Kidney storage before transplantation with the use of machine perfusion

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**ABSTRACT**

Kidney transplantation is now a recognized method of treatment for patients with end-stage renal disease in current medical practice. There are numerous reports of beneficial effects of kidneys preservation in hypothermic machine perfusion on early and long-term graft survival. It allows assessment of the organ before transplantation through an analysis of perfusion parameters and biochemical tests. Published clinical studies have demonstrated that hypothermic machine perfusion reduces the number of delayed graft function (DGF) episodes and limits the occurrence of interstitial fibrosis/tubular atrophy and chronic rejection assessed by biopsy. This is of particular importance in the case of expanded criteria donors (ECD), where the risk of DGF is markedly higher.

This article will supply a general review of the dynamic kidneys preservation methods including hypothermic machine perfusion and novel normothermic machine perfusion.
INTRODUCTION

Currently, the best method of treatment of end-stage renal disease is transplantation. One of the most important problems in transplantology is ischaemic damage to the organ associated with changes taking place in the donor’s body after brain death, in the course of kidney storage and reperfusion.

To minimise the changes related to organ damage in the course of ischaemia, it is of key importance to ensure the optimum conditions of organ storage. The objective is to ensure the best possible function of the organ after transplantation. This can be achieved with the use of hypothermia which reduces metabolic activity of tissues. At a temperature lower than 10°C, oxygen consumption by tissues constitutes only 5% of its consumption in normothermia. The consequence of blood flow stoppage after organ harvesting is activation of anaerobic metabolism resulting from an oxygen deficit, which leads to the development of metabolic acidosis. In hypothermia, the quantity and use of ATP are decreased, with consequential inhibition of activity of a protein enzyme, the sodium-potassium pump. This causes an increase of intracellular sodium levels leading to an increased water influx into the cell. This may lead to the development of cellular oedema and irreversible damaging of the membrane potential of the cell. Other consequences include activation of a number of proteolytic enzymes, free oxygen radicals, cytokines and platelet activating factor. The use of the appropriate fluid for organ perfusion, containing certain selected ingredients, and the choice of the method of organ storage may contribute to minimisation of those negative processes.

The most common method of kidney storage after harvesting from a cadaveric donor is the static method of cold storage (CS), which involves placing the organ rinsed from blood in the cold storage solution (+4°C) and a specially prepared ice container. This method was developed by G. Collins, and the solution with a reversed ratio of sodium to potassium concentration developed by him in 1969 was to resemble the intracellular fluid by its composition [1]. An alternative is the use of a dynamic method of hypothermic machine perfusion (HMP), in which cooled perfusion solution flows within an enclosed system through the vascular bed of the organ under a defined pressure.

HYPOTHERMIC MACHINE PERFUSION

The concept of hypothermic machine perfusion as a model of organ storage was proposed for the first time by Alex Carrel and dates back to the beginning of the 20th century. In collaboration with Charles Lindberg, he constructed the first perfusion machine which was to protect the kidney by delivering oxygen in the perfusion fluid. They continued their research with the use of hypothermia and their study was published by Lindbergh in 1966, already after Carrel’s death [2]. Those studies as well as the studies conducted by Lapchinsky in the Soviet Union [3] constituted the basis for the experiments of Lillehei [4] and Hoffman [5], who concentrated on the role of hypothermia in organ storage.

The first successful kidney transplantation after 17-hour HMP was performed by Belzer and Truman in a man with amyloidosis and took place in 1967 at the University of Wisconsin [6]. After that event, Belzer’s team made other successful attempts at the use of that method which was gradually introduced into clinical practice [7].

Apart from cooling down the perfused organ, the solution used in HMP maintains the appropriate oncotic pressure by delivering osmotically active substances and colloids. It also delivers nutrients and eliminates toxic metabolism products and free radicals that form in the process of ischaemia. Currently, the most popular solution in HMP is KPS-1. The introduction into practice of the UW solution of intracellular composition by Belzer and Southard made it possible to considerably prolong the duration of organ storage [8] and contributed to an even better protection of organs in the course of storage through, for example, limitation of cellular oedema owing to an addition of hydroxy-ethylated starch (HES) and adenosine indispensable for ATP re-synthesis.

A multi-centre international randomised study conducted by Moers et al. in 2009 in a group of 336 kidney pairs, one of which was preserved using the CS method and the other with the use of HMP, demonstrated a marked reduction of the risk of delayed graft function (DGF) from 26.5% to 20.8% when HMP was used and prolongation of one-year kidney graft survival (from 90% to 94%) [9]. Three-year observation of this patient group (672 recipients) confirmed a positive effect of HMP in comparison with CS on kidney transplant survival (91% vs 87%). The occurrence of DGF in the group of patients who received a CS-stored kidney markedly contributed to a reduction of three-year transplant survival in comparison with the group of patients who received the kidney stored with the use of HMP and did not experience a DGF episode. In the group of patients who received a kidney stored with the use of HMP, a DGF episode did not have such a significant effect on transplant survival [10].

