

# Ischemia and reperfusion injury in renal transplantation: hemodynamic and immunological paradigms

Lesão de isquemia e reperfusão no transplante renal:  
paradigmas hemodinâmico e imunológico

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## ABSTRACT

Ischemia and reperfusion injury is an inevitable event in renal transplantation. The most important consequences are delayed graft function, longer length of stay, higher hospital costs, high risk of acute rejection, and negative impact of long-term follow-up. Currently, many factors are involved in their pathophysiology and could be classified into two different paradigms for education purposes: hemodynamic and immune. The hemodynamic paradigm is described as the reduction of oxygen delivery due to blood flow interruption, involving many hormone systems, and oxygen-free radicals produced after reperfusion. The immune paradigm has been recently described and involves immune system cells, especially T cells, with a central role in this injury. According to these concepts, new strategies to prevent ischemia and reperfusion injury have been studied, particularly the more physiological forms of storing the kidney, such as the pump machine and the use of antilymphocyte antibody therapy before reperfusion. Pump machine perfusion reduces delayed graft function prevalence and length of stay at hospital, and increases long-term graft survival. The use of antilymphocyte antibody therapy before reperfusion, such as Thymoglobulin™, can reduce the prevalence of delayed graft function and chronic graft dysfunction.

**Keywords:** Reperfusion injury; Ischemia; Delayed graft function; Graft rejection; Perfusion/utilization

## RESUMO

A lesão de isquemia e reperfusão é um evento inevitável no transplante de rim, tendo como consequências retardo na função do enxerto, aumento no tempo de hospitalização e dos custos, aumento no risco de rejeição aguda e potencial impacto negativo na evolução a longo prazo.

Atualmente, vários fatores estão implicados na fisiopatologia da lesão de isquemia e reperfusão, podendo ser didaticamente divididos em dois paradigmas: hemodinâmico e imunológico. O paradigma hemodinâmico é classicamente descrito como a privação de oxigênio pela interrupção do fluxo sanguíneo, envolvendo diversos sistemas hormonais e pela produção de radicais livres de oxigênio após a reperfusão. O paradigma imunológico tem sido descrito mais recentemente e envolve as células do sistema imune, sobretudo as células T, como papel fundamental na lesão. De acordo com esses conceitos, novas estratégias de prevenção dos impactos da lesão de isquemia e reperfusão têm sido estudadas, especialmente formas mais fisiológicas de preservação do órgão, como a preservação em máquina de perfusão e o uso de anticorpos depletadores de linfócitos antes da reperfusão. A perfusão em máquina reduz a prevalência de retardo na função do enxerto e o tempo de hospitalização, além de melhorar a sobrevida do enxerto a longo prazo. Já o uso de anticorpos depletadores de linfócitos, como Timoglobulina®, antes da reperfusão, pode diminuir a prevalência de retardo na função do enxerto e a disfunção crônica do mesmo.

**Descritores:** Traumatismo por reperfusão; Isquemia; Função retardada do enxerto; Rejeição de enxerto; Perfusão/utilização

## INTRODUCTION

Acute kidney injury (AKI) of ischemic nature is the most common form of intrinsic renal disease in adults, and is associated with adverse clinical outcomes and high mortality rates.<sup>(1)</sup> The incidence of AKI in hospitalized patients varies from 2 to 7%, and can be higher than 10% in patients at intensive care units.<sup>(2)</sup>

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Currently, the mortality of AKI patients in native kidneys is approximately 50%, ranging from 30 to 70%.<sup>(3,4)</sup> In terms of pathophysiology, renal tubular ischemic disease was considered as resulting from tissue hypoxia.<sup>(5)</sup> When observing that glomerular filtration would not start immediately after reestablishing oxygen supply to the damaged tissue, some studies demonstrated two important concepts. First, that reperfusion after ischemia was part of the pathophysiology of the lesion, by means of generation of free oxygen species, with modifications in the cell environment of the renal tubule. Second, that a determined time interval was necessary for recovery of the tubular structure.<sup>(1,5,6)</sup> In this way, the ischemia and reperfusion injury (IRI) became the central pathophysiologic mechanism of ischemic AKI, with the participation of various hormone systems.<sup>(5,6)</sup>

