UNIVERSITA' DEGLI STUDI DI PADOVA

Dipartimento di Scienze Cardiologiche, Toraciche e Vascolari

Scuola di Dottorato di Ricerca in Scienze Mediche, Cliniche e Sperimentali

INDIRIZZO: Scienze Cardiovascolari CICLO XX

TITOLO TESI:

TEMPORARY RENAL REPERFUSION TO INCREASE SAFE ISCHEMIC TIME

Coordinatore d'indirizzo: Ch.mo Prof. Gaetano Thiene

Supervisore: Ch.mo Prof. Giovanni Deriu

Ch.mo Prof. Annalisa Angelini

Dottorando: Dott. Stefano Bonvini

ABSTRACT

Introduction

The reported rate of acute renal failure from large series of patients undergoing Thoraco Abdominal Aortic Aneurysms (TAAA) repair ranges from 5% to 40% and is associated with mortality rates of 70%. Patients who develop acute renal failure also more frequently present a worse outcome and sustain non renal complications, such as respiratory failure, central nervous system dysfunction, sepsis, and gastrointestinal haemorrhage.

The factors contributing to renal dysfunction after TAAA repair include ischemia reperfusion injury, nonpulsatile flow in perfusion systems, transfusion of blood products, pre-operatory renal impairment etc.

Ischemia (cessation of blood flow), followed by reperfusion (re-establishment of blood flow), causes characteristic injury to organs and tissues. Ischemia compromises the continuous supply of oxygen required by tissues and organs to survive and maintain normal physiological function. A rapid return of oxygenated blood (reperfusion) is therefore essential for preventing ischemic and apoptotic cell death. However, reperfusion itself also contributes to cellular injury and death by the production of free oxygen radicals and the activation of a cytokine mediated inflammatory response that, on one side, through a pro-inflammatory component expression (IL1α, IL1β, IL,2, IL6, GM-CSF, TNFα, INFγ) sustain cellular damage, and on the other side, through an anti inflammatory component expression (IL4, IL10) tends to limit it being therefore the prelude of the reestablishment of normal conditions.

The short term arterial blood reperfusion of renal arteries is one of the surgical techniques applied at the Vascular and Endovascular division of the University of Padova to prevent renal impairment. Renal blood reperfusion is obtained, once the proximal anastomosis between the proximal aorta and the Dacron graft is performed, by re-establishing pulsatile normothermic blood flow through a Pruitt-Inahara shunt. Application of the shunt may change in different surgical procedure. When the procedure requires more than 30 min to re-establish a normal flow in the renal artery, the proximal end of the

shunt is distally inserted in the vascular graft. The proximal aortic clamp is released; the shunt is blood perfused and its distal end is inserted into the open end of the renal artery. After 3 min of blood reperfusion, the aorta and the renal artery are reclamped, the shunt is promptly removed and the renal artery reconstruction completed. The reperfusion is repeated every 30 min if necessary.

Aim of my PhD thesis was to evaluate on an animal model the possibility to increase the total time of clamping ischaemia by re-establishing blood flow into renal artery for 3 or 6 minutes leading to a total ischemic period of 90 minutes, evaluating functional, morphological damage and molecular processes involved in the pathophysiology of ischemia reperfusion injury.

Materials and methods

27 Male Sprague-Dawley rats weighing 200 to 250 g, were used for the experiment. Through a midline laparotomy both renal arteries were dissected and clamped to obtain a bilateral ischemia followed by reperfusion in selected group of animals.The rats were allocated into one of the 4 experimental groups; control group, 18 rats with ischemia alone (with progressive clamping time of both renal arteries to assess organ damage at 30-45-60-90 min); group A, 4 rats (30 min of renal ischaemia followed by 3 min of reperfusion followed by other 30 min of ischemia for a total ischaemic time of 60 min), group B,4 rats (45 min of renal ischaemia followed by 3 min of reperfusion plus 45 minutes of ischemia for a total ischemic time of 90 min) and group C, 3 rats (30 min of renal ischaemia followed by 3 min of reperfusion repeated 3 times for a total ischemic time of 90min). A blood sample from inferior vena cava was drawn to evaluate serum creatinine level before the renal clamping. After 48 hours from the procedure the rats were sacrificed for kidney harvesting. Before kidney removal a blood sample from inferior vena cava was drawn to evaluate serum creatinine level.

Renal injury was assessed through evaluation of: a) renal functional parameters (serum level of creatinine at 0 and 48 hours after ischemia), b) morphological parameters on histological samples (tubular necrosis, tubular dilatation, mitosis, apoptosis, haemorrhage infiltration interstitial leukocyte infiltration, and the proliferative index (Ki67), c) pro-inflammatory (IL1α, IL1β, IL,2, IL6, GM-CSF, TNFα, INFγ) and anti-inflammatory (IL4, IL10) cytokine expression on tissue samples.

Results

Animals that underwent renal ischemia with no reperfusion exhibited increase in the serum concentrations of creatinine when the clamping ischemia was more than 45 min, and this difference becomes statistically significant when the clamping time is more than 60 minutes suggesting glomerular dysfunction. In revascularized groups creatinine serum level demonstrates no significant changes compared to the base pre-ischemic value regardless the pattern of revascularization performed suggesting the lack of glomerular dysfunction also in these groups of animals.

Morphological damages were absent in the revascularized group if the total ischemic time is less than 60 minutes. When the total ischemic time was prolonged up to 90 minutes even if no functional alteration was present histopathological analysis demonstrated a moderate renal injury in terms of necrosis and apoptosis. However a higher proliferative index is present at 90 minutes in all revascularized group with the highest increase in double reperfusion. Pro inflammatory (IL-1β, IL-2, IL-6, GM-CSF, IFM-γ ,TNF-α:) and anti-inflammatory(IL-10, IL-4) cytokines are expressed in all the different groups of animals after ischemia and after ischemia and reperfusion. Both pro and anti inflammatory cytokines are over-expressed in animals that underwent a total ischemic time of 60 minutes with a single reperfusion (30+3+30), compared to the group without reperfusion and with those with ischemia of 90 min (30+3+30+3+30; 45+3+45). The cytokine evaluation showed a prevalence of anti-inflammatory cytokine expression in the revascularized group with a statistically significant difference for IL10 over-expression.

In the reperfusion at 90 minutes we observed a decrease in both pro-inflammatory and anti inflammatory cytokines. However with double reperfusion the decrease was more pronounced for the proinflammatory cytokines (TNFα) allowing the biological effect of IL10 to be more powerful (higher proliferative index).

Discussion

The results of my PhD thesis showed that the functional, morphological and molecular results are congruent. At 60 min of ischemia there is an irreversible damage to the kidney with an increased creatinine level, necrosis and apoptosis, low proliferative response and increase in cytokines expression especially of pro-inflammatory cytokines (IL1β, IL2, IL6, TNFα and INFγ). With ischemia-reperfusion (30-3-30) we obtain a less ischemic damage, a more pronounced proliferative response and anti-inflammatory cytokines over-expression (IL-10, IL-4) compared to ischemia alone. The balance was in favour of the protective role of reperfusion. With the ischemic-reperfusion model of 90 min

of ischemia and 6 minutes reperfusion (30-3-30-3-30) we still observed the beneficial role of reperfusion since there is a reduction of necrosis, apoptosis and increasing in proliferation activity and at the end a positive ratio in favour of anti-inflammatory cytokines (IL-10). In the setting of 90 min ischemia and 3 min reperfusion, (45-3-45) we observed a similar ischemic damage in term of necrosis and less proliferative index and the ratio between pro and anti inflammatory was still in favour of anti inflammatory cytokines. However the decrease of pro inflammatory cytokine TNFα was less evident, thus counterbalancing more actively the protective role of anti inflammatory cytokine IL10.

We can state that according to our results the best model would be that of 30-3-30-3-30 which means less ischemic damage and more reperfusion benefits.

SOMMARIO

Introduzione

L'incidenza di insufficienza renale (i.r.) post-operatoria in pazienti sottoposti a chirurgia dell'aorta toraco-addominale è riportata essere dal 5 al 40 % a seconda delle casistiche associata ad un tasso di mortalità intorno al 70%. I pazienti che sviluppano una insufficienza renale acuta post-operatoria sviluppano più frequentemente complicanze non renali come ad esempio insufficienza respiratoria, disfunzioni del sistema nervoso centrale, sepsi ed emorragie gastrointestinali.

I fattori che contribuiscono al danno renale dopo chirurgia aortica includono il danno da ischemia e riperfusione, il flusso non pulsato di alcuni sistemi di riperfusione, condizione renale preoperatoria, perdite ematiche etc.

L'ischemia (interruzione del flusso ematico a livello del parenchima renale), seguita dalla riperfusione (ripristino del flusso), provocano dei danni caratteristici ad organi e tessuti. L'ischemia compromette il continuo rifornimento di ossigeno ai tessuti necessario per il mantenimento delle normali funzioni fisiologiche. La riperfusione però, necessaria per il mantenimento della vitalità cellulare, è essa stessa causa di danno cellulare attraverso la produzione di radicali liberi dell'ossigeno e attivazione di una risposta infiammatoria mediata dalle citochine che da un lato, attraverso l'espressione della componente pro-infiammatoria (IL1α, IL1β, IL,2, IL6, GM-CSF, TNFα, INFγ) sostiene il danno e dall'altro, attraverso l'espressione della sua componente anti-infiammatoria (IL4, IL10) tende ad arginarlo costituendo il preludio al ritorno alla condizione di normalità.

Attualmente la metodica chirurgica utilizzata presso la Clinica di Chirurgia Vascolare ed Endovascolare dell'Università degli studi di Padova nella prevenzione dell'insufficienza renale è rappresentata dall'utilizzo della riperfusione breve delle arterie renali che consiste nel riperfondere le arterie renali per 3 minuti, una volta completata l'anastomosi prossimale della ricostruzione aortica, attraverso degli shunt temporanei inseriti nella protesi. Tale riperfusione permette di poter clampare per ulteriori 30 minuti di ischemia le arterie renali permettendo di poter completare l'intervento. Qualora necessario è possibile inoltre riperfondere per ulteriori 3 minuti le arterie renali per ottenere ulteriori 30 minuti di ischemia.

Scopo di questo studio è dimostrare nel modello animale che è possibile incrementare il tempo di ischemia totale renale ristabilendo per 3 minuti un flusso pulsato all'interno dell'arteria renale, valutando i danni funzionali, morfologici ed i processi molecolari legati ad essi. La riperfusione breve delle arterie renali può essere ripetuta più volte raggiungendo un tempo di ischemia totale di 90 minuti.

Materiali e metodi

Lo studio è stato condotto su 27 ratti albini del ceppo Sprague-Dawley di sesso maschile e del peso di 250 gr circa .Dopo aver praticato una laparotomia mediana si è proceduto all'isolamento e al clampaggio dell'arteria renale bilateralmente ottenendo l'ischemia completa dei reni seguita da riperfusione secondo il protocollo. Gli animali sono stati suddivisi in 4 gruppi sperimentali; un gruppo di controllo, 18 ratti, (sottoposti ad un clampaggio di tempo crescente delle arterie renali per valutare la gravità del danno ischemico a tempi differenti pari a 30-35-40-45-50-55-60-75-90 minuti. Un gruppo A, 4 ratti (sottoposti a 30 minuti di ischemia seguiti da 3 minuti di riperfusione e successivi 30 minuti di ischemia per un totale di 60 minuti), un gruppo B, 4 ratti, (sottoposti a due periodi di ischemia di 45 minuti intervallati da 3 min di riperfusione per un totale di 90 minuti di ischemia); un gruppo C, 3 ratti, (sottoposti a tre periodi di ischemia di 30 minuti ognuno interrotti da 3 min di riperfusione per un totale di 90 minuti di ischemia). Prima dell'ischemia è stato eseguito un prelievo venoso dalla cava per il dosaggio della creatinina sierica. In seconda giornata post-operatoria i ratti sono stati sottoposti a riapertura della ferita chirurgica, con espianto di entrambi i reni e sacrificio dell'animale previo prelievo venoso dalla cava. Il materiale prelevato è stato inviato presso i laboratori di Anatomia Patologica per una valutazione mediante microscopia ottica.

La valutazione del danno renale è stata eseguita controllando i parametri di funzionalità renale (creatininemia a 0 e 48 ore dall'evento ischemico), i parametri morfologici di danno sugli esami istologici (la necrosi, la dilatazione tubuale, le mitosi, apoptosi, dimorfismi, stravasi ematici, infiltrato infiammatorio) e l'indice proliferativo (Ki67).

Inoltre è stata valutata l'espressione delle interleuchine pro-infiammatorie (IL1α, IL1β, IL,2, IL6, GM-CSF, TNFα, INFγ) ed anti-infiammatorie (IL4, IL10)

Risultati

La valutazione della creatinine mia a 0 e 48 ore ha evidenziato come la riperfusione temporanea sia in grado di proteggere funzionalmente il parenchima renale. A 90 minuti i

ratti riperfusi mostrano valori di creatininemia sovrapponibili ai valori di base preischemici mentre i ratti non riperfusi a 90 minuti hanno un aumento significativo della creatininemia.

Da un punto di vista morfologico la valutazione a 60 minuti documenta l'assenza di lesioni. Quando il tempo di ischemia è prolungato a 90 minuti, anche se da un punto di vista funzionale non vi è evidenza di alterazioni, l'analisi istopatologica ha dimostrato un danno seppur lieve sia in termini di necrosi che di apoptosi. Tuttavia a 90 minuti si osserva un alto indice proliferativi in tutti gli animali sottoposti a riperfusione con il più alto valore che si riscontra nei ratti sottoposti a doppia riperfusione. Le citofhine pro-infiammatorie (IL-1β, IL-2, IL-6, GM-CSF, IFM-γ ,TNF-α:) ed antiinfiammatorie (IL-10, IL-4) sono espresse dai vari gruppi di animali dopo ischemia e dopo ischemia e riperfusione. Sia le citochine pro che antiinfiammatorie sono sovra espresse negli animali sottoposti ad una ischemia totale di 60 minuti con singola riperfusione confrontati con il gruppo senza riperfusione e con quelli con ischemia di 90 minuti. La valutazione delle citochine dimostra una prevalenza di citochine antinfiammatorie nel gruppo rivascolarizzato con una differenza statisticamente significativa per IL10.

Nelle riperfusioni a 90 minuti si è osservato un decremento sia per quel che riguarda le citochine pro che antinfiammatoria . Tuttavia con la doppia riperfusione il decremento era più marcato per le citochine proinfiammatorie (TNFα) permettendo l'esplicarsi dell'attività biologica della IL10.

Discussione

I risultati della mia tesi di dottorato dimostrano come i risultati funzionali, morfologici e molecolari siano congrui. A 60 minuti di ischemia vi è la comparsa di un donno parenchimale irreversibile con incremento del dosaggio della creatininemia sierica, dei processi di apoptosi e necrosi cellulare, basso indice proliferatici ed un incremento del livello di espressione delle citochine. A tale riguardo tale incremento riguarda in particolar modo l'espressione delle citochine pro-infiammatorie (IL1α, IL1β, IL,2, IL6, GM-CSF, TNFα, INFγ). Nel caso di ischemia e riperfusione con pattern 30-3-30 è stato osservato un minor danno ischemico accompagnato ad una risposta proliferativa pronunciata ed a una sovraespressione di citochine anti-infiammatorie (IL10, IL4). Il bilancio finale è in favore del ruolo protettivo della riperfusione. Con il modello di riperfusione di 90 minuti di ischemia e 6 di riperfusione (30-3-30-3-30) si osservano ancora i risultati positivi della riperfusione con una riduzione della necrosi, ed un incremento dell'attività proliferativi con un rapporto favorevole verso la produzione di citochine antinfiammatorie (IL10). Nel

pattern dei 90 minuti di ischemia e 3 di riperfusione (45-3-45), abbiamo osservato un danno ischemico simile in termini di necrosi ed apoptosi ed un indice proliferativo più basso ed un rapporto tra citochine pro ed anti infiammatorie a favore di quelle antinfiammatorie. Tuttavia il decremento delle citochine pro-infiammatorie TNF è risultato essere meno evidente, contrastando così in maniera più attiva il ruolo antinfiammatorio di IL10.

Si può concludere,in accordo con i risultati presentati, che il miglior modello di riperfusione risulta essere quello di ischemia / riperfusione 30-3-30-3-30 accompagnato da meno danno ischemico associato ai benefici della riperfusione.

INTRODUCTION

1 - Historical aspects of cardiovascular and vascular disease of the aorta

On the whole, disease of the aorta generally has not been of much interest except to a few inquiring persons. The reasons for this are many and include the utilitarian nature of the aorta, which may simply be regarded as a large pipe connecting various organs. The aorta does not capture the imagination as does the heart or brain, and it is not a distinctive organ like the lung, liver or kidneys with fascinating discrete and complex function. Yet malfunction of the aorta is the 13th most common cause of death. In more recent times, there has been a modest increase in public awareness of aortic disease as a result of some famous person having succumbed to the condition. Albert Einstein died of the abdominal aortic aneurysm. He had previously had an abdominal aortic wrapped with cellophane. De Bakey is said to have offered to repair the aneurysm for him, but Einstein refused another operation. Eventually , the aneurysm ruptured and was diagnosed initially as being due to cholelithiasis, hence the "Einstein sign". Finally and worthy to mention is the sudden demise from aortic disease of a royal figure whose condition, one could argue , influenced the course of world events. On October 26, 1760, king George II suddenly died while moving his bowels. Nicholas, the King's physician, noted at autopsy that there was "intravasation of blood between the coats" of the aorta caused by aortic dissection. George II was succeeded by his unstable and stubborn grandson, George III, who imposed on the American colonies the taxes that led to the American War of Independence, and the rest is History.

The early history of vascular surgery is illuminated by the creative genius of physician such as Galen who based his anatomical knowledge on dissection of apes and other animals and stated, "When the arteries enlarged, the disease is called aneurysm. If the aneurysm is injured the blood gushes forth, and it is difficult to stanch it". Antylus ligated arteries proximally and distally and evacuated the blood or clot. Ambrus Paré (1510-1590) repaired arterial war injuries by ligation of arteries. Anderas Vesalius (1514-1564) published De Humani Corporis Fabrica in 1543 and a description of an aneurysm in 1555.

Although the aorta was known to anatomists, it was only when William Harvey wrote his momentous publication Exercitatio Anatomica de Motu Cordis et Sanguinis in ANimalibus in 1628 during the period known as the Enlightenment that the function of the aorta began to be understood. Harvey wrote in chapter 8 of his treatise:

When I meditated even further on the amount (i.e. of transmitted blood), and the very short time it took for its transfer,… having the veins, on the other hand, completely emptied and the arteries, on the other hand, brought to bursting through excessive inthrust of blood, unless the blood somehow flowed back again from the arteries into the veins and return to the right ventricle of the heart; I then began to wonder whether it had movement, as it were, in a circle. This I afterwards found to be true.

William Harvey proved that this connection had to exist when he conducted a simple yet ingenious experiment in which he used ligatures on the forearm and observed the direction of blood flow in the superficial veins. He also observed the direction of blood flow in the superficial veins. He also observed that the aorta had a thicker wall because " the aorta sustain a stronger inthrust of blood from the left ventricle".

In 1732, three years later after he graduated from Oxford University Nichols published his compendium of lectures on cardiovascular physiology and disease. He had recognized aortic dissection in 1728, and he wrote extensively on the physiology of arteries, the innervation of arteries, and the autonomic nervous system, blood pressure control and hypertension. His contribution to cardiovascular medicine have largely been overlooked and forgotten in the annals of history. His diagnosis of the cause of death of King George II has already been cited. Also in 1728 came Lancisi's publication of Motus Cordis et Aneurismatibus on the pathology and case reports of abdominal aneurysms.

The first attempts at aortic operations were by John Hunter (1728-1793) and Cooper (1768-1841). Nonetheless it is only during the 20th century that the successful operative repair of the aorta has been realized. The contributions of such men as Carrel to basic operative techniques and experimental use of by-pass grafts, Gibbon to the heart-lung machine, and Voorhees and De Bakey to graft prostheses will surely be landmarks in the historical annals of aortic surgery. Other milestones are the development of operative technique by Crafoord (coarctation), Dubost (infrarenal aortic repair), Debakey (ascending aorta and aortic arch repair, distal aortic arch, aortic dissection and aortic surgery generally), Colley and Debakey (ascending aorta), Morris (acute dissection, importance of profunda femoris artery in aortoiliac disease and aorto-femoral by-pass and renovascular

surgery), and Crawford (thoracoabdominal aneurysm, Marfan Syndrome, and aortic dissection surgery).

2 - Aortic aneurysm

The natural history of untreated AAA, descending thoracic and thoracoabdominal aneurysms is poor; if the aneurysm is left untreated, fatal rupture is a constant, unpredictable threat. the reported 5-year survival estimates range from 18% to 46% [1] [2].

Although emergency repair can save the life of a patient with a ruptured aneurysm, the associated morbidity and mortality remain extremely high. Elective surgical repair is the only effective treatment in eradicating the risk of aneurysm rupture improving survival. Aortic repair is a major undertaking, however, because all major organs, including the heart, lung, liver, spinal cord , intestines and kidneys are at risk during surgery. Aortic graft replacement using the inclusion technique with reimplantation of intercostal and visceral arteries forms the basis of surgical repair. During the "clamp and sew" era, the expediency of surgery was key in determining patient outcome. Next in importance for predicting patient outcome was the extent and location of aneurysm, and a classification system was devised (Fig.1).

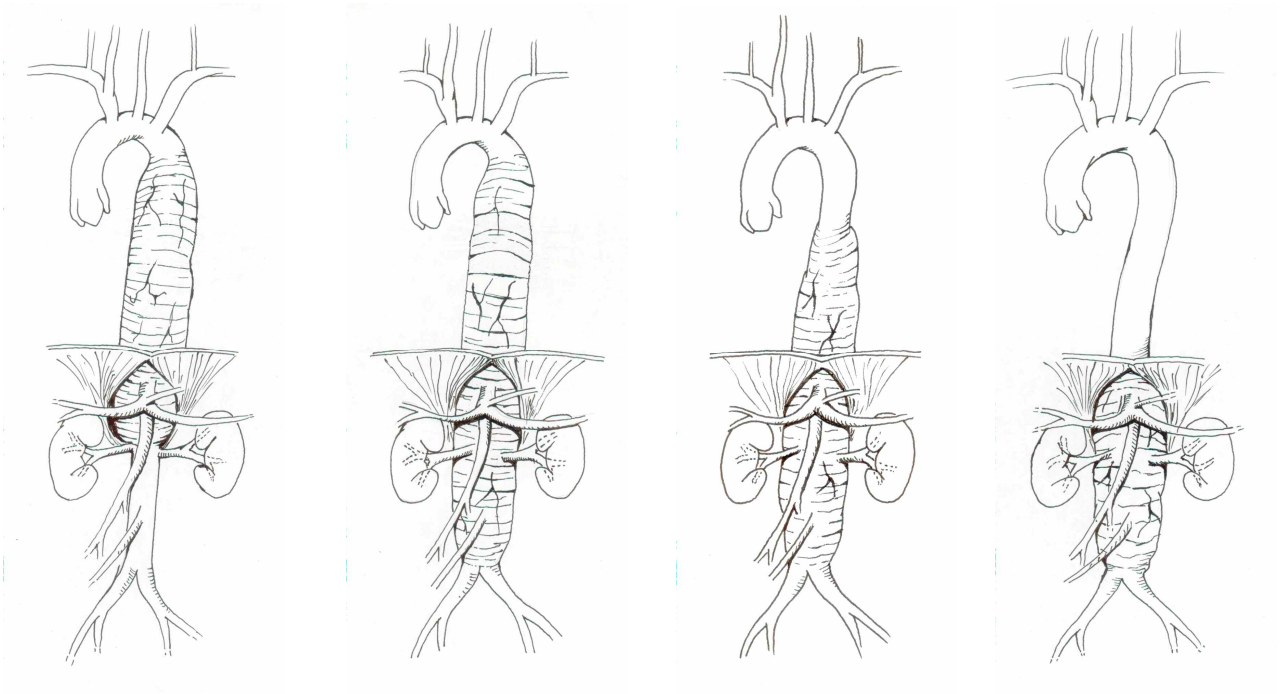


Fig. 1 - Classification of Thoraco-abdominal aneurysm

The operative mortality for thoracoabdominal aneurysms has improved from the pioneering experience of Svensson and colleagues but remains approximately 8%[2]. The incidence of severe complications is also significant with permanent spinal cord injury reported from 4.5%[3] to 15%[2] and *acute renal failure up to 20%* [4] at high-volume centers.

3 - Surgical technique

Patient is positioned in the right lateral decubitus position with the hips slightly turned to allow access to both groins. A tailor incision is performed to complement the extent of the aneurysm. The full thoracolaparotomy incision begins posteriorly between the scapula and the spinous process, curving along the sixth intercostal space to the costal cartilage, then obliquely to the umbilicus and finally in the midline above the symphysis pubis (Fig.2).

