these cytokines during episodes of acute rejection has been observed[229], as has renal function stability in patients with CAN[230]. It remains to be seen whether continuous administration during the early stages of the transplant reduces the appearance of CAN.

IMMUNOSUPPRESSIVE THERAPY AND CAN

Introduction

Chronic allograft nephropathy is a heterogeneous entity with a multifactorial etiology, and is characterized by the progressive presence of renal insufficiency accompanied by arterial hypertension and proteinuria. In histological terms, it is characterized by substitution of the renal parenchyma for fibrous tissue, and the typical lesions are proliferative arteriopathy, tubular atrophy and glomerulosclerosis. The etiology of CAN is multifactorial and involves immunological factors such as acute rejection (clinical and/or subclinical) and anti-HLA antibodies, and non-immunological factors, such as nephrotoxicity by calcineurin inhibitors (CNI), arterial hypertension, proteinuria and ischemia-reperfusion injury. The multifactorial etiology of CAN means that immunosuppressive therapy is extremely important both in the prevention and in the development of CAN. The ideal immunuosuppressive agent for preventing CAN does not yet exist and would need to be potent and selective to block not only the T response but also the B response, highly efficacious in the prevention of acute rejection, free of nephrotoxic effects and with a powerful antiproliferative effect and good safety profile, especially cardiovascular safety. To analyze the relevance of immunosuppressive therapy on the modification of the natural history of CAN, we shall review the immunosuppressive agents one by one before concluding with a summary of the impact of immunosuppressive therapy on CAN.

Corticosteroids

Corticosteroids are used in practically all kidney transplants because of their anti-inflammatory and immunosuppressive effects, and their anti-inflammatory and antiproliferative effects may play a role in preventing CAN. Nevertheless, the adverse effects of this family are well known, especially induction of cardiovascular risk factors (diabetes, dys-

lipidemia and arterial hypertension) and osteoporosis. These adverse effects generally appear in the long term and are dose-dependent. This adverse safety profile has led to several more or less successful attempts to eliminate corticosteroids from kidney transplants. One of the first studies, a multicenter Canadian study with relatively few patients at the end of the 1980s, had negative results when it revealed a greater incidence of CAN in patients whose steroid therapy was withdrawn. However, a detailed review of the study shows that the data are not as clear as they seem initially, and that the patients whose therapy was suppressed probably started with a worse functional situation than the group that continued with steroid therapy. Recent studies examining the new immunosuppressive agents show the possibility of steroids withdrawal in the short-medium term post-transplantation without increasing the risk of developing acute rejection or CAN. A rational and progressive withdrawal of steroids should be a priority objective in all kidney transplant recipients —with the exception of immunological risk patients— to avoid the morbidity and mortality associated with their chronic use. The antiproliferative and anti-inflammatory effect of steroids should be compensated by current immunosuppressive agents, especially MPAs (mycophenolate mofetil and MPA) and mTOR inhibitors (sirolimus and everolimus), with potent antiproliferative action.

Calcineurin inhibitors (cyclosporine and tacrolimus)