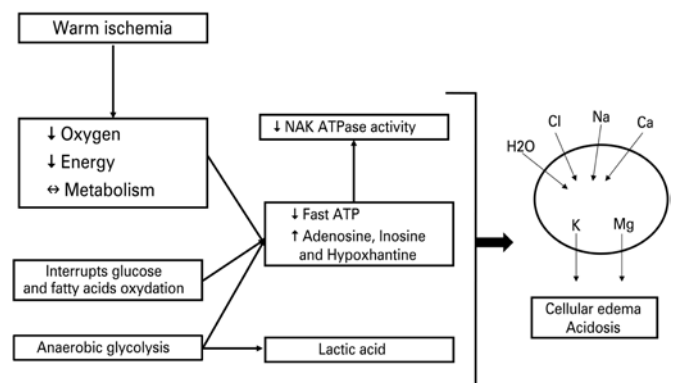
However knowledge about of the pathophysiology of IRI enhanced during the last decade, identifying components of the immune system as mediators of the lesion.<sup>(7-11)</sup> Thus, hypoxia is the initial insult and trigger of IRI, but the approach of a new paradigm for this lesion involves immune system cells, especially T cells and activation and cell adhesion molecules, that is, the immune paradigm. Despite not being frequent in native kidneys, IRI is an inevitable event in solid organ transplants. The relation between mortality and IRI is well established in native kidneys, but it is not clear in kidney transplantation. It is not possible to affirm that IRI is a factor independent from mortality in this mode of treatment, although it is related to increased risk of acute rejection (AR) and loss of renal graft in the long run. Therefore, some considerations about IRI in kidney transplant will be presented.

## HEMODYNAMIC PARADIGM

The hemodynamic paradigm of IRI in organ transplants stems from the principle that the central factors involved in IRI are the imbalance between offer and consumption of oxygen in the graft, which involves management of the donor, especially the donor with encephalic death; the variables related to the removal of the organ; the manner of preservation; the time during which the organ remains without blood circulation; and the effects of reperfusion. The clinical results with kidney transplantation can be influenced by the prolonged storage of the organ, which is correlated with the initial delay in graft function, complications with managing immunosuppression and length of hospital stay, among other outcomes.<sup>(6)</sup>

Between the removal of the dead donor organ until reperfusion in the receptor, the renal tissue is exposed to two different processes: warm ischemia and cold

ischemia. The time of warm ischemia is defined by the period between clamping of the donor's artery until perfusion with the preservation solution. This is the period in which cell damage is more evident. During warm ischemia, there is an abrupt interruption of the oxygen supply to the cells, with a consequent reduction of aerobic metabolism, suspension of glucose and fatty acid oxidation, and transfer of glycolysis to the anaerobic route, which significantly reduces the quantity of intracellular adenosine triphosphate (ATP) (Figure 1). With little ATP available, there is reduction of Na/K/ATPase activity, with a hydroelectrolyte imbalance between the intra- and extracellular space, favoring the formation of cellular edema. Additionally, the anaerobic metabolism substantially increases the quantity of lactic acid, causing a drop in intracellular pH. Studies show that kidneys exposed to more than one hour of warm ischemia become unviable, and when for more than five minutes, they are already associated with unfavorable outcomes of IRI.<sup>(1,6)</sup> The period corresponding to the graft perfusion until unclamping of the anastomosis in the receptor is the of cold ischemia time (CIT).



NAK ATPase: Na(+) and K(+) dependent ATPase; H<sub>2</sub>O: water; Cl: chlorine; Na: sodium; Ca: calcium; K: potassium; mg: magnesium; ATP: adenosine triphosphate

**Figure 1.** Impact of warm ischemia on the intracellular environment

## IMMUNE PARADIGM

There are at least five lines of evidence demonstrating the participation of T-lymphocytes and of their surface molecules in the lesion: (1) lymphocytes are found in tissues after ischemia;<sup>(10,11)</sup> (2) cytokines classically produced by these cells are amply expressed in tissue (upregulation) after the lesion;<sup>(12,13)</sup> (3) molecules such as CD11/CD18 and ICAM-1, responsible for leucocyte adhesion, are mediators of experimental IRI;<sup>(13-15)</sup> (4) the lymphocyte is the mediator of IRI in murine livers;<sup>(16)</sup> (5) blockage of lymphocyte costimulation routes, such as CD28-CD80, via CTLA4, significantly reduces the impacts of the lesion in experimental models.<sup>(17)</sup>

