Modern techniques of abdominal organ transplantation: Visceral Cold Static Organ Preservation for Transplantation

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INTRODUCTION

Abdominal organ transplantation is the optimal treatment for many patients. Retrieval, transport, and transplantation of grafts lead to ischemia-reperfusion injury. The currently accepted standard is static cold storage (SCS), in which the organ is both perfused and stored in cold preservation solution after retrieval from the donor and then removed from the ice box at the time of the implantation.

This presentation focused on the historic aspects, principles of cold static preservation of the liver and the two perfusion solutions HTK and UW solution. The history of cold static preservation started by using embalming fluids for the preservation of human bodies in Egypt, 2000 BC. The development of preservation techniques reached a peak in 1967, when huge machines were used for the normothermic preservation of abdominal organs. However, the machines were rather complex and big, often transported in a truck. Therefore, cold static organ preservation was addressed with various perfusion solutions (figure 1).

PRINCIPLES OF COLD STATIC PRESERVATION

Historical aspects

1969

Wash-out solution, hypothermic storage
Collins

1979-1988

UW-solution, hypothermic storage
Belzer

1990

HTK-solution, hypothermic storage
Bretschneider

today's products (selection)

HTK-solution
Histidine, Tryptophane, α-Ketoglutarate, Custodiol®

Euro-Collins-solution
hyperosmolar solution equivalent osmolarity to intracellular

UW-solution
University-of-Wisconsin-solution

St.-Thomas-Hospital-solution

Plegisol®

Viaspan®
Belzer UW; DuPont-Pharma GmbH, Bad Homburg

Celsior®
Imtix Sangstat, Lyon

Perfadex®
Vitrolife AB, Göteborg

Polysol®

Figure 1: The development of solutions for perfusion and storage.

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"The provision of a viable and minimally damaged homograft is undoubtedly the most important single factor in the determinant of success!"

Dr. Thomas Starzl (University of Colorado)

Hypothermia plays a critical role in the preservation:

- Decreased energy consumption
- Decreased enzymatic activity, especially the ATPases
- 0_2 consumption (as a marker for metabolism) decreases to
  - 10% at 10°C
  - 5% at 5°C
  - 3% at 0°C
- Passive transmembrane transport of ions still function

Preservation solutions should preserve the whole organ (including vessels and ductal structures). The metabolism should be reduced by hypothermia (0-4°C). Furthermore, intra- and extracellular edema formation and acidosis must be prevented. Further requirements are the protection against free radicals and the regeneration of cellular energy balance over time.

The major players of components in currently available preservation solutions are:

- Colloids (hydroxyethyl starch (HES), polyethylene glycol) for prevention of interstitial edema
- Impermeables (glucose, raffinose, mannitol, lactobionate) for the prevention of cellular edema
- Buffers (phosphate, histidine) for the prevention of intracellular acidosis
- Radical scavengers (allopurinol, glutathione, histidine, tryptophane, glycine, mannitol) for the protection against free oxygen radicals
- Energy substrates (adenosine, alpha ketoglutarate) for the regeneration of the cellular energy balance

During organ retrieval the ascending aorta is flushed for the preparation of the thoracic organs (heart, lung), down to the abdominal aorta for the abdominal organs (kidney, liver, pancreas) and in addition the portal vein especially for the procurement of the liver (figure 2).