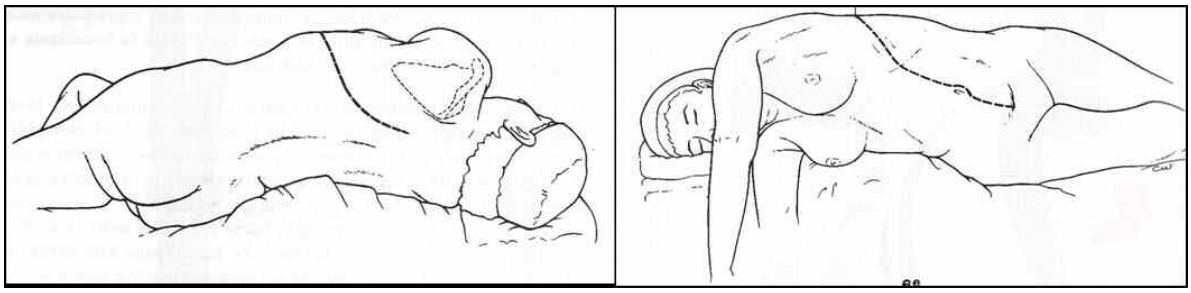


Fig. 2 - Right lateral decubitus position.

The latissimus dorsi muscle is divided and the insertion of the serratus anterior muscle is mobilized. The left lung when necessary, depending from aneurysm estension is deflated and the left thoracic cavity is entered. Resection of the sixth rib facilitates exposure and is performed routinely for all thoracoabdominal aneurysms expect extent IV.

The dissection begins at the level of the hilum of the lung cephalad to the proximal descending thoracic aorta. The ligamentum arteriosum is identified and transacted taking care to avoid injury to the left recurrent laryngeal nerve. The diaphragm is transacted near to his thoracic inserction wall to spare the left phrenic nerve. A retroperitoneal plane is developed, mobilizing the spleen, bowel loops and left kidney to the right side of the

abdominal aorta (medial visceral rotation). The pericardium is opened posterior to the left phrenic nerve to allow direct visualization of the pulmonary veins and left atrium.

Sequential clamping technique

The left atrium is cannulated through the left inferior pulmonary vein or atrial appendage. Bleeding from the site of cannulation can cause postoperative cardiac tamponade if the pericardium is not adequately opened. A bioMedicus pump (Fig. 3), with an in-line heat exchanger is attached to the left atrial cannula, and the arterial inflow is attached

to the left atrial cannula and the arterial inflow is established through the left common femoral artery or the descending thoracic aorta if the femoral artery or the descending thoracic aorta if the femoral artery is not accessible. Distal aortic perfusion is begun.

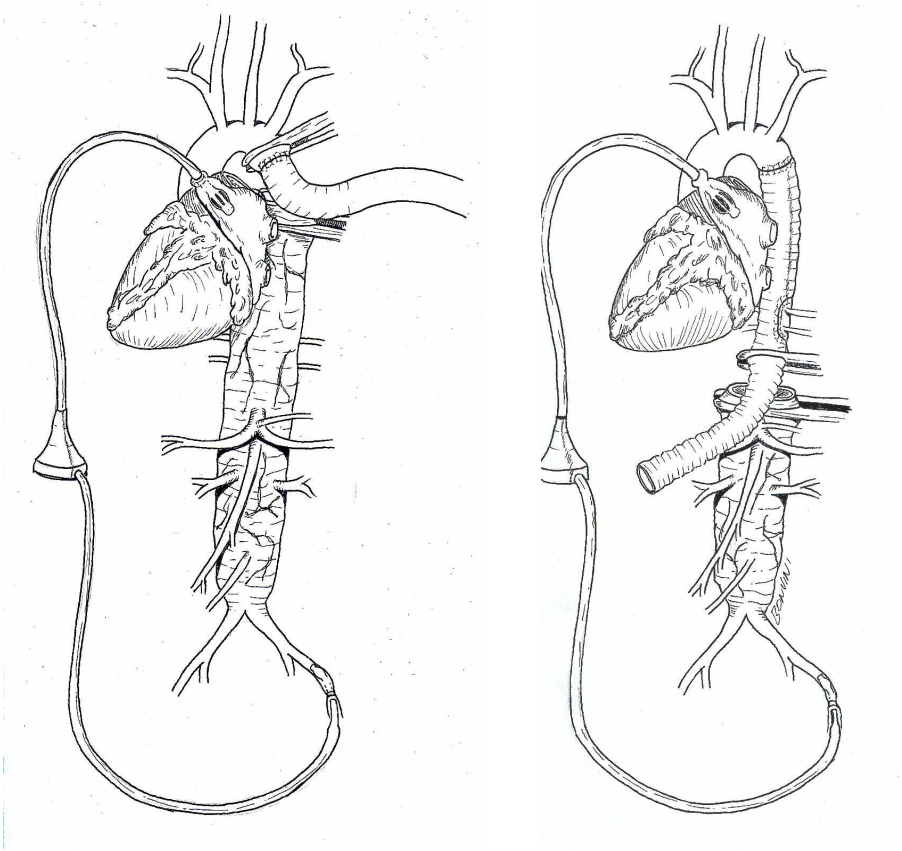


Fig. 3 - Example of Type II TAAA corrected with the clamping sequential technique with the atrio-femoral by- pass (BIOMEDICUS PUMP)

Clamps are applied to the proximal descending thoracic aorta just distal to the left subclavian artery and to the mid-thoracic aorta. When the proximal extent of the aneurysm is too close to the left subclavian artery the clamp is placed between the left common carotid artery and the left subclavian artery. A Dacron graft of correct size depending on aortic diameter is employed. The graft is then sutured in an end to end fashion to the descending thoracic aorta.

After the completion of the proximal anastomosis, the mid thoracic aortic clamp is moved distally onto the aorta just above the celiac axis to accommodate intercostal reimplantation. Reattachment of lower patent intercostal arteries is performed. After completion of intercostal reattachment the proximal clamp is released from the aorta and reapplied on the aortic graft beyond the intercostal patch, restoring pulsatile flow to the reattached intercostal arteries. The distal clamp is moved onto the infrarenal aorta and the abdominal aorta is opened.

The visceral vessel usually are reattached using the inclusion technique. In most cases an island patch accommodates reattachment of the celiac, superior mesenteric artery and right renal artery and reimplantation of the left renal artery is performed. In Marfan syndrome to avoid aneurysmatic evolution of the visceral patch a Dacron graft with side arm graft is used for separate attachment of celiac superior mesenteric right and left renal arteries.

Three potential sources of injury to cells during vascular surgery are ischemia, reperfusion, and microcirculation flow dysfunction with no reflow phenomen.

Ischemia, the lack of adequate blood flow, is the pathological process whereby the blood supply fails to keep up with tissue oxygen and metabolic substrate demands. Ischemia may be acute, as in stroke and cardiac infarction, or chronic, as in claudication. The injury substained depends on both the depth and duration of the hypoxia. The principal issues that underly the pathogenesis of cell injury in ischemia are hypoxia from lack of oxigen and the loss of energy stores.

Reperfusion, is the reestablishment of blood flow and, hence, of oxigenation to ischemic tissue. The obvious advantages of reperfusion are the restoration of the energy supply and the return to cellular homeostasis. There is however, an apparently contradictory effect in that reperfusion adds to the damage already inflicted by the

ischemia[5]. The term *ischemia-reperfusion injury* is used to describe this phenomen. This deleterious effect is attributed predominantly to oxigen-derived free radicals that arise transiently during the process of reperfusion. The highly toxic oxygen free radicals possibly are produced because of incomplete oxygenation during the initial stage of blood flow restoration. Lipid peroxidation is the chief mechanism by which oxygen free radicals injure cells.

The **no-reflow phenomenon** occurs when there is a failure in the reperfusion attempt because of blockage at the microcirculatory level, and the degree of this blockage seems proportional to the length of ischemia. What causes this effect is not clear, although evidence suggests that changes in the microcirculation may be of major consequence in postischemic damage. Nitric oxide also appears to play an important role in the regulation of the microcirculation and tissue injury.

Such damages are a frequent, complex consequence of surgery. Of particular interest to physicians dealing with aortic diseases is the deterious effect of such processes on organs at risk for hypoxia following aortic clamping include spinal cord, kidneys, intestines, and liver. It should be recalled that for any type of aortic surgery, transient ischemia occurs as a result of aortic cross-clamping or circulatory arrest. If the cross-clamping or the circulatory arrest time is prolonged, the resulting ischemia leads to considerable morbidity and often to death.

Aortic cross-clamping has profound effects on normal hemodynamics and the risks of end-organ ischemia to sensitive tissue. Several hemodinamic changes occur with cross-clamping of arch and descending thoracic aorta. The blood pressure proximal to the aortic clamp immediately rises and the blood pressure distal to it concomitaly falls precipitously to approximately 10-20 mmHg. With the sudden increase in cardiac afterload, left ventricular strain increases and cardiac preload increases.

The increased preload is reflected by an increase in central venous pressure and right heart pressure; besides the direct consequence of aortic cross-clamping, organs beyond the clamp are deprived of their blood supply and, therefore, of oxygen and metabolic substrates.

Suprarenal or more proximal aortic cross-clamping results in reduced distal organ perfusion, including renal ischemia with potential risk of acute renal failure developing.

4 - Renal failure in aortic surgery

The reported rate of acute renal failure from large series of patients undergoing TAAA repair ranges from 5% to 40%[6] and is associated with mortality rates of 70%[7 8 9]. Patients who develop acute renal failure also more frequently sustain non renal complications, such as respiratory failure, central nervous system dysfunction, sepsis, and gastrointestinal hemorrhage.

The factors that contribute to renal dysfunction after TAAA repair include ischemia reperfusion injury, nonpulsatile flow in perfusion systems, transfusion of blood products, atheroembolism, and dissection of the renal artery. The multifactorial nature of renal injury mandates a multimodality approach to renal protection.

Intraoperative changes in renal blood flow and glomerular filtration rate are common. Postoperative renal dysfunction is mainly attributed to adverse events that occur during the perioperative period, including sepsis and hypotension, or contrast administration. Renal dysfunction following major surgery (for example, abdominal aortic aneurysm repair or coronary artery bypass surgery) is one of the recognized causes of significant postoperative morbidity and mortality. Acute renal failure in the postoperative period, when it occurs, is mostly due to renal parenchymal damage and requires aggressive supportive management, including renal dialysis, fluid and electrolyte management. The reported risk of perioperative renal failure varies because of variations in patient population and the definition of renal failure[10]. The induction and maintenance of anaesthesia lowers systemic blood pressure, potentially predisposing the patient to renal ischaemia and eventual postoperative renal failure. However, in the vast majority of patients this alone rarely compromises postoperative renal function. Hence if postoperative renal dysfunction occurs, it is generally thought to be multi-factorial in nature.

In the last few decades, attempts have been made to protect the kidneys both during surgery and in the immediate postoperative period. Various regimens, such as use of low-dose dopamine, dopexamine or diuretics, have been tried. It has been suggested that there is evidence for some success with such interventions[11] ; no clear evidence of

success; or even a deterioration in renal function[12]. Invasive haemodynamic monitoring and aggressive perioperative fluid management have been found to be useful[13].

There is no clear evidence in the literature to suggest that any of the measures are effective in protecting the kidneys during surgery.

Because there is as yet no effective treatment for established ARF, the emphasis must be on preventing its development in the first place. The magnitude of this problem constitutes a challenge in perioperative care that has stimulated intensive study without significant progress to date.

Risk factor for renal failure

Risk factors for postoperative ARF have been intensively studied. A systematic review found that preoperative renal dysfunction was the single consistent predictor of postoperative renal failure[14]. The nature of the procedure also significantly affects the incidence of ARF. The unifying theme in the pathophysiology of ARF is renal ischemia, which is responsible for the bulk of cases of ARF[15]. ARF is commonly the consequence of more than one insult, and prerenal azotemia, which reflects renal hypoperfusion, is the most common predisposing factor. Patients already exposed to a chronic ischemic state such as renal artery stenosis or diabetes, or a recent acute ischemic event such as hypovolemia or radiocontrast agents, are more likely to suffer ARF when subsequently exposed to a surgical insult. Unfortunately, the patients undergoing cardiac or aortic surgery are also the most likely to have a history of diabetes, atherosclerotic disease and recent exposure to radiocontrast dye, as well as having a high incidence of pre-existing renal insufficiency. Many other conditions may predispose the kidneys to ischemic injury, mostly through the common pathway of impairment of renal blood flow. These include sepsis, cirrhosis, jaundice, hepatorenal syndrome, congestive heart failure, hemorrhage, shock, malignant hypertension, pre-eclampsia and toxemia, sickle cell anemia, collagen-vascular diseases and multiple myeloma. Many drugs may also potentiate the risk of renal injury, including angiotensin-converting-enzyme inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs), cyclosporine, tacrolimus, and amphotericin B.

Cellular basis of postoperative renal failure

By definition, renal ischemic injury is a result of inadequate blood flow to the affected cells. This may be caused by extrarenal factors such as systemic hypotension, or it may be caused by intrarenal vascular alterations. The most vulnerable region of the kidney is the outer medulla; because of a combination of high metabolic demands and low blood flow, the partial oxygen pressure of the medulla is between 10 and 20 mmHg, whereas that of the cortex is approximately 50 mmHg. This area thus has the characteristics of any vascular watershed area and is the first to manifest injury after a hypoxic event. Normal intrarenal vascular tone is characterized by a balance of vasodilatory influences, including nitric oxide, urodilatin and prostaglandin E_2, and vasoconstrictive effects, notably those of endothelin, catecholamines, and angiotensin II. Adenosine, released in all ischemic tissues as ATP is depleted and broken down, has a vasoconstricting effect in the renal cortex and a vasodilating effect in the medulla. In the setting of ischemia, decreases in nitric oxide production occur that may have a proinflammatory effect, as well as causing vasoconstriction. Normal vascular balance may be disrupted by such substances as NSAIDs, which decrease prostaglandin E_2 production or radiocontrast dye, which causes intense vasoconstriction through as yet uncertain mechanisms.

Ischemic renal injury affects the tubules at the level of the outer medulla, which include primarily the thick ascending loop of Henle (mTAL) and the S_3 portion of the proximal convoluted tubule. Tubular cell death is now understood to be characterized both by necrosis and by apoptosis, or programmed cell death. Necrosis results from profound cellular ATP depletion and is characterized by a sequence of events that begins with the loss of cell polarity and of the epithelial brush border, loss of the integrity of tight junctions, and the appearance of integrins such as intercellular adhesion molecule 1 on the cell surface. These interact with leukocyte adhesion molecules to mediate an inflammatory response with the release of cytotoxic mediators. Cells then slough into the tubular lumen and further impair already compromised filtrate flow. Tubular cell apoptosis is also triggered by ischemia through as yet uncharacterized mechanisms, also resulting in cell loss, but there is no inflammatory component, and the resultant apoptotic bodies are phagocytosed by macrophages or surviving epithelial cells. Apoptosis is also observed in

the recovery phase during epithelial proliferation, in which it probably plays a role in restoring a normal tubular structure. Clinically, the initial observation is a loss of urinary concentrating ability as the medullary gradient dissipates, followed by a decline in urine output as tubules become obstructed and denuded.

Ischemia-reperfusion (I-R) injury

Ischemia (cessation of blood flow), followed by reperfusion (re-establishment of blood flow), causes characteristic injury to organs and tissues[16 17 18 19]. Ischemia compromises the continuous supply of oxygen required by tissues and organs to survive and maintain normal physiological function. A rapid return of oxygenated blood (reperfusion) is therefore essential for preventing ischemic cell death. However, reperfusion itself also contributes to cellular injury and death—a phenomenon known as "reperfusion injury"—which is associated with the return of oxygen[20 21 22 23]. Thus, while ischemic injury is precipitated by a lack of oxygen (hypoxia), reperfusion injury is associated with the return of oxygen[24 25 26 27]. Together, I-R contributes to major organ and tissue dysfunction associated with many life-threatening conditions and diseases.

Renal ischemia-reperfusion injury

The intracellular and molecular mechanisms involved in the development of renal I-R injury are complex and not yet fully understood, but have been extensively discussed and reviewed [28 29 30 31]. A significant reduction in renal blood flow is the primary factor in the development of renal ischemia, which can be caused by a lowering of systemic blood pressure as in pre-renal ARF, or by large vessel renal vascular disease involving renal artery thrombosis, embolism, or atherosclerosis, leading to either ARF or if chronic, to CRF[32 33]. Ischemia of one or both kidneys is also a common problem experienced during aortic surgery, renal transplantation, or during cardiovascular anesthesia, leading to renal dysfunction and injury[34 35 36]. Surgical procedures involving clamping of the aorta and/or renal arteries, e.g., surgery for supra- and juxtarenal abdominal aortic aneurysms and renal transplantation, are also particularly liable to produce renal ischemia, leading to significant postoperative complications including the development of ischemic ARF[37 38]. In high-risk patients undergoing these forms of surgery, the incidence of renal dysfunction has been reported to be as high as 50%. Thus, renal ischemia remains the major cause of

ischemic ARF[39]. However, renal ischemia associated with renovascular disease involving chronic renal artery stenosis is also implicated in the development of CRF[40 41 42].

On a cellular level, the proximal tubular (PT) cells within the kidney are highly specialized both in terms of morphology and function, allowing for efficient transport of ions, water, and macromolecules across cell layers via highly selective transport mechanisms. These transport processes are governed by intracellular energy; however, during renal ischemia they are severely disrupted due to the decline in adenosine triphosphate (ATP). The resulting dysfunction of the Na^+K^+ATPase pump located on the basolateral surface of PT cells allows intracellular accumulation of Na^+ ions followed by an influx of water leading to cell swelling, intracellular disruption, and eventual cell death[43 44]. A similar dysfunction in endothelial cells leads to fluid loss from intravascular space, increased hemoconcentration with greater adhesion of blood cells, which raises blood viscosity further. Such changes interfere with the restoration of the renal microcirculation during reperfusion, leading to capillary obstruction and a lack of blood flow often referred to as 'no reflow' phenomenon.

Even when reperfusion of the kidney is established, additional renal reperfusion injury occurs. This involves the development of oxidative stress (see below), e.g., via the generation of superoxide anions $(O_2^{\bullet -})$, which has recently been measured as an indicator of I-R injury of the transplanted kidney[45]. Other cellular mechanisms involved in the development of renal reperfusion injury include dysfunctions in calcium homeostasis, phospholipase and protease activation, alterations in cellular pH, and infiltration of inflammatory cells into post-ischemic renal tissues[46 47]. These mechanisms are interrelated, leading to substantial cellular injury, and contributes to early allograft rejection subsequent to renal transplantation, which adversely affects long-term allograft survival[48 49 50]. Renal I-R also contributes to the remote injury of other non-renal organs often via mediators released into the circulation from the damaged kidney or due to biochemical alterations [51 52].

Role of reactive oxygen species (ROS) in renal I-R injury

Generation of reactive oxygen species (ROS) such as $O_2^{\bullet -}$ and the hydroxyl radical ($^{\bullet}OH$) and/or the decline of antioxidant defences lead to oxidative stress, which plays an

important part in the development of renal I-R injury and ischemic ARF[53]. Under normal conditions, ROS play a physiological role in intracellular and cell-to-cell communication[54]. However, during renal I-R, excessive ROS generation occurs as demonstrated in many biochemical and immunohistochemical studies[55]. Furthermore, both in vivo and in vitro investigations performed in the early 1990s demonstrated that ROS scavengers and antioxidants could protect against renal reperfusion-injury[56]. Within the kidney, the susceptibility of the PT to ROS-mediated damage leads to acute tubular necrosis (ATN), which plays a major role in the pathogenesis of ischemic ARF[57]. Traditionally, ROS generated during I-R have been considered to produce cell damage via a direct action on target cells[58]; however, it is now apparent that ROS can also act as signal transduction molecules to regulate gene transcription and activate transcription factors such as nuclear factor-κβ (NF-κβ) and activator protein-1 (AP-1)[59] with subsequent pathological (but also protective) consequences.

Several investigations have demonstrated the beneficial effects of pharmacological interventions, which (1) prevent ROS generation, e.g., using agents such as deferoxamine[60]; inhibit enzymes responsible for ROS generation, e.g., inhibition of xanthine oxidase by allopurinol; administration of antioxidant enzymes, which degrade ROS, such as superoxide dismutase (SOD)[61] or SOD mimetics (SODm) such as EUK-134 and ROS scavenging molecules such as mannitol, uric acid, tempol or tempone. There is also evidence for further ROS-mediated tissue injury many hours after reperfusion commences. Specifically, a recent study by Kulah and colleagues measured accumulation of oxidized low-density lipoprotein (ox-LDL) as a biomarker of oxidative stress in rat kidney tissues during I-R. Ox-LDL levels were not raised significantly 24 h after reperfusion; however, levels peaked at 48 h had declined after 72 h reperfusion, demonstrating longer term complications of reperfusion in addition to the immediate damage, which occurs within minutes or hours of reperfusion[62]. Intriguingly, there is also recent evidence that exposure of animals to hyperbaric oxygen can afford protection against renal I-R injury. Specifically, exposure of rats to 1 h of hyperbaric oxygen (2.5 absolute atmospheres) starting 15 min into the reperfusion period was able to provide a significant reduction of renal injury[63]. However, the exact mechanisms involved in the protection provided by hyperbaric oxygen remain to be determined.

In contrast, the results of some investigations have not been conclusive, leading to the suggestion by some that ROS may not be a major contributor to the development of renal I-R injury and ischemic ARF. Furthermore, although some pharmacological interventions have provided promising results against renal I-R injury, the potential benefits of systemic clinical administration of these agents have been limited due to several confounding factors. For example, the use of native SOD has faced a number of problems ranging from its rapid proteolytic degradation and immunogenicity in vivo to prooxidant and cytotoxic activities at higher concentrations[64].

Role of reactive nitrogen species in renal I-R injury

Nitric oxide ($NO^{\bullet}$), produced mainly by $NO^{\bullet}$ synthase (NOS), plays an important role in both normal and abnormal renal function[65]. All three NOS isoforms have been identified in the kidney with the endothelial and neuronal (constitutive) isoforms located in the renal vasculature and macula densa, respectively. Active inducible NOS (iNOS) is expressed in the PT after exposure to hypoxia or inflammatory cytokines[66] or in the kidney subjected to I-R, causing renal dysfunction and injury, which contributes to the development of ischemic ARF[67]. Several in vivo and in vitro investigations have also demonstrated that absence of iNOS or inhibition of its activity reduces renal I-R injury significantly, thereby confirming a cytotoxic role for high concentrations of $NO^{\bullet}$ within the kidney. In contrast, inhibition of constitutively expressed endothelial NOS (eNOS) exacerbates renal I-R injury by promoting renal vasoconstriction and microvascular thrombosis. Lower concentrations of $NO^{\bullet}$ generated from constitutively expressed NOS, therefore provides a protective effect during renal I-R via maintenance of renal vasodilatation. An imbalance between the expression and activities of eNOS and iNOS is therefore important in the pathophysiology of ARF, which involves a decline in eNOS activity with a concurrent increase in iNOS expression[68].

The interaction of $NO^{\bullet}$ with $O_2^{\bullet-}$ generates the pro-oxidant species peroxynitrite[69]. $ONOO^-$ causes significant cellular injury via DNA strand breakage and nitration of tyrosine residues on proteins[70]. This is compounded by the ability of $ONOO^-$ to generate highly damaging $^{\bullet}OH$ under the acidic conditions that prevail during I-R, causing further cellular

damage[71]. The involvement of $ONOO^-$ in the development of renal I-R injury has been confirmed[72]. $ONOO^-$ can also nitrate and deactivate antioxidant enzymes such as SOD, contributing to renal I-R injury further by promoting oxidative stress[73]. Furthermore, in reacting with $O_2^{\bullet-}$, the bioactivity of $NO^{\bullet}$ is also reduced and this promotes vasoconstriction and microvascular thrombosis and this is particularly evident when $O_2^{\bullet-}$ generation is increased in the early stages of renal I-R before generation of high concentrations of $NO^{\bullet}$ by iNOS. The role of $ONOO^-$ in the development of renal I-R injury has also been confirmed in a study in which the $ONOO^-$ scavenger ebselen provided significant renoprotection in an animal model of ischemic ARF[74].

Role of inflammation in renal ischemia-reperfusion injury

Renal inflammation also contributes to the development of renal I-R injury and associated ischemic ARF[75]. It is also implicated in the etiology of allograft rejection after renal transplantation and is involved in the development and progression of CRF leading to ESRF[76]. The renal inflammatory process involves a complex and interrelated sequence of events resulting in injury to, and the eventual death of, renal cells. Inflammation within the kidney involves the production and release of biologically active mediators such as bradykinin, histamine, and platelet-activating factor (PAF) and pro-inflammatory cytokines including interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, from migrating inflammatory cells such as polymorphonucleocytes (PMNs) or from PT and glomerular mesangial cells, which can act as pro-inflammatory cells[77]. There is also recent evidence that dendritic cells residing within the kidney are an important, if not predominant, source of some inflammatory cytokines such as TNF-α in the early stages of developing renal I-R injury[78].