Corroborating this evidence, an elegant experiment performed in knockout mice for T CD4+/CD8+ cells demonstrated that, in the absence of the CD4+ phenotype, IRI was dramatically attenuated.<sup>(18)</sup> The proposed activation mechanisms of T-lymphocytes in IRI, on the other hand, follow three lines of evidence: (1) activation of the T-lymphocyte in an independent alloantigen manner; (2) mobilization of the T-lymphocyte from the circulation to the lesion site, by means of transendothelial migration; and (3) aggression to the target tissue of the initial insult.

It is speculated that the reactive species of oxygen could modify the structure of their own molecules, making them become antigens.<sup>(19)</sup> After reaching the lesion site, the role played by the lymphocyte is not known. Lai et al., however, demonstrated that the infiltration of T cells occurs in the first hour after the injury, remaining in the tissue for about 4 hours and leaving the tissue free after 24 hours, a model that has been called hit-and-run.<sup>(11)</sup>

## CLINICAL ASPECTS

Clinically, IRI in kidney transplant is manifested as the delayed graft function (DGF), despite there not being a consensus for its clinical definition. In a study that analyzed 65 publications between 1984 and 2004, which covered the definition of DGF, the authors identified 18 different definitions based on two criteria: need for dialysis after transplant or the non-occurrence of creatinine reduction. However, the objective parameters, such as time of dialysis after the transplant or rate of creatinine reduction vary significantly (Table 1).<sup>(20)</sup> This absence of standardization in concepts has made it difficult to establish precise data, such as prevalence and its impact on short- and long-term outcomes.

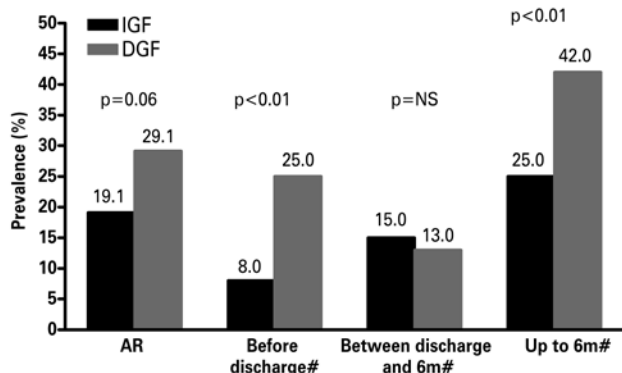
DGF is the most common complication in the immediate post-transplant period with a deceased donor, and it affects 8 to 50% of this type of transplant in the United States; in Brazil, it reaches 80%.<sup>(21,22)</sup> In a study conducted by our group with patients transplanted with deceased kidney donors at the *Hospital Israelita Albert Einstein* and *Universidade Federal de São Paulo*, between 2000 and 2005, we observed a 58.6% prevalence of DGF. When we compared these patients to those who also received grafts from dead donors, but who had immediate function, we noted that patients with more than 50 months on dialysis before the transplant had a risk of DGF 42% greater than those with less time on dialysis, and that a CIT superior to 24 hours increased

**Table 1.** Different definitions used for delayed graft function

Definitions	Studies	Patients
Definitions based on dialysis		
Need for dialysis in the first week after transplant	41	259,251
Need for dialysis in the first week after transplant, as long as the hyperacute rejection and vascular or urological complications are ruled out	2	760
Need for dialysis after transplant	2	737
Need for dialysis in the first 10 days after transplant	1	41
Absence of any type of renal function that might impose the need for 2 or more dialyses in the first week after transplant	1	547
Need for dialysis in the first week after transplant, having excluded a single dialysis immediately after transplant, if it was indicated for hyperkalemia	1	319
Return to maintenance dialysis in the first 4 days after transplant	1	263
Definitions based on creatinine		
Increase in creatinine or reduction <10% in the 3 consecutive days after transplant	5	1471
Reduction in creatinine <30% and/or urinary creatinine <1,000mg on the second day of transplant	2	401
Creatinine >2.5mg/dL on the seventh day after transplant	1	99
Time necessary for clearance of creatinine to be >10mL/min superior to 1 week	1	843
Non-reduction of creatinine in the first 48 hours, in the absence of rejection	1	291
Combination		
Non-reduction of serum creatinine at levels inferior to those pre-transplant, even with an adequate urinary volume	1	158
Increase in creatinine in the first 6-8 hours after the transplant or urinary volume <300 mL, despite adequate volemia and use of diuretics	1	143
Dialysis after transplant or creatinine >150 mcmmol/L 8 days after the transplant	1	112
Urinary volume <1.0 L in 24 hours and <25% of reduction in creatinine in the first 24 hours after transplant	1	244
Urinary volume <75 mL/hour in the first 48 hours or non-reduction in creatinine >10% in the first 48 hours	1	66
Need for dialysis in the first week after transplant or non-reduction in creatinine in the first 24 hours after transplant	1	104