We shall evaluate the role of calcineurin inhibitors (CNI) in the prevention and development of CAN, without distinguishing between CsA and tacrolimus, given their total similarity in mechanism of action and safety profile. Furthermore, most comparative studies of CNI show the same results both in the incidence of acute rejection and in the development of CNI. These drugs are an essential element in the pathophysiology of CAN. Their introduction in kidney transplant immunosuppressive therapy has been the most important advance of the last 20 years in the field of organ transplantation, with a significant decrease in the incidence of acute rejection. This decrease had a positive effect on patient and graft survival during the first year after transplant. Nevertheless, the nephrotoxic effects of these drugs, which were directly related to its mechanism of action, soon became obvious. These included severe arteriolar vasoconstriction, decrease in glomerular filtrate and presence of increasing interstitial fibrosis, with destruction of the renal parenchyma and progressive renal insufficiency. Therefore, CNI affect CAN in two ways: first, they prevent its development by preventing acute rejection and, second, they participate directly in its pathogenesis by their nephrotoxic effect and probably also by inducing cardiovascular risk factors such as diabetes, arterial hypertension and dyslipidemia. Recent data from the Australian study by Nankivell and Chapman, with protocol biopsies taken from kidney-pancreas recipients during the 10-year post-transplant period, show the universal and progressive character of CNI-associated nephrotoxicity and its importance in the development of CAN. Furthermore, data from the Hospital Ramón y Cajal in Madrid and a recent Australian study show how long-term graft survival (> 10 years) is higher in CNI-free patients treated with azathioprine compared with those who maintain therapy with CsA. After the first year of transplant, elimination of CNI leads to better renal function and longer graft survival, probably due to the absence of nephrotoxicity and poorer development of CAN. It is difficult to define the current situation of CNI in the development of CAN, as they can be combined with MMF or sirolimus. The combination of CNI with MMF or sirolimus makes it possible to minimize substantially the doses and levels of CNI that probably avoids, at least in part, their nephrotoxic and profibrotic effect and the possible development of CAN. Nevertheless, the administration of CNI is inevitably associated with nephrotoxicity, renal fibrosis and CAN, although this effect is dose-level – dependent and chronic. We must also mention that the sensitivity of the kidney graft to the nephrotoxic effect of CNI is different depending on the previous nephrological status of the kidney and, in particular, on donor age. Grafts from elderly and/or marginal donors are much more sensitive to the nephrotoxic effect of CNI than those from young or optimal donors.

Antimetabolite drugs – Purine synthesis inhibitors (azathioprine, mycophenolate mofetil and MPA)

It is difficult to define the role of antimetabolite drugs in preventing CAN, since they are generally the second line of immunosuppressive therapy in kidney transplantation. Their preventive effect can be analyzed based on five different mechanisms of action: i) Effect on the prevention of acute rejection; ii) Effect on reducing the number of CNI; iii)

Replacement of CNI in the medium term post-transplantation; iv) Absence of nephrotoxicity and non-induction of cardiovascular risk factors; and v) Direct antiproliferative effect. Their end effect in the prevention of CAN may be a combination of all five, although it is true that the introduction of these drugs, especially MMF, has had a positive impact on the long-term results of kidney transplantation, probably thanks to a very significant reduction in the incidence of acute rejection and also to a reduction in the incidence of CAN by enabling the minimization of CNI and to their antiproliferative effect. In general, the data-results of the different antimetabolite drugs could be extrapolated from one to the other, although their different historical development makes this analysis difficult. Nevertheless, it is obvious that the immunosuppressive potential of MMF is much superior to that of azathioprine, with a very significant reduction in the incidence of acute rejection when the results of both drugs in combination are compared with CsA. As far as enteric-coated MPA are concerned, despite their correct theoretical design, they do not seem to provide substantial advantages over MMF, neither in terms of efficacy nor in terms of gastrointestinal tolerance.

In order to analyze the impact of these drugs on the development of CAN, we shall comment on each of the abovementioned points:

i) Prevention in the development of acute rejection. AR is obviously a decisive factor in the development of CAN, and the introduction of antimetabolite drugs, especially MMF, significantly reduced the incidence of AR. Therefore, MMF could play a decisive role in preventing CAN via this mechanism.

ii) Effect on reducing the number of CNI. The introduction of these drugs, especially MMF, allows the number of CNI to be reduced. We have already mentioned that the nephrotoxic effect of CNI is dose-level – dependent, therefore, the substantial reduction in dose and levels of CNI and its combination with MMF should significantly reduce its nephrotoxic impact and the development of CAN.