The important role of inflammatory mediators in the development of renal inflammation is confirmed by studies in which their removal from the plasma by dialysis has provided benefit[79]. Vasoactive agents such as $NO^{\bullet}$ and arachidonic acid metabolites such as cysteinyl leukotrienes derived from 5-lipoxygenase (LOX) also contribute significantly to the pathogenesis of renal inflammation. Other pro-inflammatory proteins

such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule-1 (VCAM)-1 are also involved in the renal inflammatory process[80] as well as the activation of NF-κB and AP-1[81]. There is also recent evidence that cathepsin G plays a major role PMN-mediated inflammation and injury during renal I-R and that cathepsin-deficient mice are protected against I-R renal injury.

Renal inflammation involves a significant degree of oxidative stress[82], which contributes to the development of renal I-R injury[83]. Oxidative stress in the kidneys of mice subjected to renal I-R correlates positively with renal tissue levels of TNF-α. During reperfusion, activated pro-inflammatory PMNs attach to, and infiltrate, renal tissues where they generate $O_2^{\bullet-}$ and contribute to oxidative stress. Prevention of PMN infiltration can therefore reduce renal I-R injury and ameliorate ischemic ARF. The roles of ROS ($O_2^{\bullet-}$, $^{\bullet}OH$), $NO^{\bullet}$, and $ONOO^{-}$ have also been confirmed in studies in which reduction of the production, or scavenging, of these molecules has reduced renal inflammation significantly[84]. In contrast, there is also evidence that systemic treatment with the $NO^{\bullet}$-donor molsidomine, which generates low concentrations of $NO^{\bullet}$, can reduce renal inflammation and I-R injury.

In view of the evidence that the development of renal I-R injury is associated with intrarenal inflammation, inflammation of the kidney has become an attractive target for the development of novel drug therapies for ischemic ARF[85]. Several models have been utilized for investigation of renal inflammation associated with renal I-R injury, and these have been used to study the pathophysiological roles of inflammatory cells such as T and B cells in the development of renal inflammation and I-R injury as well as the effects of pharmacological modulators of T and B cells. Resolvins (Rv) and protectins (PD) have recently been identified as two novel families of endogenous *n*-3 fatty acid docosahexaenoic acid metabolites and in response to renal I-R injury, mouse kidneys have been reported to produce D-series resolvins (RvDs) and PD1[86]. Intriguingly, administration of RvDs or PD1 to mice before, or subsequent to, ischemia resulted in a significant reduction in functional and morphological kidney injury. Prostate apoptosis response (Par)-4, a leucine zipper protein associated with apoptosis in neuronal and prostate tissues, has recently been shown to be overexpressed in the mouse kidney after renal I-R and in a

human-derived renal cell line (HK-2) subjected to chemical I-R in which PAR-4 increased sensitivity to apoptosis. Par-4 has therefore been described as a novel and early mediator of renal cell injury after I-R and, although further research is required, it could provide a novel target by which to reduce renal I-R injury and associated ischemic ARF[87]. Further investigation and understanding of the regulation of these, and other, anti-inflammatory processes are likely to be of benefit in the development of novel treatments for the inflammatory component of renal I-R injury and ischemic ARF.

5 - Citochine pathophisiology:

In ischemic acute kidney injury, hypoxic and anoxic cell injuries occur early during the ischemic phase, followed by inflammatory responses in the reperfusion phase (Fig. 4). During reperfusion, blood containing innate immune component flows through ischemic tissues and accentuates injury. It is well established that a robust inflammatory reaction occurs following ischemia reperfusion . Renal ischemia reperfusion induces renal synthesis or activation of pro-inflammatory cytokines and chemokines, and recruits leukocytes into the post-ischemic kidneys. Functional impairment of kidney during acute kidney injury, leading to retention of fluids and nitrogenous waste products, further aggravates and sustains inflammation. The initiation signals activating the innate immune system as well as triggering inflammatory response can be classified into 4 categories:

1) factors passively released from injured cells,

2) factors actively synthesized and secreted from the cells that have undergone ischemia,

3)recognition of altered or injured cell structures

4) decreased expression of anti-inflammatory factors by injured cells .

In acute kidney injury, both endothelium and tubular epithelium participate in innate immune responses. The signaling responses in tubular epithelium during renal injury, such as signaling through toll-like receptors (TLRs), is quite similar with that during ascending urinary infection[88].

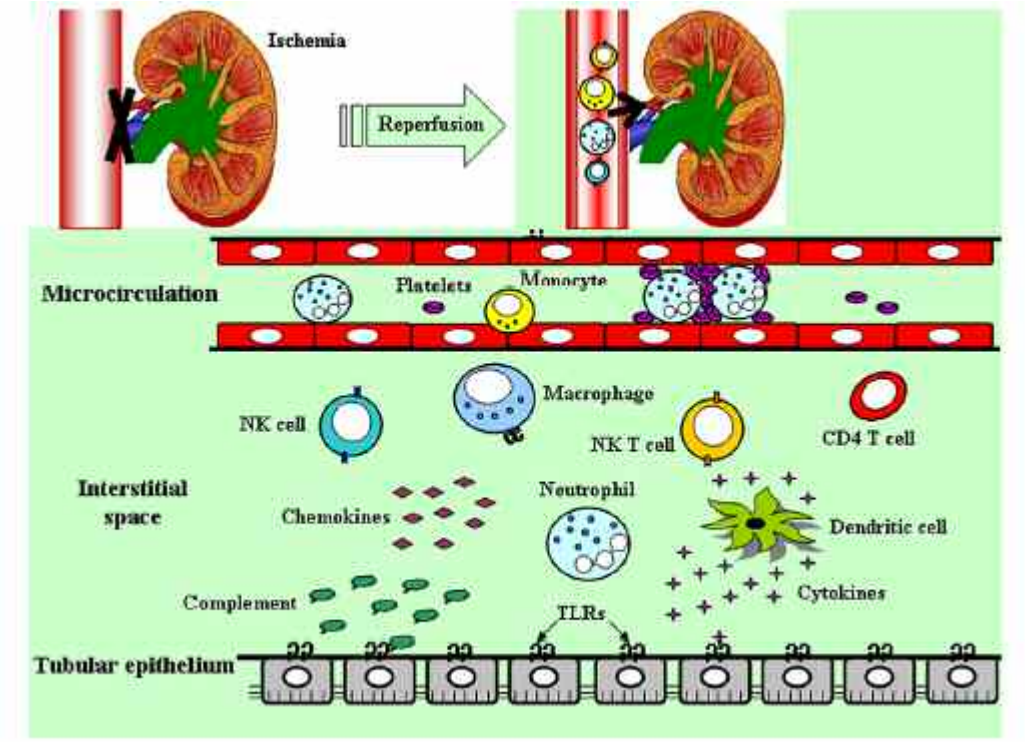


Fig. 4 - Overview of early immune response occurring in post-ischemic kidneys. In experimental ischemia reperfusion-induced acute kidney injury models, ischemic insult occurs first and then reperfusion initiates inflammation in post-ischemic kidneys with entry of blood containing major cellular components of innate immunity, plus lymphocytes. Leukocytes including neutrophils, macrophages and lymphocytes traffic into post-ischemic kidneys. Cytokines and complement system also contribute to renal injury.Early injury events take place both at the level of the microvasculature and then in the tubular interstitial space. Renal microcirculation is interfered by plugging with leukocytes and platelets and each immune component act in concert causing robus tinflammatory responses in the tubular interstitial space.

Cellular mediators of innate immunity

Macrophages, neutrophils, natural killer (NK) cells, and dendritic cells are important cells involved in innate immune responses (Tab. 1)[89]. In addition, newer data also implicates lymphocytes during these early injury responses.

Table 1 Summary of data on effector cells of innate immune system in ischemic acute kidney injury

Cells	Major findings in post-ischemic kidneys	Reference
Macrophages	• Infiltration into the outer medulla, remaining until the recovery phase	Ysebaert et al. [52]
	• Less renal fibrosis in osteopontin knockout mice, suggesting possible role of macrophages in the recovery phase	Persy et al. [54]
	• Roles in the early injury phase	Day et al. [57] Jo et al. [58]
Neutrophils	• Infiltration into post-ischemic kidneys	Chiao et al. [60]
	• Protective effects of inhibiting neutrophil infiltration	Kelly et al. [4]
	• No protection in neutrophil blockade or depletion	Thornton et al. [63] Rabb et al. [64] Nemoto et al. [61]
	• Protective effects of several adhesion molecules, PAF, CD44, and activated protein C, which are involved in neutrophil infiltration or activation	Rabb et al. [64] Riera et al. [67] Rouschop et al. [73] Turunen et al. [71]
Dendritic cells	• Increased total numbers and MHC class II expression of renal dendritic cells during ischemia reperfusion-induced acute kidney injury	Kim et al. [22]
	• TNF secretion from resident dendritic cells in early renal injury following ischemia reperfusion	Dong et al. [76]
	• Abnormal dendritic cell trafficking into transplanted kidneys	Loverre et al. [77]

Tab. 1 – Effector cells of innate immune system.

Macrophages

Macrophages play roles in both innate and adaptive immunity. Activated macrophages exhibit potent phagocytic activity and secret several important cytokines such as IL-1, IL-6, IL-8 (CXCL8), IL-12, and TNF-á. Monocyte adherence at the level of the renal microvessel, the ascending vasa recta, is observed after 2 h following reperfusion, and inhibition by anti-B7-1 antibody attenuated renal injury . Macrophages infiltrate into the outer medulla of the post-ischemic kidneys and remain into the recovery phase. Early monocyte/macrophage influx could be mediated by microvascular basement membrane heparin sulfate proteoglycans binding to L-selectin and monocyte chemoattractant protein-1 (MCP-1) . Although macrophages are suspected to play a role in renal repair after acute kidney injury, their precise role is yet to be elucidated. There are several recent reports implicating macrophages in the recovery phase of ischemia reperfusion-induced acute kidney injury and contributing to the development of renal fibrosis .However, macrophages clearly have a role in the early injury phase of ischemia reperfusion-induced acute kidney injury. Macrophage production of heme-oxygenase-1 has been shown to underlie the

protective effects of statins in acute kidney injury, though statins have quite diverse effects[90].

Neutrophils

Neutrophils play key roles in innate immune response byphagocytosis, producing reactive oxygen, nitrogen species and antimicrobial peptides. Although neutrophil infiltrationis found in ischemic acute kidney injury models and biopsies from patients with early acute kidney injury, the precise role and kinetics of neutrophil trafficking into the post-ischemic kidneys are not fully defined. Some reports demonstrated that renal injury was reduced afterischemia reperfusion when treatments inhibiting neutrophil infiltration or activity were applied, while other studies failed to find a protective effect of neutrophil blockade or depletion. Although neutrophils are less likely to cause direct renal injury compared to their effect during cardiac or skeletal muscle ischemia reperfusion injury, they likely have a contributory role by plugging renal microvasculature and releasing oxygen free radicals and proteases[91].

Many substances affecting neutrophil influx or activation, such as neutrophil elastase,tissue-type plasminogen activator, activated protein C, hepatocyte growth factor, and CD44 have been suggested toparticipate in inducing renal injury .

Natural killer (NK) cells

Natural killer (NK) cells are a class of large, granular,cytotoxic lymphocytes that lack T- or B-cell receptors. They target and kill infected cells directly as well as produce variety of cytokines including interferon (IFN)-ã and TNF-á.Little is known about the role of NK cells in acute kidney injury. However, NK cells are predicted to play a role in inducing renal injury following ischemia reperfusion because they secrete major cytokines that facilitate the inflammatory process including activation of neutrophils and macrophages.

Dendritic cells

Dendritic cells have been shown to participate in ischemic acute kidney injury in a number of studies. In a rat transplant ischemia reperfusion model, recruitment of recipient MHC class II-positive leukocytes into the kidney was demonstrated despite no sign of acute rejection, and some of them were identified as dendritic cells . Both total number and MHC class II expression of renal dendritic cells are increased after ischemia reperfusion injury. Dendritic cell–endothelial cell binding and migration seem to be facilitated during the initial inflammatory response. Resident dendritic cells are the predominant TNF-secreting cell in early ischemia reperfusion-induced acute kidney injury[92] and ischemia There are several minor lymphocytes subsets which express Therefore, they are known as innate-like lymphocytes (ILLs). Three main classes of ILLs are NK T cells, intraepithelial ãä T cells, and B-1 subset of B cells (B-1 cells).

Natural killer T cells (NK T cells)

NK Tcells, also known as NK1.1 Tcells, are lymphocytes with some of the characteristics of T cells as well as those of natural killer (NK) cells, existing both in the thymus and peripheral lymphoid organs. NK T cells exert regulatory functions by secreting cytokines such as IL-4, IL-10, and IFN-ã. NK T cells are known to traffic into the post-ischemic kidneys as early as 3 h after ischemia reperfusion injury and begin to decrease at 24 h after ischemia reperfusion injury compared to normal and sham operated kidneys. Isoflurane anesthesia significantly attenuated ischemia reperfusion-induced acute kidney injury in mice by reducing inflammation and modulating infiltration of NK T cells as well as neutrophils and macrophages. Recently, NK T cell activation was reported to contribute to acute kidney injury by mediating neutrophil IFN-ã production[93].

B-1 cells

B-1 cells are a minor subset of B cells and distinguished from conventional B-2 cells by the cell-surface protein CD5 and primary residing location. They are distributed in the peritoneal and pleural cavities. Although production of natural antibodies, immunoglobulins which arise without specific antigenic stimulation, is suggested as a primary role of B-1 cells, their precise role still remains to be defined. Natural IgM from B-1 cells has been reported to be involved in initiation of injury in murine intestinal ischemia reperfusion injury models. In a murine ischemic acute kidney injury model, ì chain-deficient mice (lacking mature B cells) were protected from renal injury, suggesting possible roles of B cells. However, there are no reports that directly reveal the role of B-1 cells in ischemia reperfusion-induced acute kidney injury.

CD4 T cells

CD4 T cells have been identified as an unexpected (based on traditional functions of CD4 T cells) mediator of acute kidney injury, functioning very early like traditional innate immunity members. The pathophysiologic role of T cells has been elucidated in the initiation phase of ischemic acute kidney injury in many studies, both directly and indirectly. However, in this work, very few infiltrating T cells were found in early (within 24 h) post-ischemic renal tissues. A "hit-and-run" hypothesis was proposed to explain the paucity of T cells during the insult phase of ischemic acute kidney injury, that T cells would rapidly infiltrate within hours, initiate damage, and then disappear soon after. This hypothesis is directly supported by two recent reports revealing early trafficking of lymphocytes into post-ischemic kidneys.

A study on CD4 T cell subsets in a murine ischemia reperfusion-induced acute kidney injury model revealed that CD4 Tcells of the Th1 phenotype are pathogenic and the Th2 phenotype can be protective[94]. This work was performed using mice with targeted deletions in the enzymes signal transducers and activators of transcription (STAT) 4 and STAT6, which regulate Th1 (IFN-ã producing) or Th2 (IL-4 producing) differentiation and cytokine production, respectively.

STAT6-deficient mice had markedly worse renal function and tubular injury, whereas STAT4-deficient mice had a mildly improved renal function. Furthermore, IL-4- deficient

mice showed similar post-ischemic phenotype with STAT6-deficient mice, suggesting that IL-4 mediates protective effect of the STAT6 pathway. One recent report supports the importance of CD4 T cells in early renal injury after ischemia reperfusion by demonstrating that inactivation of IL-16 (a T cell chemoattractant, strongly expressed in distal and proximal straight tubules of the post-ischemic kidney) by antibody therapy and IL-16 deficiency led to less CD4 T cell infiltration and prevented renal injury[95]. Despite ample data on the role for CD4 T cells in kidney ischemia reperfusion injury, as well as ischemia reperfusion injury of other organs like liver, lung, brain, and gut, the precise mechanisms underlying the role of T cells in acute kidney injury still need to be elucidated.

Soluble molecules and membrane-associated receptors

The innate immune response includes soluble molecules such as complement and cytokines. In order to initiate and generate a full-blown innate immune response, TLRs appear required.

Complement

The complement system is a group of serum and cell membrane proteins that interact with one another and with other molecules of innate and adaptive immunity to carry out key effector functions. Many studies have demonstrated that alternative and mannose-binding lectin (MBlectin) pathways are associated with ischemic injury. Alternative pathway activation occurs in a microenvironment with higher concentration of its components and lower concentrations of complement inhibitors. In murine acute kidney injury models, C3 (the first component of alternative pathway) production from tubular epithelial cells is stimulated and the complement inhibitor Crry expressed on the tubular basolateral surface is altered after ischemia reperfusion[96]. A recent report showed that C3a plays a crucial role in the CXC chemokine production by tubular epithelial cells after ischemia reperfusion, further demonstrating the role of the alternative complement pathways in post-hypoxic

injury and inflammation. Gene silencing with small interfering RNA (siRNA) targeting C3 and caspase 3 genes resulted in renal functional and structural protection. The MB-lectin pathway is also activated during acute kidney injury and implicated in tissue injury. Activation of the MB-lectin pathway is triggered by pattern recognition receptors, MB-lectin and ficolin, which bind to carbohydrates expressed on the surface of many pathogens as well as several endogenous ligands expressed on apoptotic and necrotic cells and cytokeratin exposed on hypoxic endothelial cells. MB-lectin recognizes endogenous ligands presented in the post-ischemic kidneys, resulting in complement activation within kidneys during acute kidney injury. C5b-9 complex (membrane attack complex, MAC) and C5a also contribute to ischemic acute kidney injury. MAC deposition of tubular epithelial cells is known to stimulate production of TNF-α and IL-6[97].

Toll-like receptor (TLR)

Toll-like receptors (TLRs) are a family of evolutionarily conserved transmembrane receptors and prototype of pattern recognition receptors (PRRs). The signal transduction initiated from TLRs activates effector cells of innate immune system via several kinases and NF-êB, and generates pro-inflammatory cytokines. Among the 13 known TLRs, some TLRs are activated by the endogenous ligands from damaged tissues such as hyaluronan, fibronectin, heat shock proteins (HSPs), and host DNA, resulting in the stereotypic inflammatory response seen in autoimmunediseases. Of note, TLR-2 and TLR-4 respond to HSP-60 and HSP-70. Renal tubular epithelial cells are known to express both TLR-2 and TLR-4, and expression of both TLRs is increased during acute kidney injury. TLR-2-deficient mice and mice treated with TLR-2 antisense oligonucleotides are protected from renal injury both functionally and structurally[98], possibly via production of cytokines and chemokines such as IL-1α, IL-6, KC and MCP-1, and neutrophil infiltration. Though abundant evidence implicates TLRs in experimental models of ischemic acute kidney injury, application of this knowledge to improve outcome in humans has not started.

Cytokines

Cytokines are low-molecular-weight (approximately 25 kDa) regulatory proteins or glycoproteins released from various cells, mainly from leukocytes, usually by various

activating stimuli, and regulate the development and effector functions of immune cells. Most cytokines show autocrine and/ or paracrine action and a few of them exhibit endocrine action. Ischemia reperfusion-induced acute kidney injury causes the synthesis of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α. Cytokine production following ischemia reperfusion occurs through the interaction between cytokines and the transcriptional response directly induced by hypoxia itself. For example, generation of IL-1 subsequently stimulates tubular epithelial cells to produce TNF-α and IL-6. Renal ischemia activates the transcription factors such as NF-êB, heat shock factor-1, and hypoxia-inducible factor-1 (HIF-1). Direct blockade of a number of cytokines, including IL-1, IL-6, and IL-8 has been shown to attenuate renal injury during ischemic acute kidney injury, while IL-4 and IL-10 modulation can worsen disease. Cytokines clearly play an important role in both local and distant organ effects of acute kidney injury.. Chemokines are a subgroup of cytokines, which are released by tissues and composed of 90 to 130 amino acid residues. Their basic functions are chemotaxis and activation of leukocytes. Chemokines have three subtypes according to the number of amino acids between the first two cysteines; CC, CXC, and CX3C families. Chemokine induction during the inflammatory response after ischemia reperfusion injury has been reported in several organs such as brain, heart, liver, and kidney[99]. In post-ischemic tissues, chemokines are induced by reactive oxygen species (ROS), cytokines, complement activation, TLR-mediated pathways, and the NF-êB system. Regarding the role of ROS in chemokine induction, ROS has been reported to induce chemokine production in brief myocardial ischemia in a murine model and a canine model although there are still few reports in ischemia reperfusion-induced acute kidney injury models. ROS is known to trigger cytokine and chemokine cascades through NF-êB activation. Pro-inflammatory cytokines, such as tumor-necrosis factor (TNF)-á and interleukin (IL)-1â, stimulate chemokine production in post-ischemic tissues of myocardial ischemia reperfusion injury models[100] and hepatic ischemia reperfusion injury models. Complement activation can also stimulate chemokine induction. There was a report demonstrating that a specificC5a receptor antagonist abolished up-regulation of CXCchemokines and diminished neutrophil infiltration to less than 50% of control group in an ischemic acute kidney injury model.

Interleukin (IL)-1 belongs to a group of cytokines released during the early phase of reperfusion after renal ischemia. It can promote apoptosis and also inflammatory processes. The latter is regulated through an increased expression of various pro-

inflammatory cytokines as well as adhesion molecules that mediate the infiltration of leukocytes into the injured tissue. Binding of IL-1 to the IL-1 receptor leads to anactivation of the transcription factor nuclear factor-jB (NF-jB), which results in gene transcription of pro-inflammatory factors, among them monocyte chemoattractantprotein-1 (MCP-1) and intracellular adhesion molecule-1 (ICAM-1) which are contributing to the inflammatory reaction that leads to the tissue injury afterI/R. MCP-1 and ICAM-1 play a critical role in a key step in the development of tissue damage after I/R,namely leukocyte recruitment and infiltration. NF-jB also affects cell death through apoptosis as it regulates transcriptionof apoptosis-controlling factors of the Bcl-2 family such as the apoptosis preventing Bcl-2 and the apoptosis promoting Bax. (Krisztina Rusai, Journal compilation ª 2008 European Society for Organ Transplantation 21 (2008) 572–580)

The observation that I-R may induce the activation of a powerful antiapoptotic enzyme may explain the ability of preconditioning to reduce the deleterious effect of I-R on tubular cell survival[101].

The principle of protection has been termed ischaemic preconditioning (IP). The beneficial effect of IP are most apparent when the interval between the preconditioning protocol and the subsequent sustained ischaemic insult is brief ; the number of preconditioning cycles also influences the effectiveness of IP. Structural changes in post-ischemic kidneys are characterized by vasoconstriction or necrosis with desquamation of tubular epithelial cells into the tubular lumen. The molecular mechanism underlying this renal injury are not fully understood, although it has been reported that several causal factors, such as reactive oxygen species, neutrophil infiltration, vasoactive peptides and ATP depletion, are contributive to the pathogenesis of I/R-induced ARF. Evidence has implicated proinflammatory mediators such as TNF-α in pathophysiology of ischemia-reperfusion (I/R) injuri. The signalling cascade through which renal ischemia-reperfusion induces TNF production is beginning to be elucidated. Oxidants released following reperfusion activate p38 mitogen-activated protein kinase (p38 MAP kinase) and the TNF transcription factor, NFkB, leading to subsequent TNF synthesis. In a positive feedback, proinflammatory fashion, binding of TNF to specific TNF membrane receptor can reactivate NFKB. This provides a mechanism by which TNF can upregulate its own expression of other genes pivotal to the inflammatory response. Following its production and release, TNF results in both renal and myocardial apoptosis and dysfunction. Azuma and associates demonstrated that progressive renal morphologic deterioration occurs in response to an initial ischemic

insult, and that this is temporally related to macrophage infiltration with the release of inflammatory mediators, including TNF. Azuma and colleagues suggested that TNF is involved in the late renal deterioration associated with ischemia-reperfusion injury, whereas Garcia-Criado and associates provided a direct association between renal TNF production and renal failure following the acute phase of renal ischemia-reperfusion injury. TNF reduces glomerular blood flow and glomerular filtration rate, induces the synthesis of other proinflammatory mediators, increases glomerular albumin permeability, causes glomerular fibrin deposition, and stimulates cellular infiltration. TNF reduces glomerular blood flow and glomerular filtration rate by stimulating mesangial cells to produce a variety of vasoconstrictive (platelet activating factor, endothelin-1, prostaglandins) and vasodilatory (adenosine, nitric oxide, prostaglandins) and vasodilatory (adenosine, nitric oxide, prostaglandins) mediators. TNF also stimulates the production of reactive oxygen species from mesangial cells as well as the production of other endogenous pyrogens, including IL-1. These mediators, in turn, cause cellular and organ dysfunction. Reactive oxygen species also directly disrupt the barrier function of the glomerulus and lead to increased albumin permeability. Lechener et all[102] suggested TNF-α play a major role as regulators of immune function; induced apoptosis is directed by extrinsic death receptor signalling pathway, amplifield by an intrinsic mitochondrial pathway. Renal ischemia induces tubular cell injury with decreased levels of ATP, increased levels of calcium, and alteration in membrane lipid and enzyme activity. Reperfusion of the ischemic organ exacerbates ischemic injury by producing cytotoxic oxygen species and free radicals. In addition, the deleterious role of the inflammatory response in I-R induced organ damage is suggested by an enhanced expression of adhesion molecules and proinflammatory mediators (cytokine, chemokines), activation of the complement system, priming of the coagulation cascade, and subsequent leukocyte infiltration. Renal ischemia-reperfusion injury is the leading cause of acute kidney injuri in native kidneys and delayed graft function in deceased donor kidney transplant. Loverre et al. report that I-R injuri at the renal level is characterized by two main features: apoptosis of tubular cells and interstitial inflammation .