this risk by 57%.<sup>(23)</sup> Despite the time or warm ischemia being more damaging to the organ, currently, with skilled teams, this time rarely exceeds 5 minutes, and CIT is one of the primary variables related to DGF.<sup>(21-24)</sup> There is an increase in incidence of DGF of about 8% for each 6-hour increase in CIT.<sup>(23)</sup> Other factors, such as HLA (human leukocyte antigen) compatibility, donor with expanded criterion (or isolated age of the donor), race and gender of the receptor, have also been implicated in the association with intensity of the IRI after kidney transplant.<sup>(21,23-25)</sup>

DGF causes prolonged hospitalization, increased costs and greater complexity in management of immunosuppressing drugs.<sup>(26-29)</sup> The longer length of stay add approximately US\$ 25,000 per patient to the transplant cost.<sup>(29)</sup> Another immediate consequence is the difficulty in initial management of immunosuppressing agents, especially the calcineurin inhibitors and mTOR (mammalian target of rapamycin).<sup>(30)</sup> It is also speculated that DGF is associated with the risk of AR, as well as chronic graft dysfunction (CGD). Some studies showed a correlation of prevalence between DGF and AR, in which the risk of AR may be duplicated in patients that present with DGF.<sup>(21-23,24)</sup> Figure 2 shows the associations between DGF and AR, according to time of transplant. On the left bar the incidences of AR are shown as per DGF or immediate graft function among 628 patients transplanted in the city of São Paulo (SP).<sup>(23)</sup> On the other bars (demonstrated with the symbol # on figure 2), the incidences of AR between the transplant and hospital discharge, between discharge and 6 months after transplant, and the episodes of AR that occurred after the hospital discharge and up to 6 months after the transplant are shown.<sup>(21)</sup>



IGF: immediate graft function; DGF: delayed graft function; AR: acute rejection; m: months NS: not significant.

**Figure 2.** Correlation of prevalence between delayed graft function and acute rejection

When the long-term outcomes are evaluated, such as CGD or graft survival, it is debatable as to whether DGF, alone, would have a negative impact or if the impact would be caused by the effect of the AR or of the two superimposed variables. We evaluated the influence of DGF in the kidney function of patients transplanted with a deceased donor and observed worse function, by means of creatinine clearance measures, for up to two years of follow-up.<sup>(23)</sup> It is still not yet clear if this difference in function, however, can compromise the survival of the graft. Troppman et al.<sup>(31)</sup> observed that DGF, in the absence of AR, had no influence on

the clinical outcomes. Ojo et al.,<sup>(21)</sup> however, observed a 14% reduction in graft survival one year after transplant in patients with DGF who did not have AR.

## STRATEGIES TO ATTENUATE THE EFFECTS OF ISCHEMIA AND REPERFUSION INJURY

From the hemodynamic point of view, adequate care with the donor, especially to avoid hemodynamic instability and need for large quantities of vasoactive drugs, avoid large electrolytic variations and reduce the CIT, are effective strategies in reducing the prevalence of IRI. Still as strategies of better hemodynamics, the adequate preservation of the organ has a positive impact. With the objective of attenuating the negative impact of the cellular effects of oxygen privation (Figure 1), preservation solutions and organ cooling are used. The preservation solutions try to mimic the electrolytic and osmotic intracellular environment, with the objective of stabilizing the plasma membrane, avoid edema, and reduce as much as possible the cell damage caused by ischemia and acidosis.<sup>(32)</sup> Additionally, the kidneys to be implanted are presented at temperatures as low as 4°C, which reduces the metabolism rates by 90 to 95% and with this, decreases the consumption of ATP and the deviation of the metabolism to anaerobic routs.<sup>(1,24,32)</sup>