iii) Replacing CNI in the medium-term post-transplantation. Most of the data on this area refer to switching CsA for azathioprine after the first year post-transplant, which was a fairly generalized policy in the 1980s. The development of acute rejection coinciding with this change in immunosuppressive therapy led most units to abandon the policy. Nevertheless, when the long-term results are reviewed, we can see that allograft survival is higher in CNI-free patients who have switched to azathioprine. There are few data on MMF, although in recent years, several protocols have analyzed this possibility. The first studies were also accompanied by an excessively high and intolerable incidence of AR, which led to a certain disappointment with this policy; therefore, it was not generally applied. However, recent studies in selected groups of patients show the usefulness of this practice, with a significant improvement in renal function parameters and a minimum incidence of AR. The improvement in renal function has been accompanied by an improvement in cardiovascular risk parameters. Controlled-prospective studies are necessary to determine the advantages of switching from CNI to MMF.

iv) Absence of nephrotoxicity and non-induction of cardiovascular risk factors. This is another important aspect of antimetabolite drugs, which is decisive in preventing CAN, either in combined therapy with reduced-dose CNI or in CNI-free therapy.

v) Direct antiproliferative effect. This is exclusive to MMF, and could have a positive impact on preventing CAN. The antiproliferative capacity of MMF could prevent the vascular lesions associated with CAN, which are often decisive for outcome. Recent studies show this capacity in the prevention of graft vascular disease, the clinical form of chronic rejection in heart recipients, and which is very similar to CAN of the graft.

In summary, antimetabolite immunosuppressive drugs, especially MPAs, could play a decisive role in preventing CAN by means of the abovementioned mechanisms. Nevertheless, to date, there are NO prospective studies with protocol biopsies that demonstrate this preventive effect. Histological data at three years using MMF are relatively similar to those observed previously, with no decisive impact on prevention. A high percentage of patients from the Nankivell-Chapman study were on azathioprine or MMF, and the presence of CAN was almost universal after the second year post-transplantation. We need prospective, randomized, long-term studies with protocol biopsies to confirm the preventive capacity of MMF in the development of CAN.

mTOR inhibitors (sirolimus and everolimus)

The introduction of sirolimus to immunosuppressive therapy is relatively recent, and so we have little evidence of its ability to prevent CAN. Nevertheless, at least in theoretical terms, mTOR inhibitors (sirolimus and everolimus) would be the drugs of choice in treating and preventing CAN. Their potent and selective immunosuppressive effect, their antiproliferative capacity, the absence of nephrotoxicity and a favorable cardiovascular profile mean that mTOR should change the natural history of CAN. Most available studies are with sirolimus, but the identical mechanisms of action and safety profiles allow us to extrapolate data among currently available mTOR.

Current data on the use of sirolimus in kidney transplantation and its relevance in the development and prevention of CAN can be summarized in four different categories: i) Combination with CNI; ii) Early suppression of CNI; iii) CNI-free therapy; and iv) Chronic switching to sirolimus.

i) Combination with CNI. The combination of the mTOR inhibitors sirolimus or everolimus with CNI (CsA or tacrolimus) is accompanied in the medium term (1-2 years post-transplantation) by impaired renal function compared with mTOR-free therapy (CNI + MMF), probably because of an increased nephrotoxic effect of CNI. No histological data guarantee this poorer renal function, but it is surely due to a higher degree of renal fibrosis and a greater incidence of CAN. Data from experimental models confirm the greater severity of CNI-associated nephrotoxicity when CNI are combined with mTOR inhibitors. Therefore, combination therapy with mTOR + CNI does not seem to be recommendable for long periods, despite its high efficacy in preventing acute rejection. Elimination or reduction in the number of CNI should be considered in the medium term.

ii) Early suppression of CNI. Data from study 310 (RMR) reveal the possibility of suppressing CNI during the first months after transplant for sirolimus. Suppression of CNI is accompanied by a significant improvement in renal function (GFR), which gradually increases over time, a significant improvement in chronic lesions in the renal biopsy, and, of particular importance, a significant improvement in graft survival after the fourth year post-transplantation. The slight increase in the incidence of acute rejection (< 5%) observed with suppression of CAN does not seem to negatively affect later outcome of the renal graft. Data from study 310 support the suppression of CNI and use of sirolimus as a suitable strategy for preventing CAN and for improving graft survival results in the medium-long term.