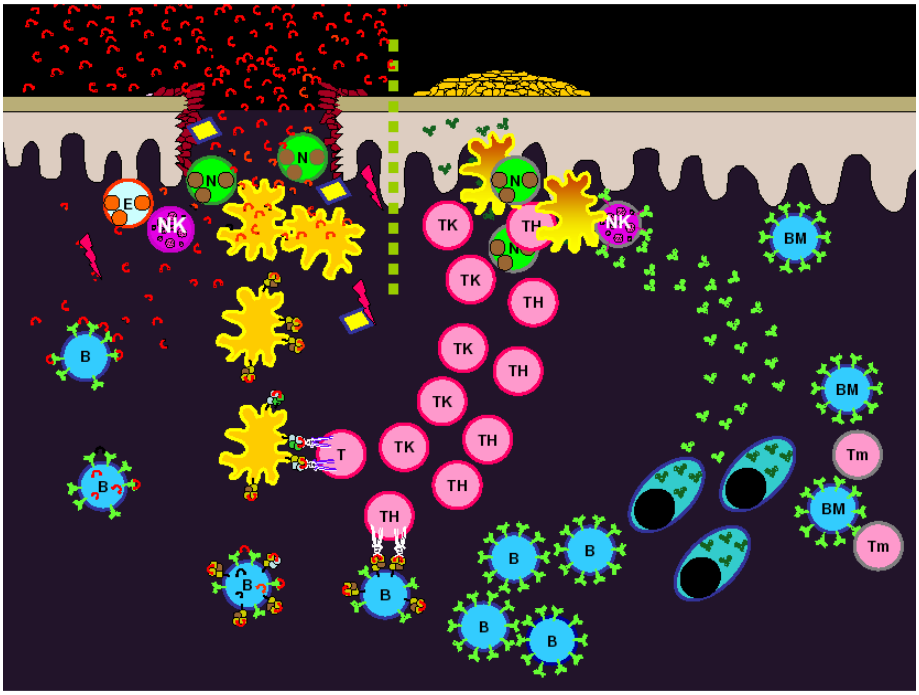


Fig. 5 - **Adaptative Immunity**

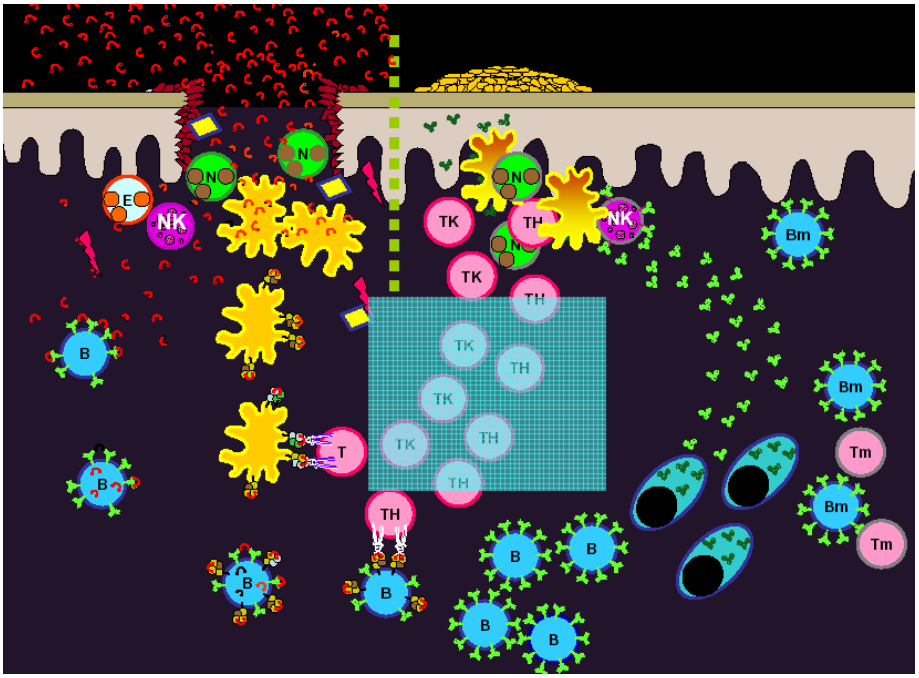


Fig. 6 - **T-Linphocite**

Apoptotic pathways in ischemic acute renal failure

The study of cell death has emerged as an important and exciting area of research in cell biology. Although two kinds of cell death, apoptosis and necrosis, are recognized, one of the major advances in our understanding of cell death has been the recognition that the

pathways traditionally associated with apoptosis may be very critical in the form of cell injury associated with necrosis.

Renal tubular epithelial cell injury from ischemia has been generally regarded as a result of necrotic form of cell death. We briefly describe recent evidence indicating that pathways generally associated with apoptosis, including endonuclease activation, role of mitochondria and caspases, are important in renal tubular injury. It is likely that the cascades that lead to apoptotic or necrotic mode of cell death are activated almost simultaneously and may share some common pathways.

The modern study of cell death began with the landmark publication by Kerr,Wyllie, and Currie in 1972[103],in which they coined the term "apoptosis" and made a distinction between necrosis and apoptosis based on morphologic criteria. Thus, in necrosis there is swelling of cell organelles, a loss of plasma membrane integrity and rupture of cell, invoking an inflammatory response. In contrast, in apoptosis, cells shrink, lose microvilli andcell junctions, and explode into a series of membrane bound condensed apoptotic bodies and affected cells are phagocytized by adjutant viable cells with little leakage of cellular contents, thus invoking no inflammation.

One of the major advances in our understanding of cell death has been the recognition that the pathways traditionally associated with apoptosis may be very critical in the form of cell injury associated with necrosis. Thus, it is now recognized that the same insult may result in apoptosis or necrosis with the mild injury generally resulting in apoptosis and severe injury in necrosis. Thus, the pathway that is followed by the cell is dependent on both nature and severity of insults. It appears likely that the cascades that lead to apoptotic and necrotic mode of cell death are activated almost simultaneously and that there are some common pathways which are shared and regulated in the two modes of cell death. Several proapoptotic signal transduction and damage pathways converge on mitochondria to induce mitochondrial membrane permeabilization and this phenomenon is under the control of Bcl-2–related proteins. The notion that a specific class of proteases, the "caspases" (cysteine aspartate-specific proteases), are involved in apoptosis, emerged from genetic studies of the nematode *Caenorrhabditis elegans .* process to occur. Caspases are a family of structurally related cysteine proteases that play a central role in the execution of apoptosis. On receiving a proapoptotic stimulus, the caspases are proteolytically processed to the active forms from their normally synthesized inactive proenzymes. At least 14 caspases encoded by distinct caspases. Caspase-3, -6, and -7 with

smaller domains are identified as effecter or executioner caspases.The executioner caspases are the major active caspases detected in apoptotic cells and are widely regarded to mediate the execution of apoptosis by cleaving and inactivating intracellular proteins that are essential for cell survival and proliferation. The specificity of downstream executioner caspases to cleave cellular proteins is unique because of their different primary sequences and different recognition sites on the target proteins. At present, there are two relatively well-characterized cell death pathways that result in the activation of the downstream executioner caspase-3. One is receptormediated and the other is mitochondrial-dependent. The receptor-dependent pathway is initiated by activation of cell death receptors Fas and tumor necrosis factor-α (TNF-α) leading to activation of procaspase-8, which, in turn, cleaves and activates procaspases-3.The mitochondrial-dependent pathway is triggered by The mitochondrial-dependent pathway is triggered by cytochrome c release from the mitochondria

There is increasing evidence for the role of caspases in hypoxic renal tubular cell injury. In our previous studies, we have demonstrated that chemical hypoxia with antimycin A results in increased caspase activity that precedes DNA damage and cell death. Examined in ischemic ARF. Caspase-1 is involved in the proteolytic cleavage of the precursor forms of proinflammatory cytokines interleukin (IL)-1b and IL-18 that result in the formation of active forms of mature cytokines. Since caspase-1 mediated formation of active IL-1b and IL-18 is associated with inflammation in renal I/R, caspase-1 may play an important role in I/R injury. A rat model of I/R injury indicated that prolonged ischemia induced proapoptotic mechanisms, including increases in the Bax/Bcl-2 ratio, caspase-3 expression, poly[adenosine diphosphate (ADP)-ribose] polymerase (PARP) cleavage,DNA fragmentation, and apoptotic cell number in renal proximal and distal tubules [48]. Recent studies from several laboratories have recognized phosphatidylinositol 3_ (PI-3) kinase/Akt phosphorylation as one of the signaling pathways that blocks apoptosis and promotes cell survival in response to diverse apoptotic stimuli in different cell types. Akt also known as protein kinase B) was originally identified phosphorylation have been proposed for cell survival. One of the well-studied molecules that mediate cell survival by Akt phosphorylation is the proapoptotic Bcl-2 family member Bad. Bad has the ability to directly interact and bind to antiapoptotic Bcl-2 and Bcl-XL and blocks their survival function. caspase-8. Similar results on activation of caspase-3 and caspase-9 were

obtained on inhibition of PI-3 kinase in hypoxia-induced injury to renal tubular epithelial cells.

6 - Drugs therapy to prevent renal failure during aortic surgery

Strategies to provide renal protection

The fundamental problem in renal ischemia is an imbalance between renal oxygen supply and demand during the ischemic period.

Supply

We are limited in our ability to improve supply, especially in the setting of suprarenal aortic cross-clamping. With the knowledge that ARF can be the result of more than one ischemic event, however, we can attempt to optimize supply, that is eliminate 'pre-ischemic ischemia', before the exposure to the ischemic insult. Obvious interventions include optimizing the patient's volume status before the ischemic period, using invasive monitoring if clinically indicated. Hyperglycemia should be corrected. Surgery should be avoided, if possible, in the immediate period after exposure to radiocontrast agents. Patients with known renal artery stenosis may benefit from pre-surgical renal artery stenting. Known renal toxins such as NSAIDs should be avoided in the perioperative period.

Demand

Having optimized supply before the ischemic period, the emphasis during the ischemic period must be on the reduction of oxygen demand. As noted above, the mTAL resides in the outer medulla, the region of the kidney most vulnerable to ischemia. The mTAL is characterized by high mitochondrial density because of the intense active transport that takes place in this segment of the nephron. Furthermore, it is known that the metabolic demands of this region can be dramatically reduced by inhibitors of active transport, notably furosemide. Similarly, the metabolic activity of the mTAL can be reduced

by reducing the amount of glomerular filtrate delivered to the tubule. Our approach, then, is to administer a generous dose of furosemide (typically 100 mg) before the anticipated ischemic period, and not to attempt to enhance glomerular filtration during the ischemic period. Urine output is thus not utilized in any way to guide therapy. The goal is thus to depress renal function acutely, on the premise that this will lead to an increased likelihood of later viability. It should be emphasized that this strategy is an extension of our knowledge of renal physiology, but has not been validated in controlled human trials.

Dopamine

This brings us to the contentious topic of 'low-dose' dopamine. Dopamine has also been utilized extensively in low doses (0.5-3 μg/kg/min) for renal protection. Studies in animals and healthy human volunteers have demonstrated increased renal blood flow and increased urine output and sodium excretion. These findings do not necessarily translate into renal protection, however. Improving renal function intraoperatively may not be protective, but rather may worsen ischemic injury by increasing renal oxygen requirements. A recent comprehensive review of the literature found no evidence of a renal protective effect of dopamine in the perioperative period. In the context of our physiological framework, does dopamine improve the supply/demand balance? The answer is unclear, not only because of contradictory data, but also because of competing intrarenal effects. Dopamine, by stimulation of the dopamine 1 receptors, causes an increase in renal cortical blood flow, leading to increased glomerular filtration, solute excretion and urine output. Dopamine 2 receptor activation decreases intrarenal norepinephrine excretion, further enhancing vasodilation and glomerular flow. These effects probably increase renal medullary oxygen requirements. On the other hand, the blockade of dopamine 1 and 2 receptors does not affect the diuretic and natriuretic effect of dopamine; this implies a separate effect of dopamine, postulated to be an inhibition of the Na/K ATPase in the proximal tubule and mTAL. Such an inhibitory effect would decrease tubular energy requirements and medullary oxygen requirements. On balance, there is no compelling argument for or against renal-dose dopamine as a renal protectant.

Mannitol

Mannitol has traditionally been employed in patients considered to be at high risk of ARF. Mannitol is felt to provide benefit by increasing tubular flow both by increasing tubular fluid volume through its osmotic effect and by reducing tubular cell swelling and hence resistance to flow. Recent identification of mannitol as a 'scavenger' of reactive oxygen species makes it potentially attractive in the setting of ischemia/reperfusion injury. Initial animal studies formed the basis for the use of mannitol as a protective strategy, and whereas no prospective controlled studies in aortic or cardiac surgical patients have convincingly demonstrated these benefits, studies of renal transplants clearly support a protective role. In aortic surgery, we use mannitol judiciously, usually in a single dose (0.5-1.0 g/kg) intraoperatively, before the application of the cross-clamp, with appropriate monitoring of volume status.

Dopexamine

Dopexamine, a pure dopaminergic agonist, has been developed as a renal protective agent on the basis of the hypothesis that it improves renal perfusion. Although one initial small study provided encouraging results, a more recent study demonstrated no effect on renal vascular resistance. Although dopexamine causes hypotension, it lacks the myocardial side-effects of dopamine and remains a theoretically attractive agent. Larger well-designed trials are needed to assess its usefulness as a renal protectant.

Calcium antagonists

Intracytosolic calcium increases with ischemic intracellular ATP depletion. In the vascular endothelium this causes vasoconstriction, and calcium may potentiate luminal epithelial cell toxicity. This has been the rationale for the use of calcium antagonists for renal protection. The use of these agents systemically has the unfortunate consequence of systemic hypotension, with obvious adverse consequences on renal perfusion. When they are utilized, these agents thus tend to be given by direct injection by the surgeon into the renal vasculature to minimize systemic effects.

Natriuretic peptides

Atrial natriuretic peptide and the related endogenous compound urodilatin have renal vasodilating and natriuretic properties, and could theoretically provide renal protective effects. An initial flurry of encouraging reports in patients after cardiac surgery has been followed by no further information. Further large investigations are warranted before the adoption of these agents as a perioperative strategy.

Many physiological and biochemical variables can be used as markers of change in renal function. Each test has significant limitations and the results of the analysis must be interpreted in the context of these limitations.

- *Plasma creatinine* is the most frequently measured marker of renal function. It makes the assumption that plasma creatinine remains constant and the clearance of creatinine is solely by glomerular filtration. Thus plasma creatinine is an indirect determinant of glomerular filtration rate (GFR). However, there has to be a greater than 50% reduction in GFR before there is a change in plasma creatinine. Plasma creatinine also reflects an individual's muscle mass and alterations in muscle mass will influence the plasma creatinine concentration, which does not reflect changes in GFR. There is a small amount of tubular excretion of creatinine which, in terms of normal GFR, is insignificant. However, with severe renal impairment tubular secretion of creatinine has a greater role and, therefore, plasma creatinine does not accurately reflect GFR. GFR is usually determined by the clearance of an inert substance which is freely filtered at the glomerulus and has no tubular secretion or reabsorption. The gold standard has been estimation of inulin clearance. Creatinine clearance correlates well with GFR. For accuracy, it is essential that creatinine clearance is determined correctly. This requires a timed and complete collection of urine along with a plasma creatinine determination. A variety of formulae have been derived using plasma creatinine, body weight, and age to estimate creatinine clearance and hence GFR. When renal function is stable these estimates correlate well with measured GFR ($r = 0.9$).

- *Urine output* is a non-specific measure of renal function. Clearly if there is no urine production then there is no glomerular filtration. However, urine output can be influenced by a number of factors that regulate renal tubular handling of water. Oliguria (less than 400 ml urine/24 hr) may just reflect excess salt and water retention by the kidney due to a low fluid intake and not necessarily impaired renal function or the effects of increased anti-diuretic hormone (ADH) release, a normal response to surgery or stress.

- In clinical practice, *renal blood flow* is rarely determined. Renal blood flow can be determined by clearance techniques using the Fick principle.

- *Free water clearance* measures urinary concentrating ability. Any form of damage to the kidney impairs urinary concentrating ability. With renal tubular injury, free water clearance is impaired. Likewise, free water clearance is modified by diuretic therapy.

- *The fractional excretion of sodium* has been used as a marker of renal function. More correctly, it reflects renal tubular reabsorption of sodium. The normal physiological response to a reduction in renal perfusion and glomerular filtration is to activate the tubular glomerular feedback mechanism leading to increased sodium reabsorption, along with water. The net effect is to increase blood pressure and hence renal perfusion. In the acute situation (the first 24 hours after an event affecting renal function), a low fractional sodium excretion (FeNa < 1%) indicates impaired renal perfusion. With any form of established renal damage, or the use of diuretics, the fractional excretion of sodium is increased and becomes impossible to interpret. It is, therefore, essential that the changes in markers of renal function that were recorded in the analysed papers are examined critically for the variables which influence the measurements reported. Conclusions drawn from the results should also be closely examined for the validity of the renal function measures that were used. The inability to correctly interpret the results prompted us not to analyse the data on FeNa but instead to provide the raw data.

7 - Surgical technique to reduce the risk of renal failure in aortic surgery

Cross clamping of the renal artery is tolerated without complications for about 30 min in physiological normothermic condition. Warm clamping ischaemia longer than 50-60 min was significantly correlated with postoperative acute renal failure. The protective effect of mannitol in preventing ischaemic renal damage has been previously studied and many surgeons use it routinely during aortic surgery. However mannitol infusion failed to show any consistent protective effect when cross-clamping time lasts longer than 60 min.

Surgical adjuncts to decrease renal failure include
1) perfusion of renal arteries with cold ringer lactate
2) various method of blood normothermic perfusion to the kidney.

Kidney hypothermia

Kidney hypothermia is a technique developed in transplant surgery. It allows to reach 48h of reversible ischaemia when the kidney is explanted and then preserved by continuous perfusion of special solution at 4°. It can be employed when bench surgery is required for complex renal artery reconstruction, but it's technically demanding. For less complex renal artery reconstruction, especially adjunctive to an aortic aneurysm, kidney hypothermia is accomplished in situ by cyclic or continuous perfusion of renal arteries with cold solutions (Ringer's lactate or saline). Ice slush can be applied on kidney surface to further ensure hypothermia, but this requires a retroperiteoneal exposure. However, hypothermia of the renal parenchyma is less reproducible when kidneys remain in situ.

Since the 1950s therapeutic hypothermia has been used extensively, predominately during cardiac surgery, for protection against I/R injury. The mechanisms of hypothermic protection during I/R are multifactorial, and as yet are not clearly defined. Several mechanisms have been proposed, including reduction in oxygen demand and consumption, oxidant injury, leukotriene production, interleukin-1β production, and

neutrophil accumulation[104]. More recently studies have focused on the molecular mechanisms of hypothermic protection, including changes in transcription factor activation and oxidative stress protein expression during I/R[105]. While local hypothermia protects against renal I/R injury in laboratory animals, its clinical efficacy is unknown[106] [107]. In a large retrospective series no significant reduction in postoperative ARF with renal perfusion with cold Ringer lactate solution during TAAA repair was identified[108].

Normothermic blood reperfusion of renal artery

- **Octopus technique with atriofemoral pump**

Is performed in presence of a pump circuit with two or more ("octopus") separate cannula inserted in each visceral artery that guarantee a continuous non-pulsatile blood flow reperfusion of renal and visceral arteries during the anastomosis technique time.

When the descending thoracic aorta is cross-clamped several cannulas are assembled from the pump to all the different visceral arteries, to guarantee a continuous non-pulsatile flow until the proximal anastomosis is performed (Fig.7)

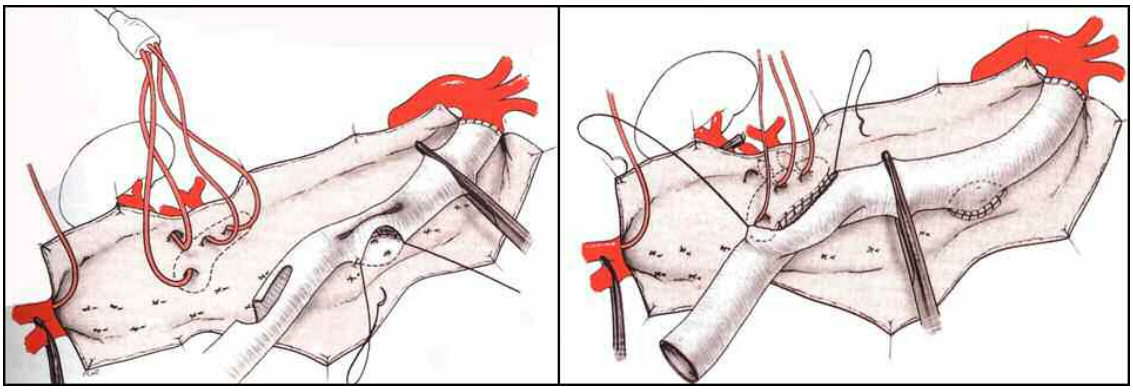


Fig. 7 – Technique with multiple separate cannula (octopus) from a pump circuit

When the proximal anastomosis is completed, the renal and visceral arteries are reattached separately while a non-pulsatile flow is maintained through the pump. When the visceral anastomosis is performed the single cannula is removed, re-establishing a physiologic perfusion.

Advantages of this technique are the low risk of kidneys and visceral ischemia and that the anastomosis can be performed with all the needed time.

Disadvantages are: non-pulsatile blood flow during all the procedure, time spending and possible complication related to incannulation or sistemic heparinization.

- **Retrogade perfusion with atriofemoral pump**

Is performed in presence of a pump circuit with segmental repair that guarantees a continuous non-pulsatile blood flow reperfusion of renal and visceral arteries during the proximal anastomosis technique time through a retrograde flow coming from the distal aorta .

A short segment of descending thoracic aorta is cross-clamped while the pump perfuses the renal and others visceral arteries through a retrograde non-pulsate flow and the proximal anastomosis is performed (Fig.8).

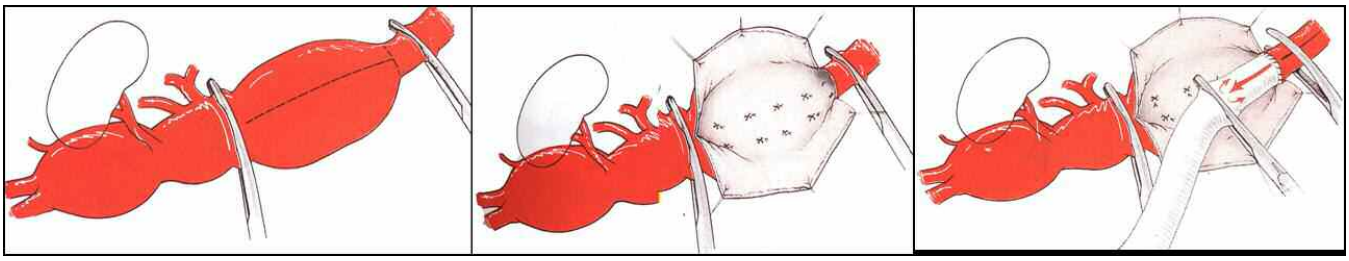


Fig. 8 – Example of retrograde perfusion during thoraco-abdominal aneurysm repair (proximal anastomosis).

After the proximal anastomosis is completed, the clamp is moved below the renal arteries and visceral branches are reattached (Fig.9).

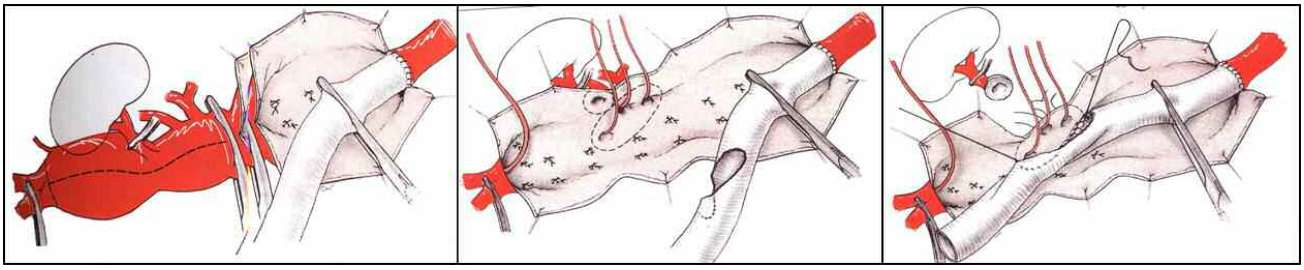


Fig. 9 - Example of retrograde perfusion during thoraco-abdominal aneurysm repair (visceral anastomosis).