Two strategies of preservation solution perfusion may be used: static, in which the kidney is preserved with hypothermic solution and stored in a thermal protection recipient, or the continuous mechanical perfusion which, besides using the hypothermic preservation solution, maintains the pulsatile flow of this solution circulating in the graft, mimicking blood flow circulation.<sup>(33,34)</sup> Mechanical perfusion seem to be associated with the reduction of negative impacts on IRI. In a study conducted by Moers et al., 672 kidneys of 336 deceased donors were paired 1:1 for two different types of perfusion.<sup>(33)</sup> There was a reduction in the prevalence of DGF (30.1% to 22.9%; p=0.03), primary absence of the graft function (4.8% to 2.1%; p=0.08), and of the time in DGF (13 to 10 days; p=0.04), that is, the results were favorable to mechanical perfusion. In a multivariate analysis, the use of this strategy reduced by 43% the risk of DGF. At the end of one-year follow-up (94% versus 90%; p=0.04) and of 3 years (91% versus 87%; p=0.04), graft survival was significantly better in the patients who received kidneys from mechanical perfusion.<sup>(33,34)</sup>

In Brazil, the prevention strategy used on a large scale is static perfusion. Recently, our group began to use a mixed strategy: we received the kidneys in static perfusion, which is the form in which they are distributed by the transplant center, and we maintained the kidney

in pulsatile mechanical preservation until the time of surgery. The rationale for placing the kidney in the machine even after the static perfusion is that, within the hemodynamic paradigm, we observe regional alterations in blood flow, which can be confirmed by the increased intrarenal vascular resistance, with resulting reduction of plasma flow.<sup>(35)</sup> Analysis of the first transplants performed with this mixed strategy (placing the kidney in the machine after static perfusion) demonstrated a significant reduction in intrarenal resistance during the first 6 hours of mechanical perfusion (Figure 3A), and consequently presenting a significant increase in the intrarenal flow (Figure 3B). In both figures, T0 was the moment in which the kidney initiated machine perfusion, that is, the end of the CIT in static perfusion and Tf was the final time of machine perfusion. The time between T0 and Tf among these patients was 9.3 hours. We noted no reduction in prevalence of DGF, but in the first cases evaluated there was significant reduction in dialysis duration after the transplant, with consequent reduction in length of hospital stay (Figure 4). We noted a reduction in hospital stay from  $21.4 \pm 12.6$  days to  $12.8 \pm 6.0$  days ( $p=0.03$ ), as well as a trend to reduced dialysis time from  $8.0 \pm 7.8$  days to  $4.4 \pm 6.1$  days ( $p=0.20$ ).

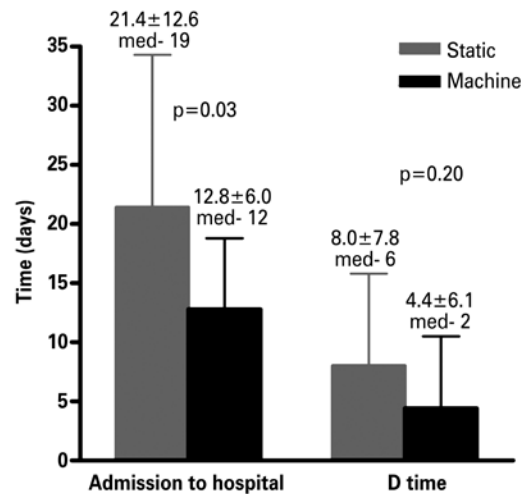
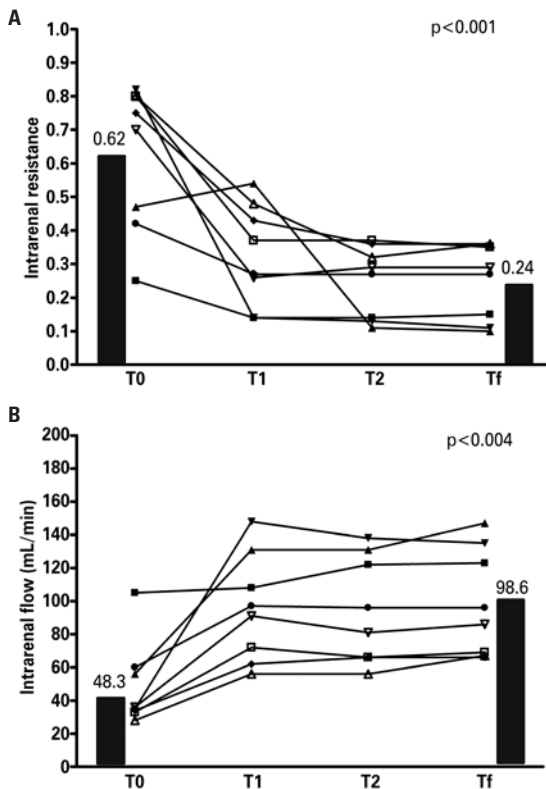