Many studies have demonstrated a relationship between a DGF episode, defined as the need for haemodialysis in the recipient within 7 days after transplantation, and the negative effect on late graft survival, an increased risk of an acute rejection episode and increased mortality in older kidney transplant recipients [11-13].

Already in 1996, Kwiatkowski et al. presented a study...
sodes in the case of kidneys stored with the use of HMP in comparison with CS, and harvested from hae-
modynamically unstable donors [14]. In a retrospec-
tive analysis of a group of 415 patients, 227 of whom received an organ stored with the use of HMP before transplantation and 188 received a CS-stored organ, the same authors demonstrated a better late graft survival and a significantly lower percentage of pa-
tients returning to dialysis treatment at 5 years after transplantation. After that period, the percentage of patients with serum creatinine concentration below 2 mg/dl was higher in the group which received the kidney stored with the use of HMP [15].

A meta-analysis and a literature review performed by Wight et al. demonstrated a significant (20%) reduction of DGF episodes associated with the use of HMP. On the other hand, no significant difference was demonstrated in one-year graft survival in com-
parison with the CS group [16].

Due to the shortage of organs for transplantation, the elongating list of patients waiting for this procedure and the improving transplantation outcomes, the eligibility criteria for organ recipients and donors have changed. Currently, many sites accept and suc-
cessfully transplant kidneys from expanded criteria donors (ECD), enlarging in this manner the pool of organ donors.

In comparison with kidneys from donors with stan-
dard criteria, kidney transplantation from ECDs is associated with an increased risk of DGF, prima-
ry non-function (PNF), an acute rejection episode and reduced late graft survival [17-19]. Although it has been demonstrated that kidney recipients who obtained an organ originating from an ECD are at a 1.7-fold greater risk of transplant loss [20], the use of such organs has a significantly positive effect on recipient survival as compared with the survival of dialysed patients [21], especially those over the age of 60 [22].

The study by Stratta et al., analysing 141 kidneys harvested from ECDs and stored before transplantation with the use of HMP or CS, demonstrated a reduced DGF rate among recipients of the HMP group (11%) in comparison with the CS group (37%), despite prolongation of the cold ischaemia time (CIT) in the first group [23]. Similar results were presented by Matsuoka et al. who analysed the results of 4618 transplanted kidneys obtained from expanded criteria donors, 912 of which were stored with the use of HMP. Delayed graft function episodes in the HMP group were noted less frequently than in the CS group (26% vs 36%). However, they did not demonstrate a difference between those groups in an analysis of 3-year survival of the transplanted kidney [24].

In 2013, Baoping et al. published a meta-analysis based on seven studies comparing the results of kidney transplantation from ECDs, stored with the use of HMP (n=2374) and CS (n=8716). As they found, the use of mechanical perfusion significantly decreases the risk of DGF and is associated with increased one-
year graft survival in this group. However, no inter-
group differences were noted in PNF episodes and one-year survival of transplant recipients [25].

In a situation of shortage of organ donors, kidneys procured from donors after cardiac death (DCD) con-
stitute an important additional source of transplanta-
ble organs. However, the kidneys obtained from such donors are associated with a significantly greater DGF risk, which is associated with ischaemic dama-
g to the organs, and especially with prolonged warm ischaemic phase. In 2010, Jochmans et al. published a study comparing the results of transplantation of kidneys harvested from 82 DCDs, stored with the use of HMP and CS. They observed a reduced rate of DGF episodes from 69.5% to 53.7% in the group of recipients of perfused kidney transplants and a shortened duration of haemodialysis treatment by 4 days. One-year survival of grafts and recipients were similar in both groups. The authors suggest that HMP should be a routine method of storage of such kid-
nneys [26]. Similar results were obtained by Plata-Mu-
noz et al. on the basis of a comparison of two groups of 30 recipients of kidneys from DCDs stored with the use of HMP and CS. In the HMP group, the number of DGF episodes was significantly lower and the duration of patient hospitalisation after the transplan-
tation procedure was shorter [27]. Other published papers also report that the use of HMP in the case of kidneys originating from DCDs contributes to an improvement of early function and may increase graft survival [28-29].

Hypothermic machine perfusion also enables an as-
sessment of ischaemic damage to the organ through the analysis of perfusion parameters such as vascular flow and renal resistance (RR) and biochemical para-
meters measured in the perfusion solution (including levels of lactate dehydrogenase, lactate, alanine aminotransferase) [30-31]. As demonstrated by Jo-
chnams et al., RR (mmHg/ml/min) measured at the end of perfusion may be helpful in the assessment of DGF risk and one-year graft survival [32]. According to some authors, the RR value measured at four hours of perfusion may be a parameter enabling potential disqualification of kidneys for transplantation [33]. Nevertheless, it should be borne in mind that this is one of many parameters and should not be used alone for kidney disqualification, and such a decision should be taken by analysing a number of factors.