iii) CNI-free therapy. It seems reasonable that CNI-free therapy should be the best strategy for preventing CAN, as long as it is accompanied by a low incidence of acute rejection and a good safety profile. To date, there has been little evidence in this respect, but the work of S. Flechner indicates that the combination of sirolimus and MMF with loading regimens has a very low incidence of acute rejection (< 10%), a good safety profile, better renal function (GFR) than traditional regimens of CNI and MMF, although the most spectacular advantage at two years post-transplantation is that the incidence of CAN is significantly lower in the CNI-free group than in the CNI + MMF group (25 vs. 70%). Furthermore, the study shows a greater activation of profibrotic genes in the CNI group than in the SRL + MMF group. The data are preliminary and only include 65 patients, although, there are three large-scale studies in progress that could confirm this hypothesis: the Symphony study, with a CNI-free arm (SRL + MMF + daclizumab), the Orion study (SRL + MMF + basiliximab) and Study 318 (SRL + MMF + basiliximab). In all three studies, there is a control group with conventional CNI therapy (CsA or TAC) + MMF. The studies last two years, after which time some of them carry out protocol biopsies that could confirm a lower incidence of CAN in the CNI-free arms. These three studies should confirm the benefits of CNI-free therapy in the prevention of CAN, with improvement in graft survival in the medium-long term.

iv) Chronic switch to SRL. Finally, this could be a good strategy in the prevention and/or treatment of CAN. With respect to the switch to SRL once CAN has already been defined, there are few histological data to support involution of chronic CAN lesions post-switch. Studies published to date DO NOT PERFORM kidney biopsies after switching, and only show a significantly improved renal function in a sub-

group of patients (75% of the total). Recent series of switches to SRL due to CAN show the need to make the switch early, with moderate impairment of renal function, and especially in the absence of massive proteinuria. We must wait for data from the CONVERT macrostudy (# 318), which carries out biopsies at baseline and at two years, to confirm the regression and/or improvement in renal histology after suppressing CNI, compared with the group remaining on CNI. In some American centers, the usual practice is to replace CNI a few months after the transplant, once the immediate transplant period has finished, and acute rejection, surgical problems and delayed renal function have been avoided. Nevertheless, it is still early to affirm that this strategy is accompanied by a significant reduction in the incidence of CAN. In general, it seems reasonable to consider the possibility of switching CNI to mTOR, although this should always be done early, without waiting for severe CAN lesions, which are irreversible in many cases. Furthermore, in cases of developed CAN lesions, switching to SRL could be contradictory, as it may increase the magnitude of proteinuria and probably speed up progression to chronic renal insufficiency.

Inducion therapy: monoclonal and polyclonal antibodies

There is little evidence on the role of loading therapy in preventing CAN. The basic objective and indication for loading therapy with monoclonal or polyclonal antibodies is to prevent acute rejection. Most studies on loading therapy merely refer to a decrease in the incidence of acute rejection, without presenting long-term data on graft and patient survival or on the incidence of CAN. It is obvious that AR favors the development of CAN; therefore, preventing AR should reduce the incidence of CAN. Nevertheless, loading therapy is administered during very short periods, just after transplant, and it is during this period when it protects patients from the development of AR. There are some data (although not validated) which state that thymoglobulin, especially at high doses, could produce tolerance, which in turn would lead to a low incidence of CAN by eliminating the immunological component of CAN and offering the possibility of stopping immunosuppressive therapy. Another advantage of loading therapy is the possibility of avoiding and/or minimizing administration of CNI in the immediate post-transplant period, thus avoiding its nephrotoxic effect and ischemia-reperfusion injury in an extremely sensitive graft in this phase. Loading regimens allow CNI-free therapy, using the combination of SRL + MMF, with a very low incidence of AR and an acceptable safety profile. In summary, it is difficult to define the role of loading therapy in the development of CAN, but correct use significantly reduces the incidence of AR and makes it possible to use CNI-free regimens, which should be accompanied by a lower long-term incidence of CAN.