After completion of the visceral artery repair, the clamp is moved on the graft below the renal arteries and a physiological flow is re-established (Fig.10).

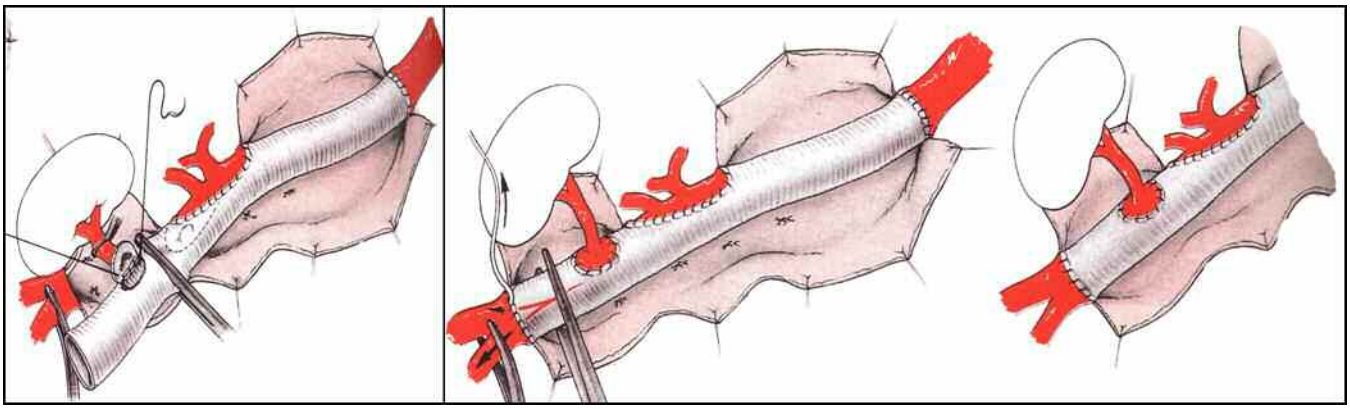


Fig. 10 - Example of retrograde perfusion during thoraco-abdominal aneurysm repair (distal anastomosis).

Advantage of this technique are the possibilities of vascular fluid reuptake and the continuous flow.

Disadvantages are the non-pulsatile flow to visceral arteries that many authors claim to be a possible cause of renal injury (because of alterations in human factors, especially the renin angiotensin system, during cross-calmping)[109]; possible complication related to incannulation or sistemic heparinization,

- **<u>Anterograde perfusion with pulsatile blood flow (shunts Pruitt-Inahara)</u>**

Is performed without a pump circuit. Perfusion is guaranteed directly by an anterograde pulsatile flow coming from above the proximal clamp (descending aorta) during the anastomosis time.

When the proximal anastomosis is performed between the thoracic aorta and a branched graft several Pruitt-Inahara shunts are inserted in each branch on the graft side (Fig. 11). The clamp is moved below the graft branches and a physiological flow is re-established through the shunts into the visceral arteries.

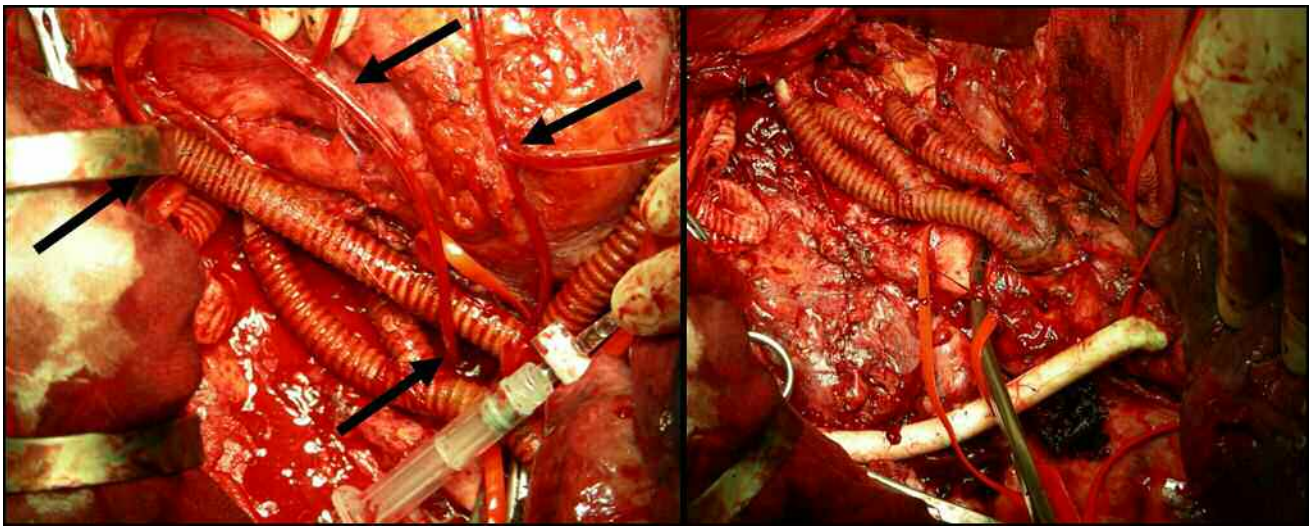


Fig. 11 - Intraoperative images of octopus technique. Shunts are indicated by arrows

Advantages of this technique are the physiological pulsatile blood flow during all the procedure with low risk of visceral and kidneys ischemia and that the anastomosis could be performed with all the needed time.
Disadvantage are time spending, technically complex.

Short term arterial blood reperfusion of normothermic kidney

Renal blood reperfusion is obtained, once the proximal anastomosis between the proximal aorta and the Dacron graft is performed, by re-establishing pulsatile normothermic blood flow through the a Pruitt-Inahara shunt (500-50-9 F Ideas for Medicine TM, Cryolife® Comp, St. Petersburg, FL, U.S.A.). Application of the shunt may change in different surgical procedure. When the procedure requires more than 30 min to re-establish a normal flow in the renal artery, that always happen in all thoraco-abdominal reconstruction, the proximal end of the shunt is distally inserted in the vascular graft. The proximal aortic clamp is released; the shunt is blood perfused and its distal end is inserted into the open end of the renal artery. After 3 min of blood reperfusion, the aorta and the renal artery are reclamped, the shunt is promptly removed and the renal artery reconstruction completed. The reperfusion is repeated every 30 min if necessary.

This technique is particularly interesting in all those cases in which a less extensive surgical approach is possible avoiding the atrio-femoral pump such as in type IV TAAA or in suprarenal aneurysms.

Fig. 12 - *Introduction of Fogarty catheter into visceral and renal arteries to obtain emostasis.*

Fig. 13- *The proximal aorta anastomosis need a 1° time of renal arteries less than 30 min. (27 in the Fig. below). The proximal branch of the Pruitt-Inahara shunt is introduced on the graft and the ballon is inflated with 1.5 cc of phisiologic solution.*

Fig. 14 - *The 1° ischemical time of the right kidney is follow by a 3 min. temporary reperfusion by arterial blood from the sistemic circle trought the shunt.*

Fig. 15 a-b-c - *After the first temporary reperfusion of the right and left renal arteries the patch is performed, including the celiac trunch, the superior mesenteric artery and the right renal arteries. At the end of the anastomosis, a temporary reperfusion of the left renal artery is performed and after that reimplanted on the prothesic graft.*

Fig.12

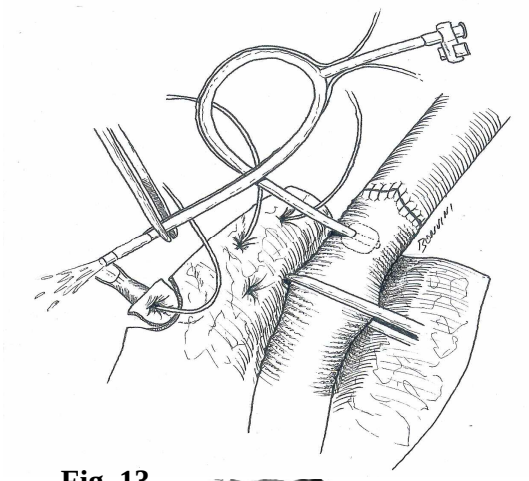


Fig. 13

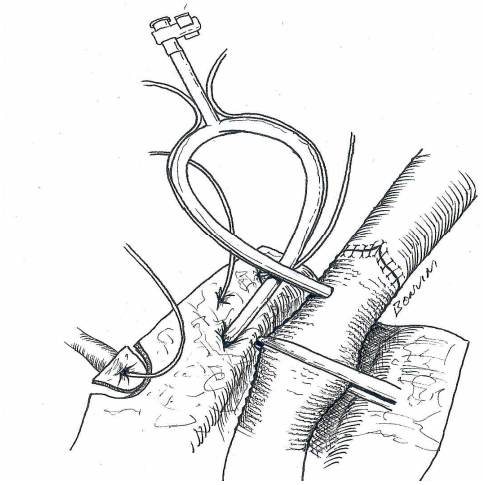


Fig. 14

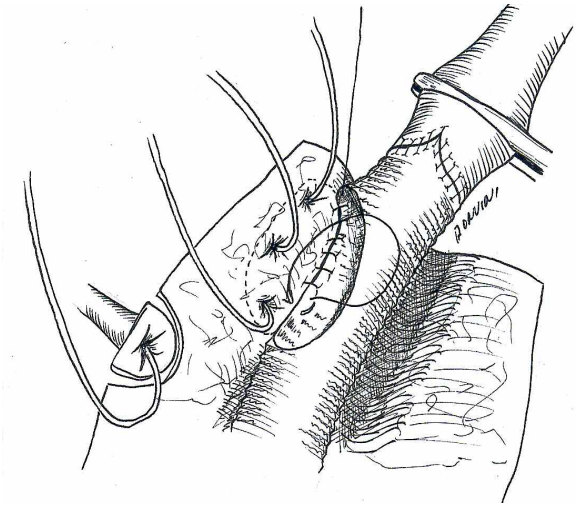
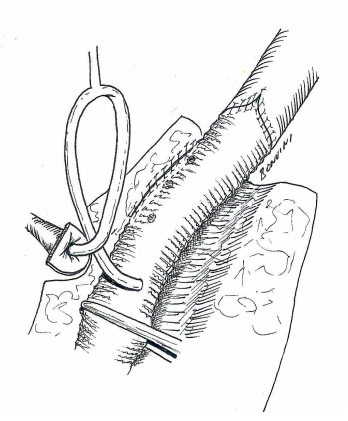


Fig. 15°a.



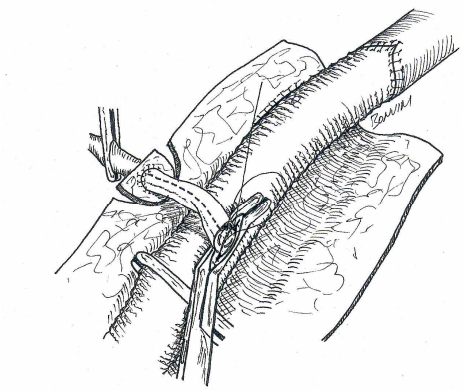


Fig. 15 b.-c.

Renal function deterioration is responsible for morbidity and mortality in patients undergoing surgical repair of abdominal aorta and renal arteries. On of the main cause of renal dysfunction is usually the result of cross clamping ischaemia or ischaemia reperfusion injury. Prolonged renal clamping ischaemia may become necessary during reapir of complex renal artery occlusive disease or renal artery occlusive disease with an abdominal aortic aneurysm and in pararenal or thoracoabdominal aortic aneurysms.

In this study we demonstrate on an animal model that is possible to increase the total time of clamping ischaemia by re-establishing blood flow into renal artery for 3 minute. Short term arterial blood reperfusion can be safely repeated twice leading to a total ischemic period of 90 min.

Materials and methods

In the present study we used an in vivo model of repeated ischemia reperfusion injury. We compared the control group of animal that underwent a renal ischemia of 60 minutes with a group of animal that underwent two period of 30 minutes of ischemia with 3 minutes of blood reperfusion for a total ischemic time of 60 minutes, and a group of control animal that underwent a renal ischemia of 90 minutes with 2 groups of animal that underwent a total ischemic time of 90 minutes but in one group with a reperfusion of 3 minutes after 45 minutes of ischemia and the second group with 3 periods of 30 minutes of ischemia followed by 3 minutes of reperfusion to assess whether this technique is correct and to be able to tell which is the best pattern of reperfusion to apply.

Male Sprague-Dawley rats (10 weeks of age) weighing 200 to 250 g, were used for the experiment. The experimental protocols were approved by the Animal Ethics Committee of the University of Padova**.** The rats were acquired from the university vivarium sources and were housed in individual cages in a temperature and light-dark cycle-controlled environment with free access to food and water. All rats received humane care, in compliance with the "Principles of Laboratory Animal Care" prepared by the Institute Experimental Surgery Laboratory of the University of Padova.

Experimental Groups

The rats were allocated into one of the 4 experimental groups; control group, 18 rats (with progressive clamping time of both renal arteries to assess organ damage-30-45-60-90 min); group A, 4 rats (30 min of renal ischaemia followed by 3 min of reperfusion followed by other 30 min of ischemia for a total ischaemic time of 60 min), group B,4 rats (45 min of renal ischaemia followed by 3 min of reperfusion repeated twice for a total ischemic time of 90 min) and group C, 3 rats (30 min of renal ischaemia followed by 3 min of reperfusion repeated 3 times for a total ischemic time of 90min).

Surgical technique

The rats were anesthetized with ketamine hydrochloride (50 mg/kg intramuscular) and anesthesia was maintained with supplementary intramuscular injections of ketamine hydrochloride. The rats were placed supine under a heating lamp. The skin was aseptically prepared and a midline laparotomy was done. Ten mL of warm normal saline was instilled into the peritoneal cavity to help maintain fluid balance. The abdominal aorta was exposed by gently deflecting the loops of intestine to the left with moist gauze swabs and the origin of both renal arteries were isolated (the left renal artery separated by the renal vein) (Fig.16).

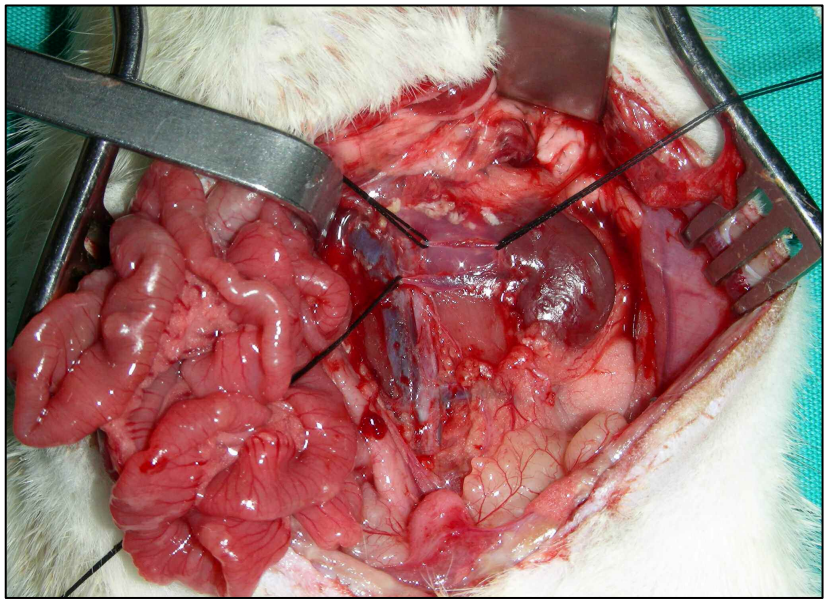


Fig. 16 – Left renal artery exposure.

A blood sample from inferior vena cava with a 31 gauge needle was drawn to evaluate serum creatinine level. Atraumatic microvascular clamps (Vascu-Statts II, midi straight 1001-532; Scanlan Int., St. Paul, MN) were placed at the origin of both renal arteries and both kidney were controlled to assure the total ischemic condition. When a complete parenchimal ischemia was impossible to achieve due to anatomical vascular anomalies rats were excluded by the study. A wet and warm gauze was placed in the peritoneum cavity to minimize heat and fluid losses. In group A after 30 min, microvascular clamps on both renal arteries were removed and both kidneys were reperfused for other 30 min checking the complete reestablishment of normal parenchimal condition. This

procedure was repeated three times for a total ischemic time of 90 min. In group B microvascular clamps were removed after 45 min for 3 min and then replaced for other 45 min for a total ischemic time of 90 min. The abdomen at the end of the procedure was closed and the rat was let recovery replaced in his cage under heat lamp with free access to food and water.

After 48 hours recovery the rats were anesthetized again with ketamine hydrochloride (50 mg/kg intramuscular) and reopened the abdomen for kidney harvesting. Before kidney removal a blood sample from inferior vena cava was drawn to evaluate serum creatinine level. levels were measured as a marker of renal function, using a 557A Creatinine kit (Sigma Diagnostics, St. Louis, MO) and analyzed on a Cobas Mira S Plus automated analyzer (Roche Diagnostics, Indianapolis, IN).

All rats were killed under anesthesia and both kidneys were carefully removed en bloc from the abdomen. The specimens were harvested, stored and sent separately in physiological solution for anatomo-pathological evaluation.

Blood samples were analysed by the Central Laboratory Analysis of the University of Padova

Histological examinations

For conventional histological analysis one section of right kidney, 5 mμ thickness, was cut from each paraffin block and hematoxylin-eosin stained. Histological assessment was semiquantitatively performed according to a previously published guideline. Renal morphological changes were graded on a 0 to 3 scale in relation to the extent of kidney alterations: 0 none; 1 up to 20%; 2 from 20 to 50%; 3 more than 50%. Six morphologic changes were assessed in the cortex and in medulla: (*1*) neutrophi linfiltration, (*2*) tubular necrosis (that is, the presence of necrotic cells, apparently denuded areas of tubular basement membranes, or ruptured tubular basement membranes), (3) tubular dilatation, , and(*4*) interstitial leukocyte infiltration . Two independent observers examined the slides by light microscopy in a blinded fashion.

Immunohistochemistry

The immunostaining was processed in 5mμ paraffi-nized sections. Briefly, after deparaffinization, endogenous peroxidase activity was blocked with 3% H2O2 for 10 min at room temperature. Sections were then subjected to microwave irradiation in citrate buffer to enhance antigen retrieval. Sections were then incubated with the primary antibodies against K67i (mouse anti-rat) (Dako) Specific labelling was detected with appropriate biotin-conjugated secondary antibodies and avidin-biotin peroxidase complex (Vector Laboratories, Burlingame, CA). The color reaction was developed with 3,3-diaminobenzidine (Dako) and sections were counterstained with hematoxylin.

Evaluation of all sections was performed by an experienced renal pathologist, who was unaware of the origin of the sections. K67i cells were quantitatively measured by counting 20 randomly selected highpower fields (HPF; 400×) per section in the cortex and outer medulla areas

Cytokines analysis in Kidney Tissue from Rat

Cytokine analysis. kidney samples containing 500 μg/ml proteins per sample were homogenized with cell lysis buffer (Cell Lysis Kit, Bio-Rad Laboratories, Hercules, CA) containing protease inhibitor cocktail (Bio-Rad) and 3_l of a stock solution containing 500mM phenylmethylsulfonyl fluoride in dimethyl sulphoxide both from Sigma, St. Louis, MO). Tissue was disrupted by drawing samples up and down through a 1 ml pipette tip (cut back to a 2mm opening) 20 times followed by orbital agitation for 20 min at 300 rpm and 4 ◦C. Tissue homogenates were centrifuged at 12,000 rpm for 15 min at 4°C, and supernatants were collected and used for the cytokine multiplex bead-based kit Bio-Rad Laboratories).

Total protein concentration was determined using a DC Protein Assay Kit (Bio-Rad) and spectrophotometer (SmartSpec 3000TM; Bio-Rad). All tissue samples were diluted with cell lysis buffer as needed to a final total protein concentration of 500 ug/ml.

Proteins assayed included measure 9 cytokines (proinflammatory cytokine : IL-1α, IL-1β, IL-2, IL-6, granulocyte monocyte colony stimulating factor GM-CSF, IFN-γ ,TNF-α and antiflammatory cytokine : IL-4 and IL-10 .Cytokine assay plate layout consisted of eight standards in duplicate (32,000 to 1.95 pg/ml), two blank wells (for background fluorescence subtraction), and each sample in duplicate wells. The cytokine multiplex assay uses microbead and flow-based protein detection system (Bio-Plex Suspension Array System, Bio-Rad Laboratories) based on the Luminex xMAP technology. In this quantitative assay, surfaces of fluorescence- coded microbeads were conjugated to specific antibodies directed against each cytokine or chemokine. Each fluorescence-coded microbead type was conjugated to one specific capture antibody and consequently one specific target analyte. Each supernatant sample was first incubated with a mixture of all microbead types for 90 min at room temperature. Samples were then washed, incubated with a mixture of secondary biotinylated detection antibodies also directed against each target for 30 min at room temperature, washed again, and incubated with a streptavidin-coupled phycoerythrin reporter system for 10 min at room temperature. After a final wash, the samples were resuspended in buffer and subjected to flow cytometric analysis. The Bio-Plex system instrument uses fluidics, laser excitation, fluorescence detection, and digital signal processing for individual scanning and microbead identification. Each bead taken from the sample was identified based on its internal fluorescence signature, and the phycoerythrin reporter signal associated with that bead was quantified. The data were analyzed using Bio-Plex Manager 3.0 software (Bio-Rad).

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) studies were performed on sections fixed in 10% formalyne. Sections were incubated with proteinasi K (20 µg/ml , washed and incubated with TUNEL solution containing terminal deoxynucleotidyltransferase (Boehringer Mannheim, Mannheim, Germany)and biotin-16-DUTP. Sections
were washed with stop/wash buffer followed by washing and incubation with avidin-biotin-complex (VECTOR laboratories Burlingame, CA) Antibody binding was visualized

using DAB chromogen (DAKO). The sections were counterstained with hematoxylin. All positive tubular epithelial cells in each section were counted at a magnification of 400× and related to the number of view fields per section.

Statistical Analysis

The main goals of statistical analysis were focused on

(i) the detection of significant trend for each one of the 32 endpoints;

(ii) the univariate and the multivariate comparison (also for intermediate class of endpoints)

a. between the 60 min. and the 30/3/30;

b. among the 90 min., the 30/3/30/3/30 and the 45/3/45;

c. between each one of the 30/3/30 the 30/3/30/3/30 and the 45/3/45 with the observed trend of 0-30-45 min.

As statistical methodology we proposed the application the Nonparametric Combination (NPC) of Dependent Permutation Tests. The importance of the permutation approach in resolving a large number of inferential problems is well-documented in the literature, where the relevant theoretical aspects emerge, as well as the extreme effectiveness and flexibility from an applicatory point of view[110 111 112].

The complexity of the experimental design and the relatively small sample size advised for the use of nonparametric statistics. The statistical analysis were conducted using the Nonparametric Combination Test methodology, which is an inferential multivariate nonparametric method which frees the researcher from the stringent assumptions of parametric methods and allows a more flexible analysis both in terms of specification of multivariate hypotheses and in terms of the nature of the variables involved. One of the most important advantages of applying nonparametric tests like permutation tests instead of traditional parametric tests such as t and F test is that permutation tests allow to relax the assumption of normality, which is not always satisfied in behavioural data. Moreover, when considering a multivariate and/or multistrata problem, this approach frees the researcher from the stringent assumptions required by parametric methods (like MANOVA for example), such as multivariate normality and linear regression relationships among variables, and allows a more flexible analysis by specifying both multivariate and univariate hypotheses[113]. One of the most relevant features of the NPC

Test is that it does not need a modelling for dependence among variables and it is not affected by the problem of loss of degrees of freedom when the number of variables is large compared to sample size. Furthermore, simulation studies have shown that with relatively small sample sizes, as in the context of life sciences, the power of permutation tests is quite similar to that of parametric tests when normality holds, but it can be considerably better when there are different distributions[114].

Considering a *k*-dimensional hypothesis testing problem, the NPC solution is processed in two steps: firstly, a suitable set of *k* unidimensional permutation tests, called partial tests, are defined. Each partial test examines the marginal contribution of any single response variable in the comparison made among phases or between groups. The second step is the nonparametric combination of dependent tests into one second order combined test, which is suitable for testing possible global differences between the multivariate distributions of two or more groups. When there is a stratification/classification variable, two combination levels are expected: the partial tests combination in a set of second order combined tests, within the stratum/class, and a further combination of the tests in a single third order combined test.

As far as the problem of detection of a significant trend of each endpoint is concerned, we considered the permutation approach as well. More in details, the method we applied can be referred to a special case of the so called stochastic ordering problem where the main goal is to find out, in a sequential set several samples, whether several possible treatment peaks there exist. The proposed solution involves testing for stochastic ordering of continuous variables and the nonparametric combination methodology. If the peak groups were known, then the trend alternative could be detected by combining together two partial tests for simple stochastic ordering alternatives. Since the seek of the peak is generally unknown, it can be detected by repeating the testing procedure for known peak to every group in the study. The global significance (i.e. the significant presence of a trend) is then evaluated by nonparametrically combining together the tests for known peak.