In an analysis developed by Hall, Bhangoo et al., glu-
tathione S-transferase (GST) levels were compared in the perfusion solution of kidneys stored with the use of HMP and harvested from cadaveric donors. The GST levels measured were measured at two time points, at the beginning and at the end of HMP. It was demonstrated that an increase in the level of the pi isoenzyme of that biomarker (piGST) measured in
the perfusion solution was independently associated with an increase in the DGF risk. This shows that the use of HMP and an analysis of various biomarkers measured, for example, in the perfusion solution makes it possible to assess the stored organ and may enable the estimation of early outcomes of transplantation [34].

The use of HMP for perfusion of kidneys from donors infected with hepatitis C virus makes it possible to reduce the number of virus copies, and thus reduce the risk of infection transfer to the recipient [35].

Renal perfusion also influences the microscopic changes in a biopsy specimens of the organ. Ischemic organ damage may lead to intensified interstitial fibrosis of the graft. In a retrospective study presented by Kwiatkowski et al., a lower rate of interstitial fibrosis/tubular atrophy (IF/TA) and less frequent chronic rejection were demonstrated in renal biopsy specimens taken more than 7 years after transplantation in a group of recipients who received an organ stored with the use of HMP [36]. A positive effect of HMP is associated with its protective effect on vascular endothelium. Continuous flow of the perfusion solution through the vessel and mechanical contact and stress generated in this manner stimulate the endothelium to the synthesis of nitric oxide responsible for vascular relaxation [37]. This in turn contributes to a better regulation of the vascular tone, and thus to better distribution of the perfusion solution to renal cells and lowering of vascular resistance, which is usually observed in the first hours after perfusion initiation.

The use of HMP also influences the expression of genes associated with organ damage due to ischaemia and reperfusion. In the study presented by Wszola et al., the results of kidney biopsies performed 30 minutes after reperfusion were analysed [38]. The activity of mRNA for hypoxia-inducible factor 1-alpha (HIF-1-alpha) was significantly lower after reperfusion for the group of kidneys that were stored with the use of HMP in comparison with the CS group. HIF-1-alpha activation is strongly associated with ischemic organ damage and may have an effect on late changes in the organ, for example through activation of the transforming growth factor-beta (TGF-beta) participating in chronic graft fibrosis processes [39].

Currently, two systems of mechanical renal perfusion in hypothermia are commonly used in clinical practice: Waters Medical Systems, Rochester, UK (RM3) and Organ Recovery Systems, Chicago, IL, USA (LifePort). In both these systems, it is possible to modify systolic perfusion pressure and to monitor blood flow, vascular resistance and diastolic and mean perfusion pressure. In the study presented by Wszola et al., comparing these systems, a significantly higher IF/TA rate assessed one year after transplantation was shown in biopsies of the kidneys that were stored with the use of RM3, Waters Medical Systems. No inter-group differences in DGF episodes were found but DGF duration in the LifePort, Organ Recovery Systems group was shorter [40].

NORMOTHERMIC MACHINE PERFUSION

Another promising method of organ preservation which is still under investigation is normothermic machine perfusion (NMP). This method seems to enable good assessment of ischemic organ damage and reduced hypothermia-related damage. The first report on transplantation of a human kidney stored with the use of normothermic perfusion was published by Hosgood et al. in 2011 [41]. In their subsequent paper, they presented a comparison of the outcomes of transplantation of 18 kidneys harvested from ECDs, stored with the use of normothermic perfusion with the outcomes of transplantation of the control group, which were 42 kidneys obtained from ECDs preserved in cold storage [42]. Normothermic perfusion involved passing a plasma-depleted erythrocyte concentrate through the kidney, oxygenated and warmed to 32-36°C, for 60 minutes immediately before transplantation. A significantly lower rate of DGF episodes was demonstrated in the NMP group (5.6%) in comparison with the CS control group (36.2%). No inter-group differences in one-year graft survival and recipient survival were noted.

CONCLUSIONS

Kidney storage with the use of machine perfusion is currently becoming a standard modality used in numerous centres. It allows assessment of the organ before transplantation through an analysis of perfusion parameters and biochemical tests. It has a proven beneficial in limiting the number of DGF episodes and the development of chronic changes assessed by biopsy, and thus contributing to good long-term outcomes of renal transplantation. This is of particular importance in the case of ECDs, where the risk of DGF is markedly higher. Therefore, the use of HMP in the case of kidneys obtained from these donors should be considered mandatory.

Attempts at storing other organs with the use of machine perfusion are currently subject to numerous studies, and the use of normothermic perfusion in the case of kidneys seems to be a new very promising technique which may be introduced into clinical practice in the near future.

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