New immunosuppressive drugs

There are few data on the role of new immunosuppressive drugs in preventing CAN. CAN is a chronic process that usually takes place after the third or fourth year post-transplant, and which requires protocol biopsy for an early diagnosis. To date, no drug being developed has shown the capacity to prevent CAN. Studies on the development of new immunosuppressive drugs merely show their usefulness and efficacy in preventing AR, but do not have prevention of CAN as an objective. These studies are usually very short (6-24 months), which prevents us from analyzing their influence on the development of CAN. The only drug of those currently being developed with any capacity to prevent CAN is belatacept (LEA29Y), a potent co-stimulation blocker (anti-CD28), with high efficacy in preventing AR, no nephrotoxic effects and a good safety profile. Its only limitation is that it must be administered parenterally.

Recent data on belatacept confirm its ability to prevent AR with a good safety profile and significant improvement in renal function one year after transplant compared with CNI + MMF. Long-term prospective studies on this and other immunosuppressive drugs should show their ability to prevent CAN.

Summary and conclusions

Immunosuppressive therapy plays a decisive role in the development of CAN, by preventing acute clinical or subclinical AR, and because of the nephrotoxic effect associated with CNI therapy. Despite this important role, there is little evidence to indicate which is the best therapeutic option in preventing

CAN. The need for long-term studies due to the chronic nature of CAN and the use of protocol biopsies to diagnose CAN have noticeably limited the role of CAN as the primary or secondary objective in most clinical trials analyzing immunosuppressive drugs[15,17,100-102,151,152,231-243].

5. Recommendations

Chronic Allograft Nephropathy	
Recommendation	**Level of evidence**
IMPACT OF CAN ON THE OUTCOME OF THE TRANSPLANT	
Chronic allograft nephropathy is the first cause of graft loss after the first year post-transplant. It is a clinical-pathological entity with a multifactorial origin characterized by tubulo-interstitial and vascular damage accompanied by a progressive impairment of renal function, hypertension and proteinuria.	A
Graft half-life has improved during the last ten years, and is now longer in live-donor transplants, followed by cadaveric transplants with a standard donor, and lastly a cadaveric transplant with expanded criteria.	B
NON-IMMUNOLOGICAL RISK FACTORS: GENETIC FACTORS	
Some genetic polymorphisms of the donor and recipient of the molecules which are involved in the pathogenesis of CAN can play a pathogenic role in the development and outcome of this entity.	C
NON-IMMUNOLOGICAL RISK FACTORS: OTHER FACTORS	
The non-immunological factors that predispose to CAN are:	
Nephrotoxicity induced by calcineurin inhibitors	B
Donor age	B
Brain death process	B
Ischemia-reperfusion injury	B
Inadequate nephron mass	B
Delayed intial graft function	B
Obesity	B
The non-immunological risk factors that accelerate the progression of CAN are:	
Nephrotoxicity due to calcineurin inhibitors	B
Proteinuria	B
Poor renal function	B
Hypertension	B
Post-transplant hyperglycemia	B
CMV infection	C
HCV infection	C
Lipid alterations	C
Smoking	C
IMMUNOLOGICAL RISK FACTORS	
The degree of HLA incompatibility between the cadaveric donor and the recipient only has an influence when we compare situations between maximum and minimum compatiblity, i.e. 0 and 6 HLA antigens.	B