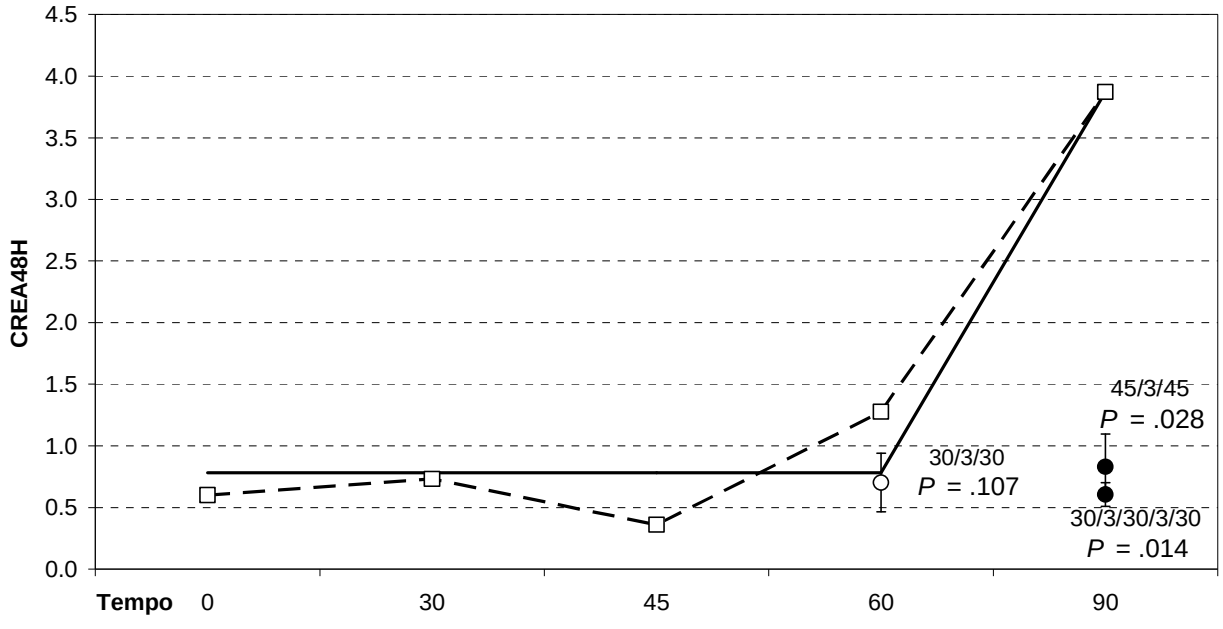
Results

All results are presented in table 2 express in term of permutation p-values when we compare the results of revascularized group to a trend work out from the non revascularized group for all the parameters analyzed.

Tab. 2 Permutation *p*-values by type of statistical analysis (abbreviation: IL, interleukin, IFN, interferon, TNF, tumor necrosis factor, GMCSF, granulocyte/macrophage colony stimulating factor, crea creatinine, Dila tubular dilatation, necro necrosis, mito mitosis, dismor dismorphism, strava haemorrhage, infilt neutrophil infiltration, apopto apoptosis, MIB proliferative index, pr proximal, ds distal)

Endpoint	Trend	Alternative hypothesis	60 min. vs 30/3/30	90 min. vs 30/3/30/3/30	90 min. vs 45/3/45
CREA48H	**.002**	<	**.099**	**.033**	**.014**
DILAPR	.184	<	.768	.999	.999
NECROPR	**.003**	<	**.059**	**.089**	.128
MITOPR	**.023**	>	.986	.789	.869
NECRDS	**.003**	<	.316	.715	.502
MITODS	**.007**	>	.983	.424	.885
DILADS	**.005**	<	.686	**.088**	.787
DISMOR	**.031**	<	.999	.718	.139
STRAVA	.709	<	.356	.702	**.056**
INFILT	**.005**	<	.233	.884	.502
APOPTO	**.034**	<	**.021**	.999	.678
MIB	**.008**	>	**.007**	**.033**	**.056**
IL-1 a	.121	≠	.952	.342	.124
IL-1 b	**.077**	≠	**.025**	.428	.408
IL-2	**.007**	≠	.862	**.087**	.543
IL-4	**.037**	>	.771	**.033**	.200
IL-6	**.005**	≠	.906	.103	.315
IL10	**.030**	>	**.007**	**.091**	**.075**
GMCSF	**.029**	≠	.500	.173	.625
IFN-ε	.460	≠	**.025**	.803	.402
TNF-α	**.087**	≠	**.061**	.362	.603

Fig. 17 – Creatinine values at 48 hours from ischemia.

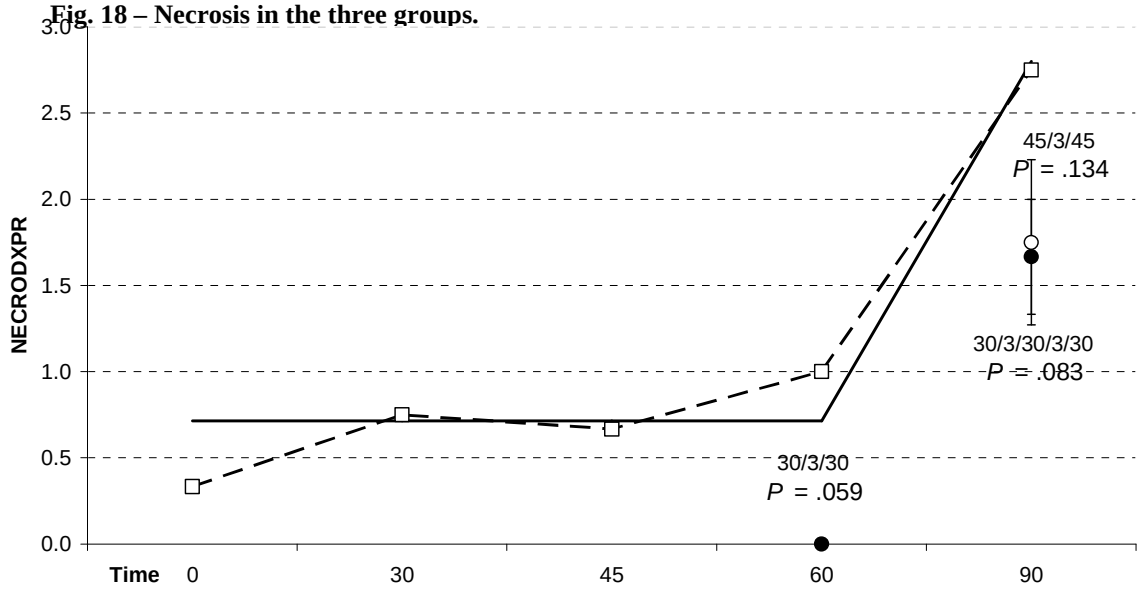


Animals that underwent renal ischemia with no reperfusion exhibited increase in the serum concentrations of creatinine when the clamping ischemia is more than 45 min, and this difference becomes strongly significant from a statistical point of view when the clamping time is more than 60 min suggesting a significant degree of glomerular dysfunction. In revascularized groups Creatinine serum level demonstrates no significant changes compared to the base pre-ischemic value regardless the pattern of revascularization performed as shown in Fig. 17 suggesting in this groups of animal that no glomerular dysfunction happens.

The histopathological analysis (Fig. 19, 20, 21, 22, 23 and 24) focused on the presence of necrosis, like shown in Fig. 18.. At 90 minutes the group of animal that underwent the short term reperfusion evidenced a statistically significant lower score if considered the 3 reperfusion after 30 min of ischemia ($p<0.01$). This difference is less significant for the group that underwent a single reperfusion after 45 min of ischemia.

In the group of animal that underwent a total ischemia of 60 minutes we have the same result with a statistically significant lower presence of necrosis in the group reperfused after 30 minutes of ischemia ($p<0.1$). Generally the section of kidneys obtained from animals that underwent no renal revascularization demonstrated the recognized features of severe acute tubular damage with a heavy presence of necrosis defined with swelling of cell organelles, loss of membrane integrity and rupture of cells. The short term revascularization technique helps preserving the normal morphology of the kidney. This fact is more evident if the total ischemic time is 60 min. When this time is prolonged to 90 min the benefit, even if still present, is less evident.

Fig. 18 – Necrosis in the three groups.



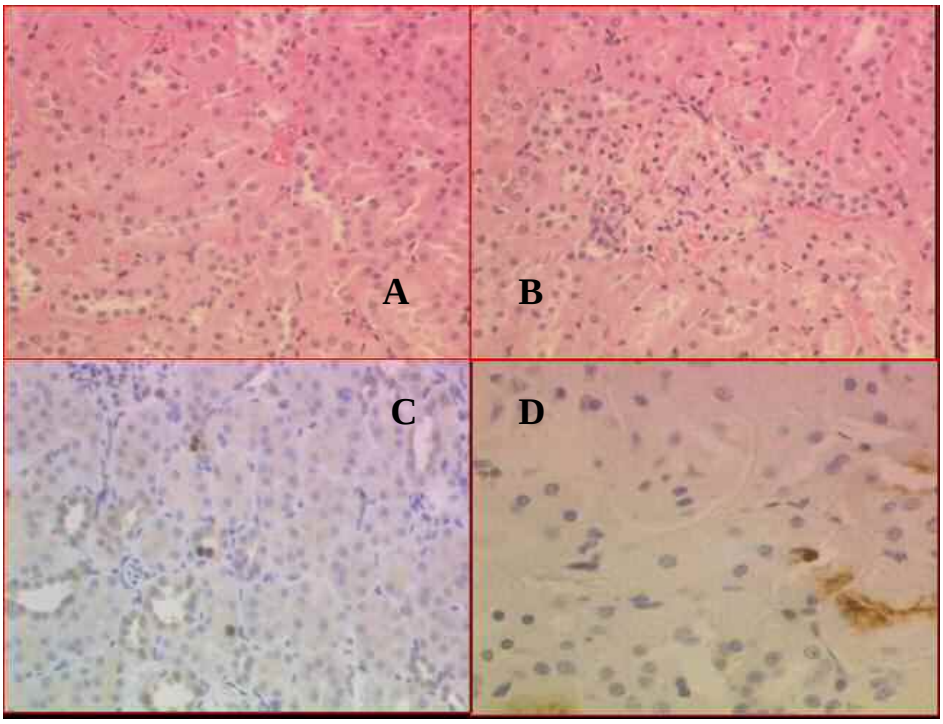


Fig. 19 - Sham operated. Kidney without pathological abnormalities (20x A,B). Proliferative cells Mib1 (20x C) and apoptosis (in situ hybridization TUNEL 40x , D) are present normaly in a small percentage of epithelium.

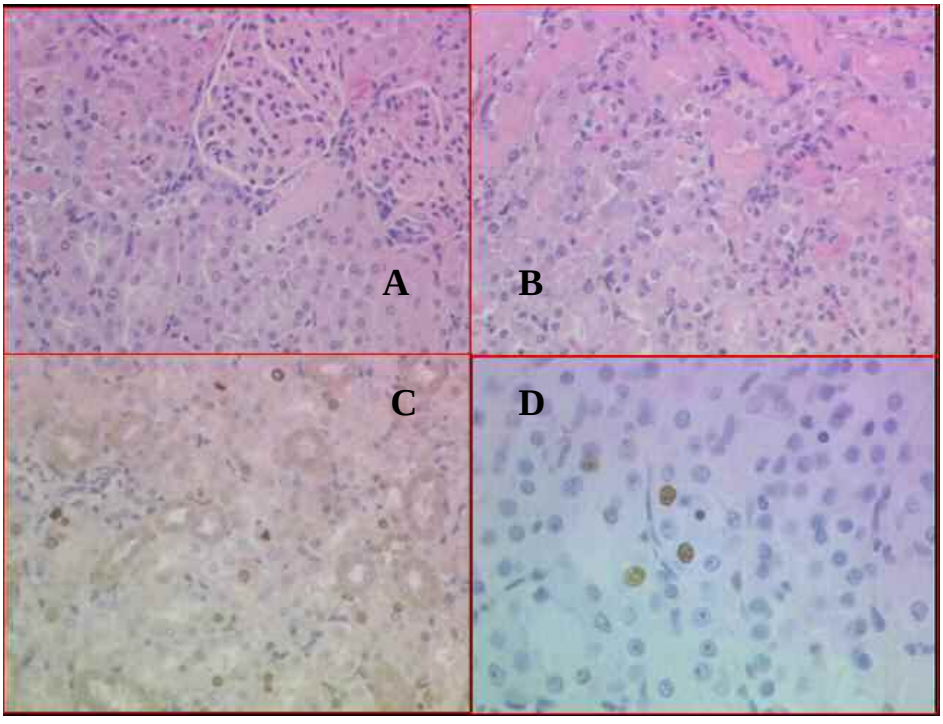


Fig. 20 – Time of kidney ischemia 60 min. A(10x) and B(20x) show initial tubular necrosis mainly in proximal tubuli. Proliferative index (MIB1) and apoptotic celles (TUNEL) are increased (20xC, 40x D)

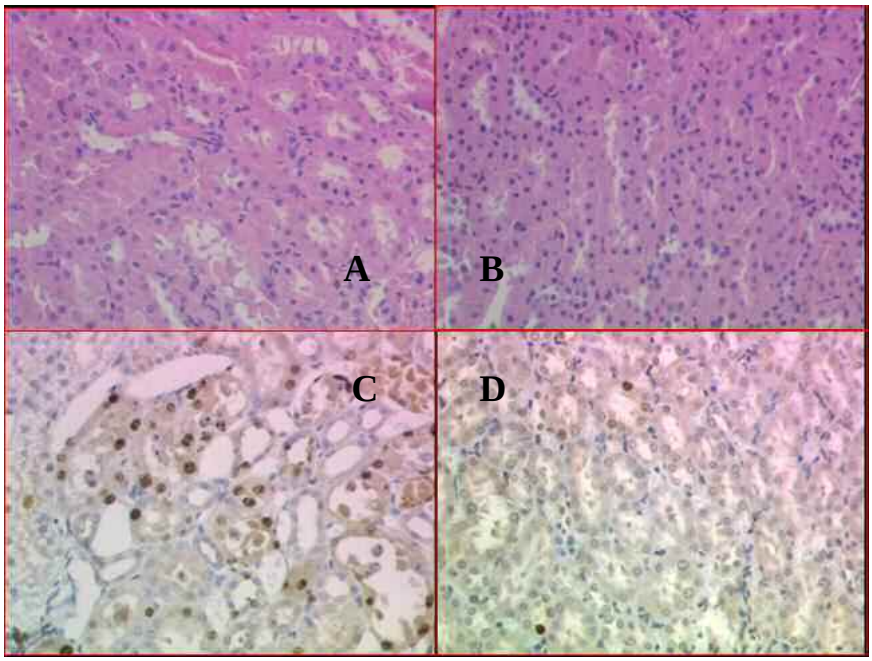


Fig. 21 -Time of ischemia 60 minutes with 3 minutes of reperfusion (30'-3'-30'). Histological evidence of small areas of tubular necrosis (20x A,B). Proliferative index (20x C) and apoptotic cells are more increased in relation to both sham and 60 minutes ischemia (40x D)

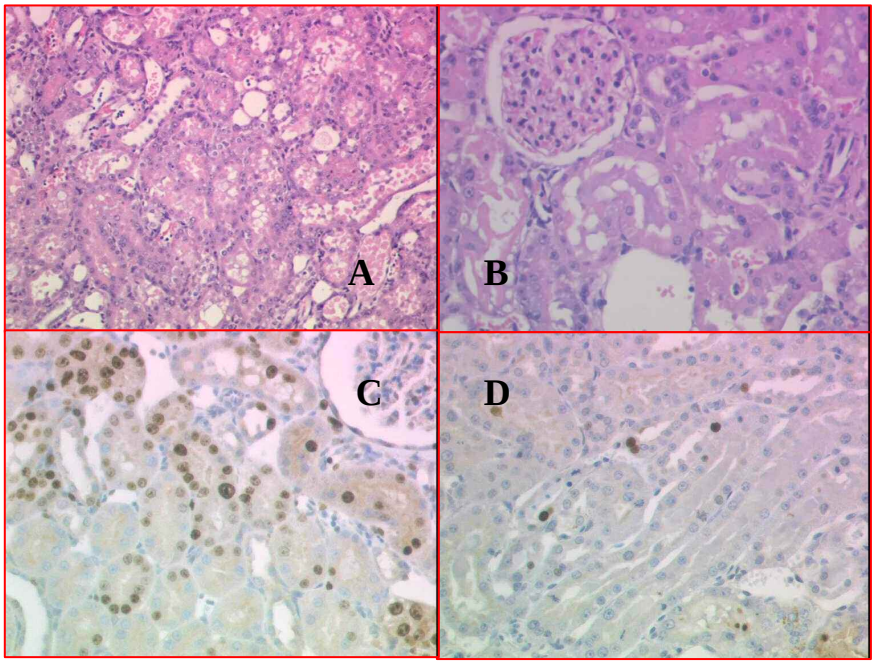


Fig. 22 - Time of kidney ischemia is 90 min. Evidence of extensive tubular necrosis (10xA, 40x B) without an increase of proliferative index (40x C) and of apoptosis (40x D)

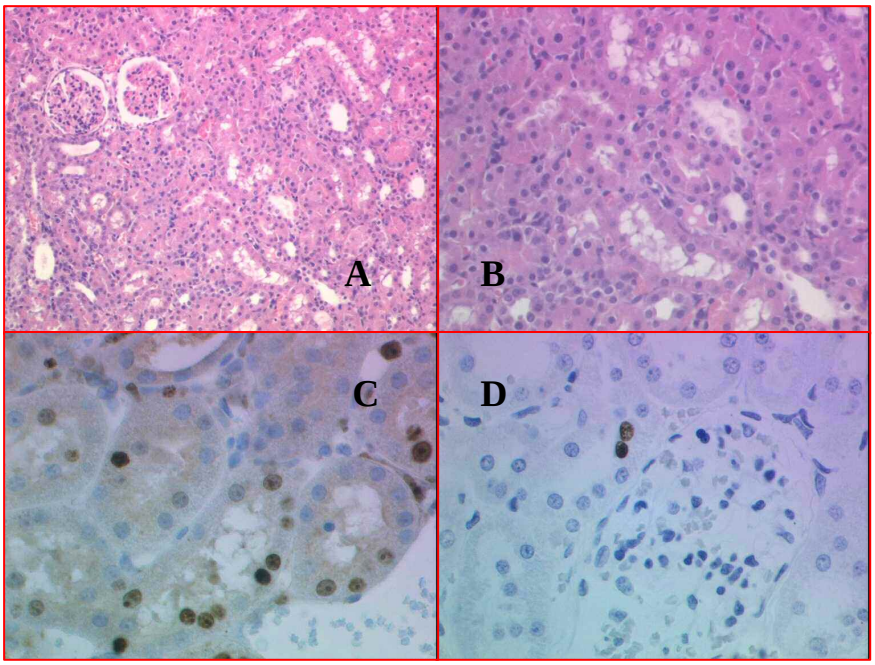


Fig. 23 - Time of ischemia 90 minutes with 3 minutes of reperfusion (45'-3'-45'). Tubular necrosis and tubular dilation decrease respect to kidney with 90' of ischemia (10x A, 20x B). Mild increase in proliferation index and number of apoptotic cells (40x C,D)

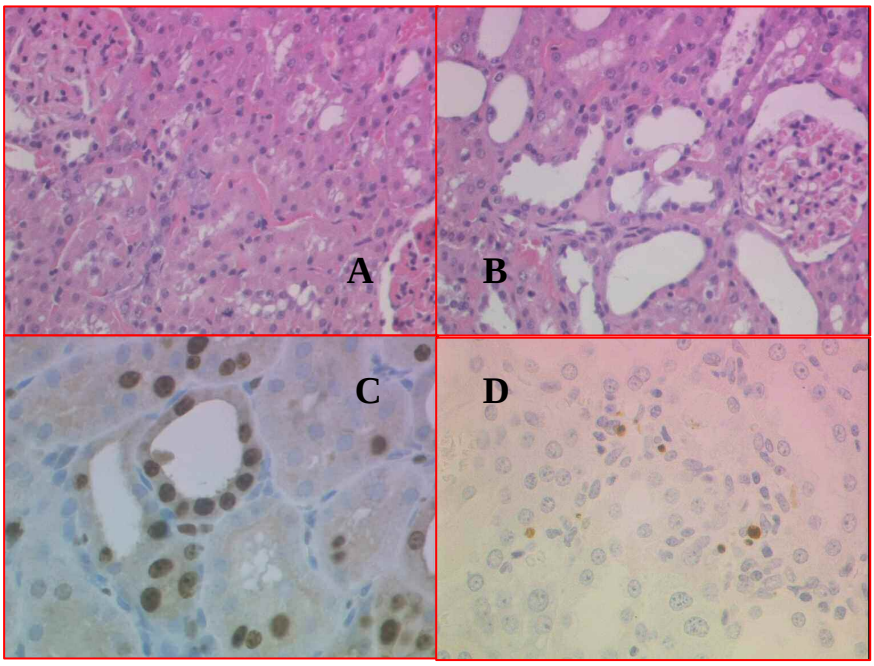


Fig. 24 - Time of ischemia 90 minutes with 6 minutes of reperfusion (30'-3'-30'-3'-30'). Histological features are not different from kidney with ischemia-reperfusion damage of 3 minutes reperfusion (20x A,40x B). We observed a significant increase of proliferative index (40x C) and apoptotic cell number (40x D).

The short term revascularization technique induces a significant increase in the count of proliferating cells as shown in the graphic (Fig.25). This increase is more evident in the group that underwent a double reperfusion (p=.056) when compared to the single reperfusion (p=.033). When the groups exposed to a 60 minutes of ischemia are compared the same results are present demonstrating that the reperfusion technique stimulate, regardless the ischemia time, a proliferative response.

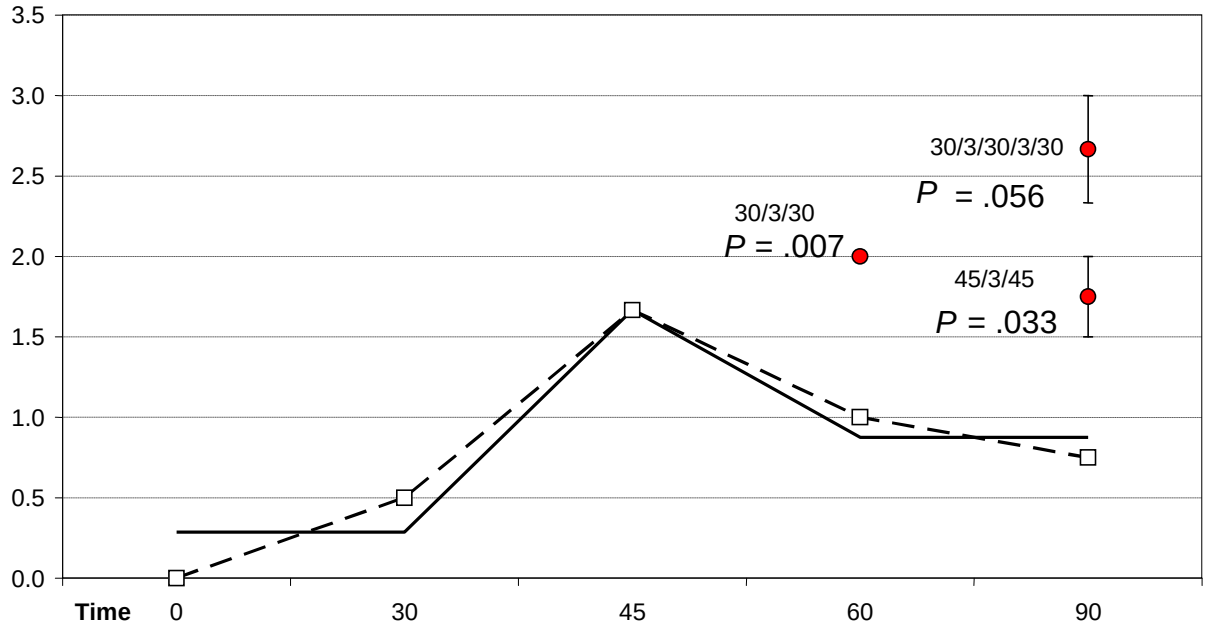
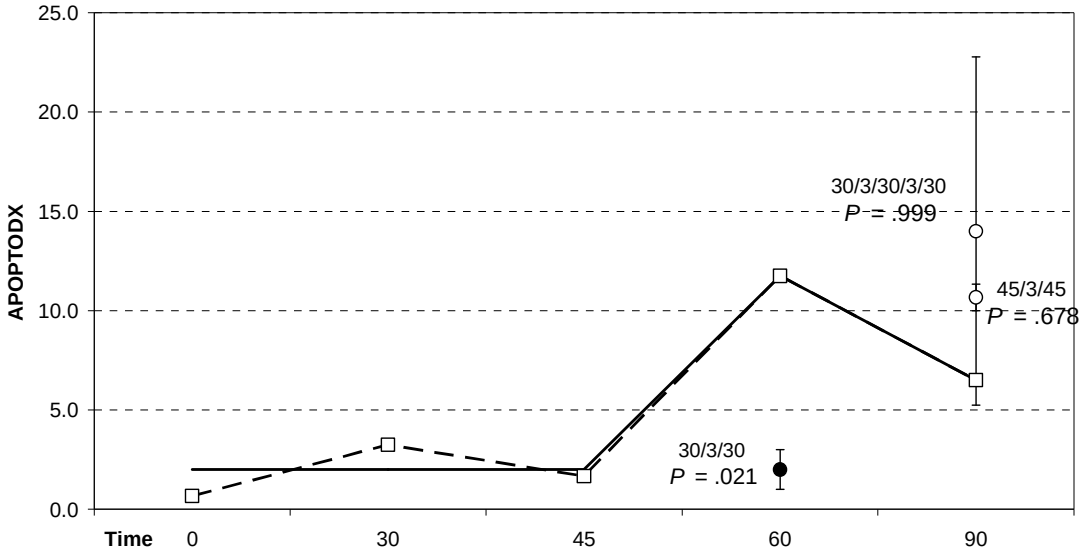


Fig. 25 - Significant increase in the count of proliferating cells with short term revascularization technique

In apoptosis cells shrink, lose microvilli and cell junctions and explode into a series of membrane. At 48-hours post-ischemia, there was a significant decrease in the number of TUNEL positive cells in the group that underwent a single reperfusion with a total ischemic time of 60 min. No significant difference was present in the groups that underwent a total ischemic time of 90 min regardless the pattern of reperfusion (Fig. 26).