Figure 4. Length of hospital stay and dialysis time (D time) after transplant with the perfusion machine

Within the context of the immune paradigm, a series of studies, both experimental and clinical, demonstrated the benefit of using drugs that deplete leukocytes or antibodies directed against adhesion molecules, attenuating the effects of the IRI. Based on these concepts, Yokota et al., in an experimental study, demonstrated that the use of antibodies that deplete T CD4+ cells in mice attenuated IRI, and that this effect was potentiated by performing thymectomy prior to the lesion.<sup>(36)</sup> Since knowledge evolved on the primary mechanisms of action of the ALA antilymphocyte antibodies, especially in lymphocytic depletion and as T cell modulators, in parallel with the growing knowledge of the role of T cells in IRI, there has been interest in evaluating the benefit of these antibodies. The polyclonal antithymocyte antibody (ATG) is the most studied polyclonal antibody and has the characteristics of rapid action, antigen specificity, with consequent depletion and modulation of the immune response. The benefits of use of ATG in IRI would be supported by the reduction of the mass of circulating lymphocytes and by the blockage of the machinery necessary for the migration of the lymphocyte to the lesion site.<sup>(37)</sup>

Beiras-Fernandez et al., using a model of IRI in non-human primates, demonstrated that the use of ATG significantly reduced the infiltration of leukocytes in connective tissue, vessels, perivascular tissue, and muscle itself.<sup>(38)</sup> These findings were translated into lower lesion scores, with lower rates of necrosis, hemorrhage areas, and areas of diffuse infiltration, both in muscle tissue and in connective tissue of animals that used the drug. All these concepts gave support to the use of ATG with the objective of reducing the effects of IRI. Goggins et al. confirmed this hypothesis demonstrating that the



T0: moment when the kidney initiated perfusion on the machine; Tf: final time of machine perfusion. Figure 3. Intrarenal hemodynamics after the use of perfusion machine

use of the dose previous to unclamping the vascular anastomosis significantly reduced the occurrence of DGF (14.8% versus 35.5%), even though these results were not reproduced in other studies.<sup>(39)</sup> We compared two cohorts of paired patients, in which one received induction with Thymoglobulin® and the other, not, and we noted no reduction in the prevalence of DGF.<sup>(40)</sup>

## CONCLUSION

The ischemia and reperfusion injury in kidney transplantation is currently understood in light of two different paradigms: hemodynamic and immune. In addition to the classic privation of circulation, with reduction of aerobic activity and of the lesion caused by the oxygen-free radicals in reperfusion and in involvement of hormone systems, the involvement of the immune system in the genesis of ischemia and reperfusion injury has been discussed, especially the central role of T cells and its surface molecules. The adequate management of donors, reduction in warm and cold ischemia time, and the more physiological strategies of storage of the kidneys are related to smaller impact of the ischemia and reperfusion injury in the evolution of kidney transplant. Likewise, the immune system block by means of activated T cell deprivation, as well as the neutralization of the surface molecules of these cells, with the use of polyclonal antibodies that deplete lymphocytes, may have a protective role on the grafts. This is still a field for further investigation on exploration to improve the short- and long-term outcomes, especially in kidney transplant of a deceased donor.

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