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Chronic Allograft Nephropathy	
Recommendation	**Level of evidence**
Acute rejection is associated with poorer survival, as is its number and clinical and histological intensity.	B
Post-transplant appearance of anti-HLA antibodies has a negative influence on long-term graft survival.	A
In patients diagnosed with CAN, it is recommended not to determine donor-specific antibodies.	D
Determination of anti-HLA antibodies is recommended in the case of impaired renal function, serum creatinine > 150 micromol/l (1.7 mg/dl) or before changes in immunosuppression.	D
DIAGNOSIS	
The clinical suspicion of CAN requires histological confirmation.	A
Biopsy is indicated in patients with proteinuria > 1g/24h, slow and progressive increase in serum creatinine of at least 15% during the last 3 months and/or suboptimal renal function defined as serum creatinine > 150 micromol/l (1.7 mg/dl).	C
Protocol biopsies allow early diagnosis of CAN.	B
CAN diagnosed in protocol biopsies is an independent predictor of graft survival.	B
There is controversy surrounding the relationship between subclinical acute rejection and CAN.	D
The coexistence of CAN and subclinical acute rejection in a protocol biopsy implies a worse prognosis than the presence of only one of these lesions.	B
Transplant glomerular disease is a factor of poor prognosis in the outcome of CAN.	B
MODIFICATION OF NON-IMMUNOLOGICAL FACTORS: ARTERIAL HYPERTENSION	
AHT is very prevalent after kidney transplant. It is a clinical marker of CAN and contributes to graft loss and morbidity and mortality.	B
The therapeutic objective is to maintain blood pressure at £130/80 in patients who do not have proteinuria, and at £125/75 in those with post-transplant proteinuria.	B
Patients with proteinuria should begin therapy with ACE inhibitors or ARA.	C
In patients with uncontrolled AHT and/or impaired renal function, other causes of AHT should be ruled out, especially renal graft arterial stenosis.	C
Withdrawal of steroids and/or switching CsA to tacrolimus or an mTOR inhibitor can reduce blood pressure.	B

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Chronic Allograft Nephropathy	
Recommendation	**Level of evidence**
MODIFICATION OF NON-IMMUNOLOGICAL FACTORS: PROTEINURIA	
Proteinuria is common after kidney transplant and is associated with graft loss and mortality and morbidity of patients with a renal graft.	B
The drugs of choice are ACE inhibitors and ARA. Therapy should aim to maintain figures at <0.5 g/day.	B
MODIFICATION OF NON-IMMUNOLOGICAL FACTORS: HYPERLIPIDEMIA	
Modification of immunosuppression can help to improve the lipid profile after a kidney transplant.	B
There is some controversy over the role of lipid-lowering therapy (statins and fibrates) in the prevention of CAN.	D
MODIFICATION OF NON-IMMUNOLOGICAL FACTORS: HYPERGLYCEMIA	
Changes in immunosuppression can help to minimize this post-transplant complication.	B
MODIFICATION OF NON-IMMUNOLOGICAL FACTORS: OVERWEIGHT	
In the post-transplant period, the following are recommended: moderate exercise, dietary control and, in cases of morbid obesity that does not respond to these measures, bariatric surgery should be considered.	C
Withdrawal of steroids can help improve post-transplant obesity.	C
MODIFICATION OF NON-IMMUNOLOGICAL FACTORS: ANTIPROLIFERATIVE DRUGS AND OTHER MEASURES	
ACE inhibitors/ARA II can prevent appearance of CAN, due to their antiproteinuria and antifibrosis effects.	C
There is no evidence that omega-3 fatty acids curb progression of CAN.	A
IMMUNOSUPPRESSIVE THERAPY	
The incidence of CAN is practically the same with CsA and tacrolimus as basic immunosuppression.	A
The early withdrawal of CsA followed by therapy with sirolimus in patients with a low immunological risk is associated with a lower incidence of CAN and better graft survival that continuous therapy with both drugs.	A
Prolonged therapy with CNI is associated with a greater incidence of CAN than CNI-free therapy.	B
Suppression of steroids in the medium-long term is not accompanied by a negative effect on graft survival.	B
Prolonged therapy with MMF is accompanied by a reduction in the incidence of CAN compared with prolonged azathioprine therapy.	B

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