Fig. 26 - Apoptosis at 48 hours compared between the three groups



legend: □ apoptotic cells values in non reperfused rats;
○ apoptotic cells in reperfused rats

Cytokine Measurements *by multiplex suspension array*

A total of 29 sample of renal tissue were evaluated. We analyzed cytokine expression by suspension bioplex array evaluating the of IL1α-IL1β-IL2-IL4-IL10-TNFα-INFγ-GMcsf .

In our measurements cytokines panel are often categorized as principally either pro-inflammatory (i.e., IL-1α, IL-1β, IL-2, IL-6, GM-CSF, IFN-γ, and TNF-α) or anti-inflammatory (i.e., IL-4 and IL-10) mediating signalling molecules (Oppenheim and Feldmann, 2001). Our intent here is not to detail mechanisms for their actions in renal tissue. Instead, as with other cytokines, these 9 cytokines are individually pleiotropic and variably pleiotropic in combination with other cytokines. Thus, their simultaneous quantification from individual samples was used as an example of the potential utility of multiplexed proteomic measurements.

Renal tissue levels of GM-CSF was too low to measure.

The only statistically significant difference that we noticed regards IL10 whose value is increased in all the groups of animal that are reperfused (Fig. 27), IL4 that is increased in the group that underwent 90 minutes of ischemia with 3 reperfusion after 30 minutes (Fig.

28), IL 2 that is overexpressed in the group that underwent 90 minutes of ischemia with 3 reperfusion (Fig. 29). IL1β, TNFα, INFγ are increased in the group of animal that underwent 60 minutes of ischemia with a single reperfusion (Fig. 30 a-b-c).

Even if there is no statistically significance we noticed a trend in all cytokine expression to return to basal value at 90 minutes in the group that underwent the reperfusion.

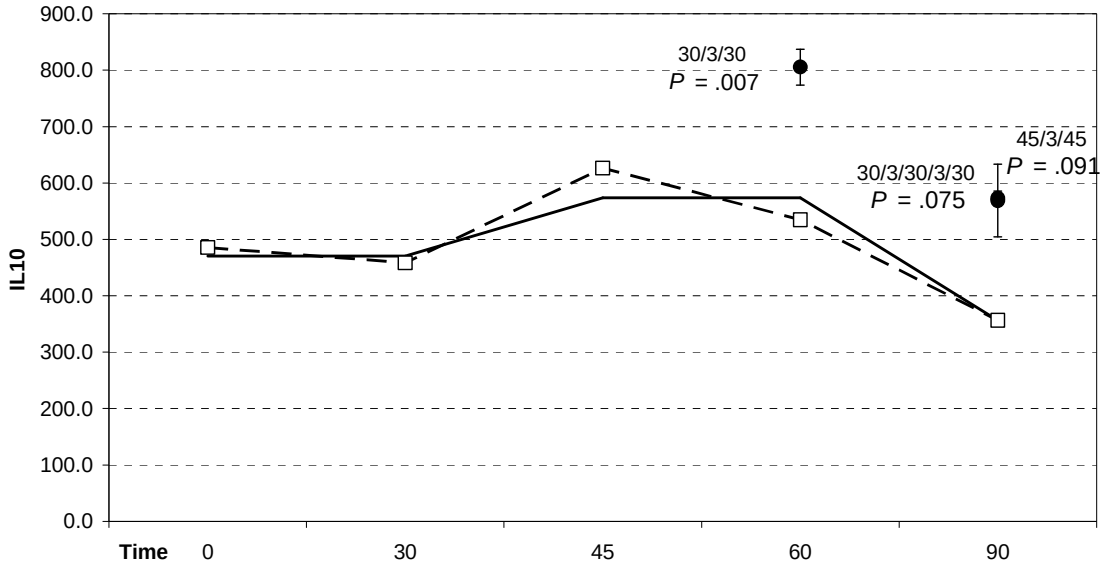


Fig. 27 - Statistically significant difference regards IL10 whose value is increased in all the groups of animal that are reperfused

legend: □ IL values in non reperfused rats;

o IL values in reperfused rats

Fig. 28 - IL4 increased in the group that underwent 90 minutes of ischemia with 3 reperfusion after 30 minutes

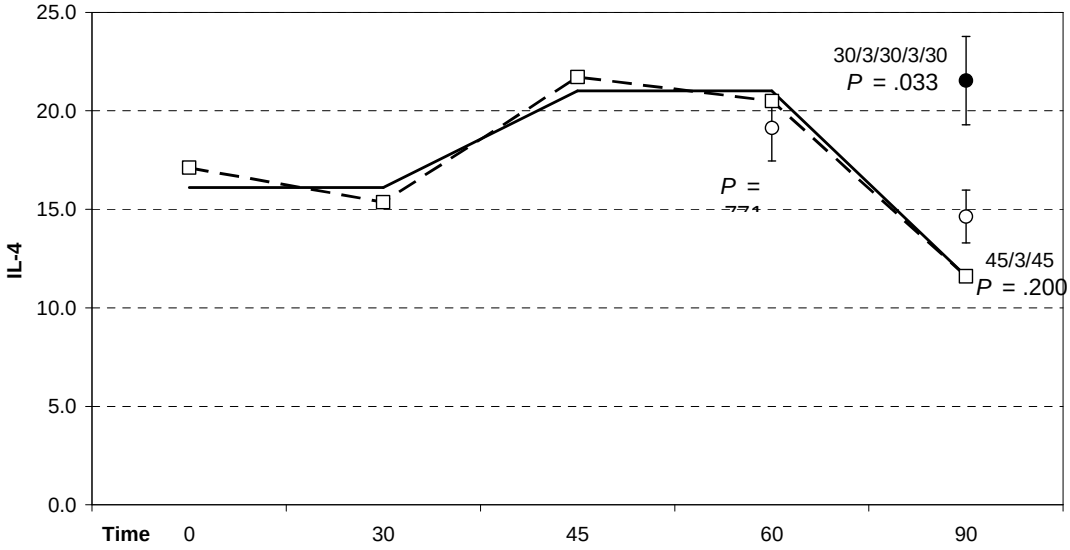
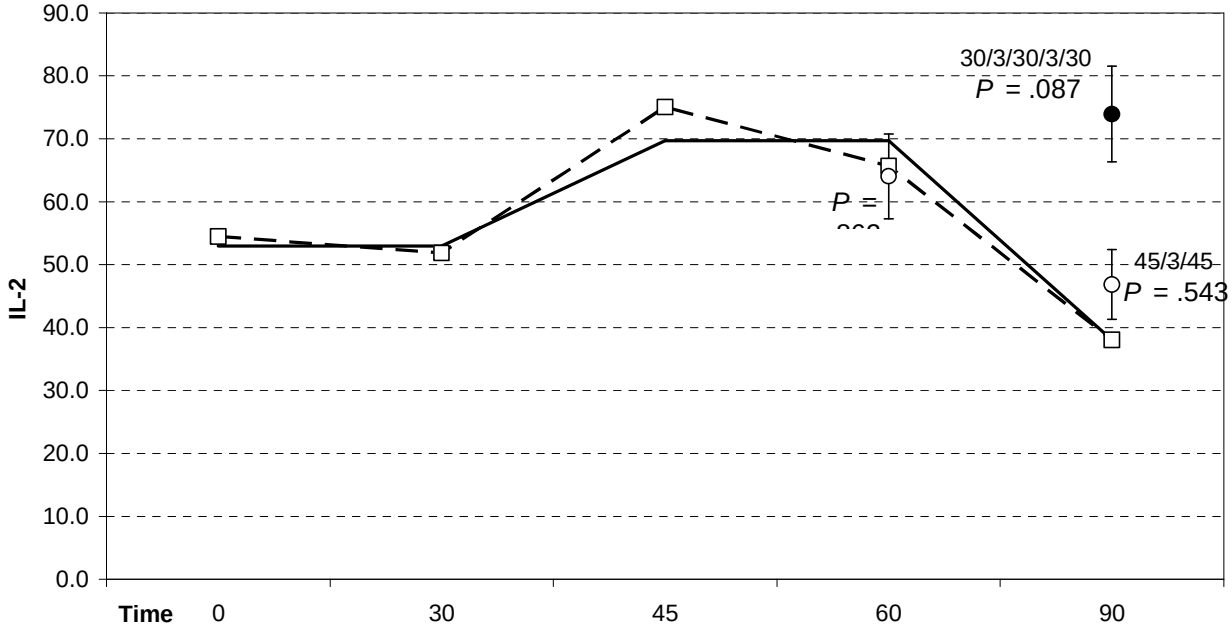
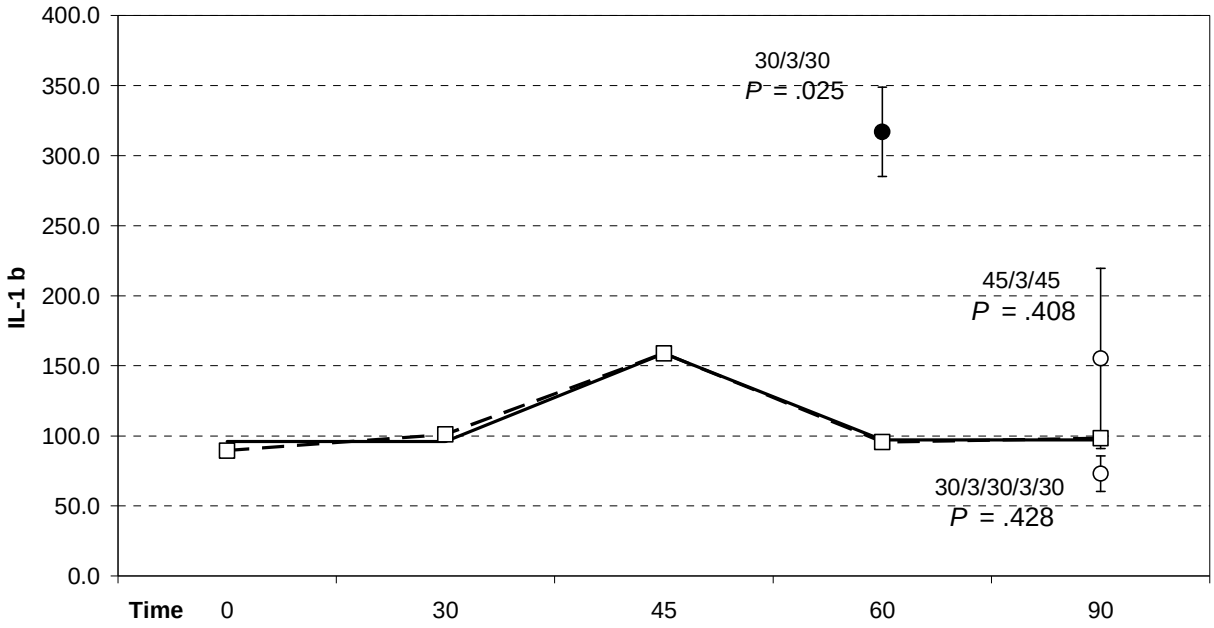


Figura 29 IL 2 is overexpressed in the group that underwent 90 minutes of ischemia with 3 reperfusion



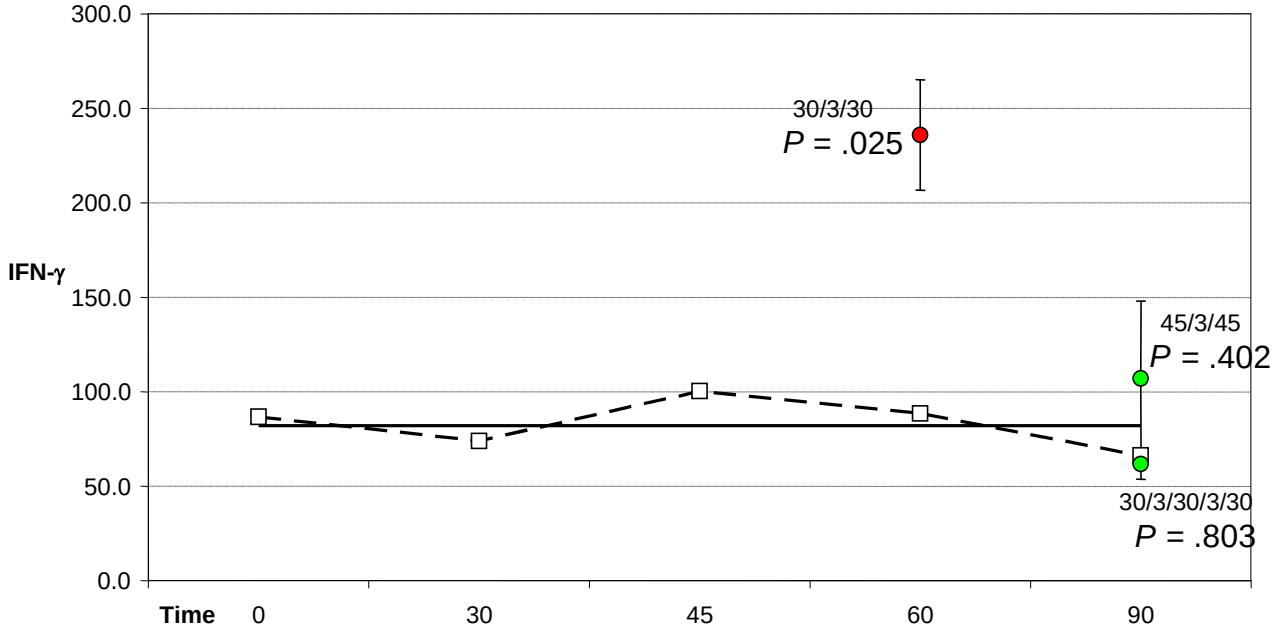
legend: □ IL values in non reperfused rats;
o IL values in reperfused rats

Figura 30 a. - IL1β increased in the group of animal that underwent 60 minutes of ischemia with a single reperfusion



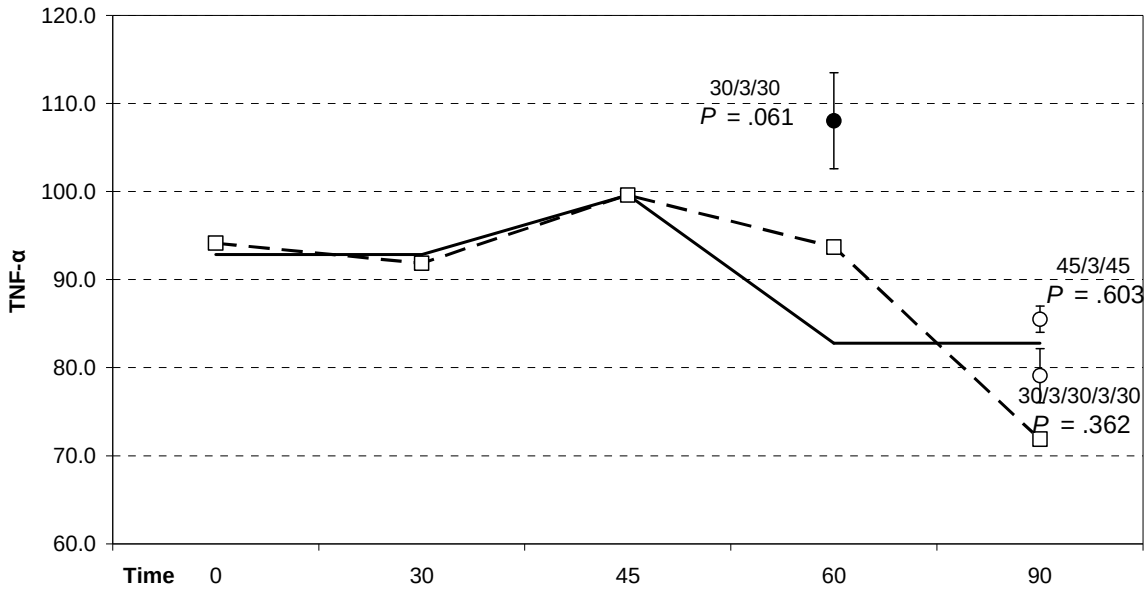
legend: □ IL values in non reperfused rats;
o IL values in reperfused rats

Fig. 30 b. - INFγ increased in the group of animal that underwent 60 minutes of ischemia with a single reperfusion



legend: □ IL values in non reperfused rats;
o IL values in reperfused rats

Fig. 30 c. - TNFα increased in the group of animal that underwent 60 minutes of ischemia with a single reperfusion



legend: □ IL values in non reperfused rats;
oIL values in reperfused rats

Discussion

The patho-physiology following renal I/R injury is not well established. All the surgical techniques and pharmacological tools reducing the risk of acute renal failure employed until now, have not demonstrated any convincing result . The choice is still dependent more on surgeon's habits and personal confidence on the different technique adopted than on scientific results.

The crucial role of inflammation and the immune response in the pathogenesis of ischemic ARF are well established, with many pro-inflammatory cytokines/chemokines being up-regulated in the kidney. Most cytokines show autocrine and/ or paracrine action and a few of them exhibit endocrine action. Ischemia reperfusion-induced acute kidney injury causes the synthesis of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α.

In my PhD thesis we used for the first time an in vivo animal model of repeated ischemia reperfusion injury with peculiar time frames for ischemia-reperfusion to reduce injury and to expand the time for surgical manoeuvre. At difference with previous experiments we compared the control group of animal that underwent a renal ischemia of 60 minutes with a group of animal that underwent two period of 30 minutes of ischemia with 3 minutes of blood reperfusion for a total ischemic time of 60 minutes, and a group of control animal that underwent a renal ischemia of 90 minutes with 2 groups of animals that underwent a total ischemic time of 90 minutes but in one group with a reperfusion of 3 minutes after 45 minutes of ischemia and the second group with periods of 30 minutes of ischemia followed by 3 minutes of reperfusion. The aim is to assess whether this technique is feasible and to identify which is the best pattern of reperfusion to be applied and to transfer these results in human.

The principle on which we based our study was that a short period of revascularization (3 minutes) delivered before the onset of the irreversible ischemic damage, is able to block the cascade of events leading to kidney damage. This idea was supported by our clinical experience on patients operated with this technique. In a large series of patients operated for TAAA with the short term arterial blood reperfusion scheme we had no development of renal dysfunction based on serum creatinine assessment.

However at the moment no patho-physiological data were available on our model of repeated ischemia-reperfusion.

In this study we compared different aspects of renal injuries to correlate the functional parameter (serum creatinine), morphological parameters on histological sample

(neutrophil infiltration, tubular necrosis, tubular dilatation); on immuno-histochemistry (evaluation of proliferation with Ki67) and molecular parameter (evaluation of apoptosis with terminal dUTP nick end labelling ,TUNEL), and cytokine expression. With the introduction of multiplex suspension array, multiple analyses can be quantified simultaneously and rapidly in individual sample. This technique has better reproducibility and sensitivity than the traditional ELISA) .

In our study, considering creatinine serum dosage at 48 h, when the animals were sacrificed, animals subjected to brief period of renal reperfusion (3 or 3+3 min) didn't developed any clinical renal impairment regardless the total ischemic time (60 or 90min) with creatinine in the range of normality. Our functional data were clearly supported by the morphological results. In fact when total ischemic time is 60 minute plus 3 min of reperfusion the histological alterations appeared to be minimal and very close to those presented in the animals that underwent the shame operation with less necrotic index, less apoptosis and more proliferation index. When the total ischemic time is prolonged to 90 minutes (30+3+30+3+30 and 45+3+45) even if no functional alteration was present histopathological analysis showed a moderate renal injury still demonstrating a protective effect of reperfusion on the ischemic damage. The time period at which we are able to detect morphological irreversible injury is 60 min. The histological analysis of the kidneys of rats that underwent IRI revealed few hyperaemic and necrotic areas with dilated tubules and a background of mildly interstitial inflammatory cells at difference with the total ischemic time of 90 minutes which showed necrosis of tubular epithelial cells and the presence of tubular casts as the most severe alterations, with large and irregular confluent areas of parenchymal hemorrhage and necrosis. The reperfusion technique is able to start a global proliferative process which is considered to be the prelude to parenchimal repair as reported by other studies with different time frames[115]. This positive protective effect seems to be related to the number of reperfusion performed in agreement with our results which showed that there was a statistically significant difference between the two groups of animals with double (30+3+30+3+30) and single reperfusion (45+3+45).

The accurate and reproducible measurement of cytokines in serum, plasma, or tissue culture supernatants is important for studies involving disease pathogenesis, treatment, and prognosis. Cytokines can be measured by a variety of assay formats, including enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, bioassays, and mass spectrometry. In recent years, multiplexed assays have been developed that are

capable of simultaneously measuring multiple cytokines in a variety of matrices. These multiplex assays have the advantage of requiring small sample volumes and providing a quick turnaround time for analysis. Multiplex assays have been based primarily on bead-based Luminex-type platforms, which utilize antibodies applied as a coating on microbeads in suspension to detect the target cytokines. The development of plate-based multiplex assays is a more recent technology. The present study was designed to examine 9 kit cytokines (IL 1α, 1IL1β, IL2, IL4, IL6, IL10, GM-CSF, IFM-γ ,TNF-α) in renal tissue after different patterns of ischemia reperfusion[116 117]. The inflammatory response activated by the reperfusion seems to move toward a protective global effect. Pro inflammatory (IL-1β, IL-2, IL-6, GM-CSF, IFM-γ ,TNF-α:) and anti-inflammatory(IL-10, IL-4) cytokines are expressed in all the different groups of animals after ischemia and after ischemia and reperfusion. Both pro and anti inflammatory cytokines are over-expressed in animals that underwent a total ischemic time of 60 minutes with a single reperfusion (30+3+30), compared to the group without reperfusion and with those with ischemia of 90 min (30+3+30+3+30; 45+3+45). After ischemia and reperfusion there is a prevalence of anti-inflammatory cytokine expression that is able to spare healthy tissue (IL-10, IL-4). At 90 minutes of total ischemic time plus reperfusion (30+3+30+3+30; 45+3+45) there is a trend in reduction of cytokine compared to the higher expression detected at 60 min (30+3+30) probably as a result of the loss of healthy parenchyma that we noticed in all groups at 90 minutes. Moreover also in the 90 minutes ischemia and reperfusion there is a prevalence of anti-inflammatory cytokines (IL-10, IL4) on the pro-inflammatory ones . Loverre et al. report that I-R injury in kidney is characterized by two main features: apoptosis of tubular cells and interstitial inflammation and reparative cell regeneration. Pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α can affects renal tubular function by inducing apoptotic cell death. Maximum cell regeneration occurred during phases in which an anti-inflammatory environment (represented by maximum levels of IL-10) prevailed. In accordance with these results we have detected that increases in the proliferative markers are concomitant with significant increases in the anti-inflammatory cytokine IL-10 that is clearly stimulated by reperfusion. Even though the ischemia reperfusion-induced acute kidney injury causes the synthesis of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α which produce apoptotic cell death together with anti inflammatory cytokines (IL10-IL4) which enhance the proliferation response, the balance is in favour of the anti-inflammatory cytokines.

The results of my PhD thesis showed that the functional, morphological and molecular results are congruent. At 60 min of ischemia there is an irreversible damage to the kidney with an increased creatinine level, necrosis and apoptosis, low proliferative response and increase in cytokines expression especially of pro-inflammatory cytokines (IL-1-β, IL-2, IL-6, TNF-α and TNF-α). With ischemia-reperfusion (30-3-30) we obtain a less ischemic damage, a more pronounced proliferative response and anti-inflammatory cytokines over-expression (IL-10, IL-4) compared to ischemia alone. The balance was in favour of the protective role of reperfusion. With the ischemic-reperfusion model of 90 min of ischemia and 6 minutes reperfusion (30-3-30-3-30) we still observed the beneficial role of reperfusion since there is a reduction of necrosis, apoptosis and increasing in proliferation activity and at the end a positive ratio in favour of anti-inflammatory cytokines (IL-10). In the setting of 90 min ischemia and 3 min reperfusion, (45-3-45) we observed a similar ischemic damage in term of necrosis and apoptosis and a less proliferative index and the ratio between pro and anti inflammatory was still in favour of anti inflammatory cytokines. However the decrease of pro inflammatory cytokine TNFa was less evident, thus counterbalancing more actively the protective role of anti inflammatory cytokine IL10. Our methods of cytokines detection confirm the utility of bead-based immunoassays to provide sensitive, accurate and reproducible measurement of nine exemplary cytokine proteins from rat renal tissues. Such informations may be useful in understanding the pathobiology of ischemia reperfusion[118].

We can state that according to our results the best model would be that of 30-3-30-3-30 which means less ischemic damage and more reperfusion benefits.

References

[1] R.B. Griepp, M.A. Ergin and J.D. Galla *et al.*, Natural history of descending thoracic and thoracoabdominal aneurysms, *Ann Thorac Surg* 67 (1999), pp. 1927–1930.

[2] L.G. Svensson, E.S. Crawford, K.R. Hess, J.S. Coselli and H.J. Safi, Experience with 1509 patients undergoing thoracoabdominal aortic operations, *J Vasc Surg* 17 (1993), pp. 357–370

[3] J.S. Coselli, L.D. Conklin and S.A. LeMaire, Thoracoabdominal aortic aneurysm repair: review and update of current strategies, *Ann Thorac Surg* 74 (2002), pp. S1881–S1884

[4] H.J. Safi, C.C. Miller 3rd and M.S. Subramaniam *et al.*, Thoracic and thoracoabdominal aortic aneurysm repair using cardiopulmonary bypass, profound hypothermia, and circulatory arrest via left side of chest incision, *J Vasc Surg* 28 (1998), pp. 591–598

[5] McCord JM. Oxigen-derived free radicals in post-ischemic tissue injury.N. Engl.Jour. Med.(1985);312:159-63

[6] N. Singri, S.N. Ahya and M.L. Levin, Acute renal failure. *JAMA* 289 (2003), pp. 747–751.

[7] C.A. Schmidt, M.N. Wood, K.A. Gan and A.J. Razzouk, Surgery for thoracoabdominal aortic aneurysms. *Am Surg* 56 (1990), pp. 745–748

[8] L.M. Hollier, S. Monet, T. Naslund, D.C. Procter, W.C. Buhman and R.J. Marino, Risk of spinal cord dysfunction in patients undergoing thoracoabdominal aortic replacement. *Am J Surg* 164 (1992), pp. 210–214

[9] V.S. Kashyap, R.P. Cambria, K. Davison and G.J. Renal failure after thoracoabdominal aortic surgery. *J Vasc Surg* 26 (1997), pp. 949–957

[10] Kellen M, Aronson S, Roizen MF, Barnard J, Thisted RA. Predictive and diagnostic tests of renal failure: a review. *Anesthesia and Analgesia* (1994);78:134-42

[11] Welch M, Newstead CG, Smyth JV, Dodd PD, Walker MG. Evaluation of dopexamine hydrochloride as a renoprotective agent during aortic surgery. *Annals of Vascular Surgery* (1995);9:488-92

[12] Lassnigg A, Donner E, Grubhofer G, Presterl E, Drubl W, Hiesmayr M. Lack of renoprotective effects of dopamine and furosemide during cardiac surgery. *Journal of American Society of Nephrology* (2000);11:97-104.

[13] Wahbah AM, el-Hefny MO, Wafa EM, el-Kharbotly W, el-Enin AA, Zaglol, A, et al.Perioperative renal protection in patients with obstructive jaundice using drug combinations. *Hepato-gastroenterology* (2000);47:1691-4.

[14] J.S. Coselli and S.A. LeMaire, Left heart bypass reduces paraplegia rates after thoracoabdominal aortic aneurysm repair, *Ann Thorac Surg* 67 (1999), pp. 1931–1934

[15] H.J. Soukiasian, S.S. Raissi and T. Kleisli *et al.*, Total circulatory arrest for the replacement of the descending and thoracoabdominal aorta, *Arch Surg* 140 (2005), pp. 394–398.

[16] McCord JM, Oxygen-derived free radicals in post-ischemic tissue injury. N Engl J Med (1985) 312:159–163

[17] Anaya-Prado R, Toledo-Pereyra LH, Lentsch AB, Ward PA Ischemia/reperfusion injury. J Surg Res (2002) 105:248–258

[18]Grace PA Ischaemia-reperfusion injury. Br J Surg (1994) 81:637–647

[19] Anaya-Prado R, Toledo-Pereyra LH, Lentsch AB, Ward PA Ischemia/reperfusion injury. J Surg Res (2002) 105:248–258

[20] McCord JM Oxygen-derived free radicals in post-ischemic tissue injury. N Engl J Med (1985) 312:159–163

[21] Zimmerman BJ, Granger DN Reperfusion injury. Surg Clin North Am(1992) 72:65–83

[22] Ar'Rajab A, Dawidson I, Fabia R Reperfusion injury. New Horiz (1996) 4:224–234

[23] Homer-Vanniasinkam S, Crinnion JN, Gough MJ Post-ischaemic organ dysfunction: a review. Eur J Vasc Endovasc Surg (1997) 14:195–203

[24] Granger DN, Hollwarth MA, McCord JM Ischemia reperfusion injury: role of oxygen-derived free radicals. Acta Physiol Scand (1986) 548:47–63

[25] Bodwell W Ischemia, reperfusion and reperfusion injury: role of oxygen free radicals and oxygen free radical scavengers. J Cardiovasc Nurs (1989) 4:25–32

[26] Kloner RA, Przyklenk K, Whittaker P Deleterious effects of oxygen radicals in ischemia/reperfusion. Resolved and unresolved issues. Circulation (1989) 80:1115–1127

[27] Toledo-Pereyra LH, Lopez-Neblina F, Toledo AH Reactive oxygen species and molecular biology of ischemia/reperfusion. Ann Transplant (2004) 9:81–83

[28] Bonventre JV Mechanisms of ischemic acute renal failure. Kidney Int (1993) 43:1160–1178

[29] Paller MS The cell biology of reperfusion injury in the kidney. J Invest Med (1994) 42:632–639

[30] Weight SC, Bell PR, Nicholson ML Renal ischemia-reperfusion injury. Br J Surg (1996) 83:162–170

[31] Versteilen AM, Di Maggio F, Leemreis JR, Groenveld AB, Musters RJ, Sipkema P Molecular mechanisms of acute renal failure following ischemia/reperfusion. Int J Artif Organs (2004) 27:1019–1029

[32] Thadhani R, Pascual M, Bonventre JV Acute renal failure. N Engl J Med (1996) 334:1448–1460

[33] Gilbert RE, Kelly DJ, Atkins RC Novel approaches to the treatment of progressive renal disease. Curr Opin Pharmacol (2001) 1:183–189

[34] McCombs PR, Roberts B Acute renal failure following resection of abdominal aortic aneurysm. Surg Gynecol Obstet (1979) 148:175–178

[35] Troppmann C, Gillingham KJ, Benedetti E, Almond PS, Gruessner RW, Najarian JS, Matas AJ Delayed graft function, acute rejection, and outcome after cadaver renal transplantation. The multivariate analysis. Transplantation (1995) 59:962–968

[36] Aronson S, Blumenthal R Perioperative renal dysfunction and cardiovascular anesthesia: concerns and controversies. J Cardiothorac Vasc Anesth (1998) 12:567–586

[37] Zanardo G, Michielon P, Paccagnella A, Rosi P, Calo M, Salandin V, Da Ros A, Michieletto F, Simini G Acute renal failure in the patient undergoing cardiac operation. J Thorac Cardiovasc Surg (1994) 107:1489–1495

[38] Chertow GM, Levy EM, Hammermeister KE, Grover F, Daley J Independent association between ARF and mortality following cardiac surgery. Am J Med (1998) 104:343–348

[39] Lameire N, Van Biesen W, Vanholder R Acute renal failure. Lancet (2005) 365:417–430

[40] Gilbert RE, Kelly DJ, Atkins RC Novel approaches to the treatment of progressive renal disease. Curr Opin Pharmacol (2001) 1:183–189

[41] Murphy TP, Rundback JH, Cooper C, Kiernan MS Chronic renal ischemia: implications for cardiovascular disease risk. J Vasc Interv Radiol (2002) 13:1187–1198

[42] Rundback JH, Murphy TP, Cooper C, Weintraub JL Chronic renal ischemia: pathophysiologic mechanisms of cardiovascular and renal disease. J Vasc Interv Radiol (2002) 13:1085–1092

[43] Venkatachalam MA, Bernard DB, Donohue JF, Levinsky NG Ischemic damage and repair in the rat proximal tubule. Differences among the S_1, S_2 and S_3 segments. Kidney Int (1978) 14:31–49

[44] Sheridan AM, Bonventre JV Cell biology and molecular mechanisms of injury in ischemic acute renal failure. Curr Opin Nephrol Hypertens (2000) 9:427–434

[45] Masztalerz M, Wlodarczyk Z, Czuczejko J, Slupski M, Kedziora J Superoxide anion as a marker of ischemia-reperfusion injury of the transplanted kidney. Transplant Proc (2006) 38:46–48

[46] Paller MS The cell biology of reperfusion injury in the kidney. J Invest Med (1994) 42:632–639

[47] Weight SC, Bell PR, Nicholson ML Renal ischemia-reperfusion injury. Br J Surg (1996) 83:162–170

[48] Cecka JM, Cho YW, Terasaki PI Analyses of the UNOS scientific renal transplant registry at 3 years—Early events affecting transplant success. Transplantation (1992) 53:59–64

[49] Troppmann C, Gillingham KJ, Benedetti E, Almond PS, Gruessner RW, Najarian JS, Matas AJ Delayed graft function, acute rejection, and outcome after cadaver renal transplantation. The multivariate analysis. Transplantation (1995) 59:962–968

[50] Shoskes DA, Halloran PF Delayed graft function in renal transplantation: etiology, management and long-term significance. J Urol (1996) 155:1831–1840

[51] Awad AS, Ye H, Huang L, Li L, Foss FW Jr, Macdonald TL, Lynch KR, Okusa MD Selective sphingosine 1-phosphate 1 receptor activation reduces ischemia-reperfusion injury in mouse kidney. Am J Physiol Renal Physiol (2006) 290:F1516–F1524

[52] Hassoun H, Grigoryev DN, Lie M, Liu M, Cheadle C, Tuder RM, Rabb H Ischemic acute kidney injury induces a distant organ functional and genomic response distinguishable from bilateral nephrectomy. Am J Physiol Renal Physiol (2007) 204:921-925

[53] Nath KA, Norby SM Reactive oxygen species and acute renal failure. Am J Med (2000) 109:655–678

[54] Nose K Role of reactive oxygen species in the regulation of physiological functions. Biol Pharm Bull (2000) 23:897–903

[55] Chatterjee PK, Cuzzocrea S, Brown PAJ, Zacharowski K, Stewart KN, Mota-Filipe H, Thiemermann C Tempol, a membrane-permeable radical scavenger, reduces oxidant stress-mediated renal dysfunction and injury in the rat. Kidney Int (2000) 58:658–673

[56] Greene EL, Paller MS Oxygen free radicals in acute renal failure. Miner Electrolyte Metab (1991) 17:124–132

[57] Venkatachalam MA, Bernard DB, Donohue JF, Levinsky NG Ischemic damage and repair in the rat proximal tubule. Differences among the S_1, S_2 and S_3 segments. Kidney Int (1978) 14:31–49

[58] Kloner RA, Przyklenk K, Whittaker P Deleterious effects of oxygen radicals in ischemia/reperfusion. Resolved and unresolved issues. Circulation (1989) 80:1115–1127

[59] Bowie A, O'Neill LA Oxidative stress and NF-κB activation: a reassessment of the evidence in the light of recent discoveries. Biochem Pharmacol (2000) 59:13–23

[60] Pincemail J, Defraigne JO, Detry O, Franssen C, Meurisse M, Limet R Ischemia-reperfusion injury of rabbit kidney: comparative effects of desferrioxamine and *N*-acetylcysteine as antioxidants. Transplant Proc (2000) 32:475–476

[61] Baker GL, Corry RJ, Autor AP Oxygen free radical induced damage in kidneys subjected to warm ischemia and reperfusion. Protective effect of superoxide dismutase. Ann Surg (1985) 202:628–641

[62] Kulah E, Tascilar O, Acikgoz S, Karadeniz G, Tekin IO, Can M, Gun B, Barut F, Comert M Oxidized LDL accumulation in experimental renal ischemia reperfusion injury model. Ren Fail (2007) 29:409–415

[63] Solmazgul E, Uzun G, Cermik H, Atasoyu EM, Aydinoz S, Yildiz S Hyperbaric oxygen therapy attenuates renal ischemia/reperfusion injury in rats. Urol Int (2007) 78:82–85

[64] McCord JM, Edeas MA SOD, oxidative stress and human pathologies: a brief history and a future vision. Biomed Pharmacother (2005) 59:139–142

[65] Kone BC, Baylis C Biosynthesis and homeostatic roles of nitric oxide in the normal kidney. Am J Physiol (1997) 272:F561–F578

[66] Chatterjee PK, Kvale EO, Patel NS, Thiemermann C GW274150 inhibits nitric oxide production by rat proximal tubular cells. Med Sci Monit (2003) 9:BR357–BR362

[67] Weight SC, Nicholson ML (1998) Nitric oxide and renal reperfusion injury: a review. Eur J Vasc Endovasc Surg (1998) 16:98–103

[68] Goligorsky MS, Brodsky SV, Noiri E - NO bioavailability, endothelial dysfunction, and acute renal failure: new insights into pathophysiology. Semin Nephrol (2004) 24:316–323

[69] Pryor W, Squadrito G - The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. Am J Physiol (1995) 268:L699–L772

[70] Radi R, Beckman JS, Bush KM, Freeman BA - Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys (1991) 288:481–487

[71] Beckman JS, Beckman TW, Chen J, Marshalland PA, Freeman BA - Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA (1990) 87:1620–1624

[72] Chatterjee PK, Patel NSA, Kvale EO, Cuzzocrea S, Brown PAJ, Stewart KN, Mota-Filipe H, Thiemermann C - Inhibition of inducible nitric oxide synthase reduces renal ischemia reperfusion injury. Kidney Int (2002) 61:862–871

[73]Yamakura F, Taka H, Fujimura T, Murayama K - Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. J Biol Chem (1998) 273:14085–14089

[74] Noiri E, Nakao A, Uchida K, Tsukahara H, Ohno M, Fujita T, Brodsky S, Goligorsky MS - Oxidative and nitrosative stress in acute renal ischemia. Am J Physiol (2001) 281:F948–F957

[75] Kelly KJ - Acute renal failure: much more than a kidney disease. Semin Nephrol (2006) 26:105–113

[76] Kaysen GA - Inflammation and oxidative stress in end-stage renal disease. Adv Nephrol Necker Hosp (2000) 30:201–214

[77] Thurman JM - Triggers of inflammation after renal ischemia/reperfusion. Clin Immunol (2007) 123:7–13

[78] Dong X, Swaminathan S, Bachman LA, Croatt AJ, Nath KA, Griffin MD - Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia-reperfusion injury. Kidney Int (2007) 71:619–628

[79] Montoliu J - Clearance of inflammatory mediators through continuous renal replacement therapy. Blood Purif (1997) 15:305–308

[80] Burne-Taney MJ, Rabb H - The role of adhesion molecules and T cells in ischemic renal injury. Curr Opin Nephrol Hypertens (2003) 12:85–90

[81] Viedt C, Dechend R, Fei J, Hansch GM, Kreuzer J, Orth SR - MCP-1 induces inflammatory activation of human tubular epithelial cells: involvement of the transcription factors, nuclear factor-kappaB and activating protein-1. J Am Soc Nephrol (2002) 13:1534–1547

[82] Salvemini D, Doyle TM, Cuzzocrea S - Superoxide, peroxinitrite and oxidative/nitrosative stress in inflammation. Biochem Soc Trans (2006) 34:965–970

[83] Bonventre JV, Zuk A - Ischemic acute renal failure: an inflammatory disease? Kidney Int (2004) 66:480–485

[84] Schindler R - Causes and therapy of microinflammation in renal failure. Nephrol Dial Transplant (2004) 19:V34–V40

[85] Bonventre JV - Pathophysiology of acute kidney injury: roles of potential inhibitors of inflammation. Contrib Nephrol (2007) 156:39–46

[86] Duffield JS, Hong S, Vaidya VS, Lu Y, Fredman G, Serhan CN, Bonventre JV - Resolvin D series and protectin D1 mitigate acute kidney injury. J Immunol (2006) 177:5902–5911

[87] Xie J, Guo Q - Par-4 is a novel mediator of renal tubule cell death in models of ischemia-reperfusion injury. Am J Physiol Renal Physiol (2007) 292:F107–F115

[88] P. Chowdhury, S.H. Sacks, N.S. Sheerin, Minireview - functions of the renal tract epithelium in coordinating the innate immune response to infection, Kidney Int. (2004) 66 1334–1344.

[89]Hye Ryoun Jang, Hamid Rabb - The innate immune response in ischemic acute kidney injury Clinical Immunology (2009) 130: 41–50

[90] F. Gueler, J.K. Park, S. Rong, T. Kirsch, C. Lindschau, W. Zheng, M. Elger, A. Fiebeler, D. Fliser, F.C. Luft, H. Haller, Statins attenuate ischemia–reperfusion injury by inducing heme oxygenase-1 in infiltrating macrophages, Am. J. Pathol. (2007) 170:1192–1199.

[91] J.J. Friedewald, H. Rabb, Inflammatory cells in ischemic acute renal failure, Kidney Int (2004) 66: 486–491.

[92] X. Dong, S. Swaminathan, L.A. Bachman, A.J. Croatt, K.A. Nath, M.D. Griffin, Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia–reperfusion injury, Kidney Int. (2007) 71: 619–628.

[93] L. Li, L. Huang, S.S. Sung, P.I. Lobo, M.G. Brown, R.K. Gregg, V.H. Engelhard, M.D. Okusa, NKT cell activation mediates neutrophil IFN-gamma production and renal ischemia–reperfusion injury, J. Immunol. (2007) 178:5899–5911.

[94] N. Yokota, M. Burne-Taney, L. Racusen, H. Rabb, Contrasting roles for STAT4 and STAT6 signal transduction pathways in murine renal ischemia–reperfusion injury, Am. J. Physiol. Renal. Physiol. (2003) 285:F319–F325.

[95] S. Wang, H. Diao, Q. Guan, W.W. Cruikshank, T.L. Delovitch, A.M. Jevnikar, C. Du, Decreased renal ischemia–reperfusion injury by IL-16 inactivation, Kidney Int. (2008) 73: 318–326.

[96] J.M. Thurman, D. Ljubanovic, P.A. Royer, D.M. Kraus, H. Molina, N.P. Barry, G. Proctor, M. Levi, V.M. Holers, Altered renal tubular expression of the complement inhibitor Crry permits complement activation after ischemia/reperfusion, J. Clin. Invest. (2006) 116:357–368.

[97] S. David, L. Biancone, C. Caserta, B. Bussolati, V. Cambi, G. Camussi, Alternative pathway complement activation induces proinflammatory activity in human proximal tubular epithelial cells, Nephrol. Dial. Transplant. (1997) 12:51–56.

[98] J.C. Leemans, G. Stokman, N. Claessen, K.M. Rouschop, G.J. Teske, C.J. Kirschning, S. Akira, T. van der Poll, J.J. Weening, S. Florquin, Renal-associated TLR2 mediates ischemia/reperfusion injury in the kidney, J. Clin. Invest. (2005) 115:2894–2903.

[99] K. Furuichi, T.Wada, Y. Iwata, S. Kokubo, A. Hara, J. Yamahana, T. Sugaya, Y. Iwakura, K. Matsushima, M. Asano, H. Yokoyama, S. Kaneko, Interleukin-1-dependent sequential chemokine expression and inflammatory cell infiltration in ischemia– reperfusion injury, Crit. Care Med. (2006) 34:2447–2455.

[100] B. Chandrasekar, J.B. Smith, G.L. Freeman, Ischemia–reperfusion of rat myocardium activates nuclear factor-KappaB and induces neutrophil infiltration via lipopolysaccharide-induced CXC chemokine, Circulation (2001) 103: 2296–2302.

[101]Antonia Loverre, Pasquale Ditonno, Antonio Crovace, Loreto Gesualdo, Elena Ranieri, Paola Pontrelli, Giovanni Stallone, Barbara Infante, Antonio Schena, Salvatore Di Paolo, Carmen Capobianco, Michele Ursi, Silvano Palazzo, Michele Battaglia, Francesco Paolo Selvaggi,

Francesco Paolo Schena and Giuseppe Grandaliano Ischemia-Reperfusion Induces Glomerular and Tubular Activation of Proinflammatory and Antiapoptotic Pathways: Differential Modulation by Rapamycin J Am Soc Nephrol (2004)15: 2675-2686, American Society of Nephrology

[102] Judith Lechner, Nadia Malloth, Thomas Seppi, Bea Beer, Paul Jennings, and Walter Pfaller IFN-□ induces barrier destabilization and apoptosis in renal proximal tubular epithelium *Am J Physiol Cell Physiol* (2007)294: C153-C160, 2008. November 21,

[103]Young C.; Tenkova T.; Dikranian K.; Olney J.W. Excitotoxic Versus Apoptotic Mechanisms of Neuronal Cell Death in Perinatal Hypoxia / Ischemia Volume 4, Number 2, March (2004) , pp. 77-85(9)

[104] S.A. Tisherman, A. Rodriguez and P. Afar, Therapeutic hypothermia in traumatology. *Surg Clin North Am* (1999), 79:pp. 1269–1289

[105] H.T. Hassoun, R.A. Kozar, B.C. Kone, H.J. Safi and F.A. Moore, Intraischemic hypothermia differentially modulates oxidative stress proteins during mesenteric ischemia/reperfusion. *Surgery* (2002) 132: pp. 369–376.

[106] M.D. Carattino, F. Cueva, A. Zuccollo, J.L. Monti, M. Navarro and O.L. Catanzaro, Renal ischemia-induced increase in vascular permeability is limited by hypothermia. *Immunopharmacology* (1999) 43:pp. 241–248.

[107] R.A. Zager, D.J. Gmur, C.R. Bredl and M.J. Eng, Degree and time sequence of hypothermic protection against experimental ischemic acute renal failure. *Circ Res* (1989) 65:pp. 1263–1269

[108] L.G. Svensson, J.S. Coselli, H.J. Safi, K.R. Hess and E.S. Crawford, Appraisal of adjuncts to prevent acute renal failure after surgery on the thoracic or thoracoabdominal aorta. *J Vasc Surg* (1989) 10:pp. 230–239

[109] Vikram S. Kashyap MD, Richard P. Cambria MD, J.Kenneth Davison MD and Gilbert J. L'Italien PhDRenal failure after thoracoabdominal aortic surgery Eastern Vascular Society, (1997)May 2–4,.

[110] Basso, D., Pesarin, F., Salmaso, L., Solari, A. *Permutation Tests for Stochastic Ordering and ANOVA: Theory and Applications with R*. Springer, Heidelberg. (2009).

[111] Pesarin, F. *Multivariate Permutation Tests: With Applications in Biostatistics*. Wiley, Chichester. (2001).

[112] Edgington, E.S., Onghena, P. *Randomization tests* (4th edn). Chapman and Hall, London. (2007).

[113] Corain, L., Salmaso, L.,. Multivariate and Multistrata Nonparametric Tests: the NPC method. *Journal of Modern Applied Statistical Method,* (2004)3 :(2), 443–461.

[114] Blair, R.C., Higgins, J.J., Karnisky, W., Kromrey, J.D.,. A study of multivariate permutation tests which may replace Hotelling's T2 test in prescribed circumstances. Multivariate Behavior Research, (1994) 29: 141–163.

[115] Vinuesa E, Hotter G, Jung M, Herrero-Fresneda I, Torras J, Sola A – Acute Heart Inflammation: ultrastructural and functional aspects - J Pathol. 2008 Jan;214(1):104-13.

[116] E. Hulse, P. E. Kunkler, J. P. Fedynyshyn and R. P. Kraig -Optimization of multiplexed bead-based cytokine immunoassays for rat serum and brain tissue- Journal of Neuroscience Methods Volume 136, Issue 1, 15 June 2004, Pages 87-98

[117] Gary Toedter,* Karen Hayden, Carrie Wagner, and Carrie Brodmerkel -Simultaneous Detection of Eight Analytes in Human Serum by Two Commercially Available Platforms for Multiplex Cytokine Analysis Clin Vaccine Immunol. 2008 January; 15(1): 42–48.