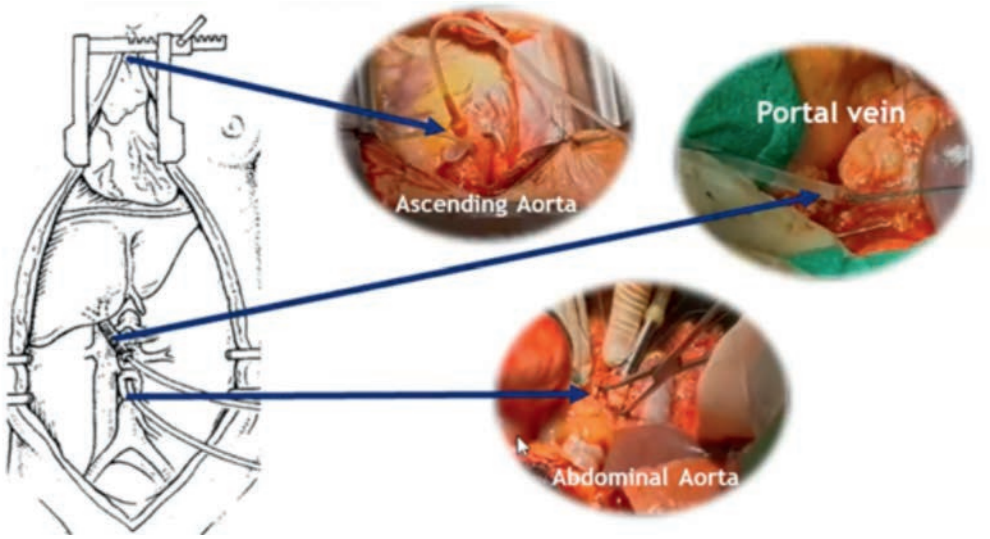


Figure 2: Organ retrieval: Donor operation and organ flush.

The initial flushing step is very important. This can be achieved by either using 5-10 liters HTK **(CUSTODIOL®)** or 3 liters UW solution at a perfusion pressure (aorta) of 80-100 cm H_2O (gravity) to rapidly cool down the organs. A clear rinse leaves a pale organ, which is an indication for a well-done perfusion. In addition, abdominal organs are further protected by topical cooling using crushed ice. Organs are then packed according to the so called 3 bag method which is standard in the Eurotransplant region: The organ floats in the preservation solution in the inner bag. The inner bag is floating in normal saline in a second bag. The third bag protects the others from the outside.

Low temperatures decrease the lysis of cell membrane and protect the organ. Therefore, static preservation requires a storage temperature of 0-4°C. Less than 0°C should be avoided to prevent from freezing of the tissue. Endothelial cells are more susceptible to cold injury as compared to parenchymal cells. 4°C is the standard storage temperature today, allowing the organs to survive for rather long cold ischemia times:

- Kidney 24 (-40) h
- Pancreas 12 (-36) h
- Liver 12 (-36) h
- Small bowel 8 (-12) h
- Lung 8 (-12) h
- Heart 4 (-6) h

It is important to keep the cold ischemia time as short as possible, because the quality of the organ decreases over time.

The most common preservation solutions are UW and HTK. In Germany, HTK is used in more than 90 % of cases for cold storage of organs (information provided by the DSO, www.dso.de). In the USA, the use of HTK started in 2004. In 2008 around 70% of UW and 30% of HTK were used (Klein et al., 2010). The UW solution (Belzer, ViaSpan®) can be used for the preservation of the liver, pancreas, and kidney.

In situ volumes used are 2000 – 4000 ml in the aorta and 1000 ml into the portal vein (liver). Ex situ rinses can be done at the back table with 1000 ml for the liver (portal vein, bile duct) respectively 300 - 500 ml for the pancreas and kidney. These volumes are given for adults. 50 ml/kg are given in children.

The main differences are the high potassium concentration in UW (125 mM versus 9 mM) and high buffer concentration in HTK (198 mM versus 25 mM). Besides electrolytes, UW contains components shown in figure 3.

Components		Guiding principles of the UW solution
Potassium lactobionate: Raffinose:	100 mM 30 mM	Osmotic concentration maintained by the use of metabolically inert substances to prevent cell swelling
Hydroxyethyl starch (HES):	50 g/L	to minimize interstitial edema
Adenosine:	5 mM	stimulation of ATP production
Glutathione: Allopurinol:	3 mM 1 mM	stimulation of ATP production, free radical scavenger decreased free radical formation
KH_2PO4: MgSO4:	25 mM 5 mM	buffer to maintain intracellular Mg^{2+} concentration

Figure 3: Components of UW (Belzer, ViaSpan®) and their function.

Next to the advantages listed in figure 3, there are also disadvantages to the UW solution:

- High viscosity
- Solution cannot be released into circulation (high K+ content)
- Particles of 100 µm in diameter contained in the UW storage solution
  - In line filtration with 40µm pore size is mandatory! Particles caught in capillary bed of perfused organs, resulting in vascular constriction, impeded reperfusion, and reduction of functional recovery (Tullius et al., 2002)

HTK (**CUSTODIOL®**), on the other hand, can be used for all organs (liver, pancreas, kidney, heart, combined heart-lung). Recommended volumes for in situ perfusion are 8-10 liters via the aorta. Additionally, in situ rinsing is done using 1 liter for the liver, including portal vein and bile ducts. Pancreas and kidney grafts are rinsed with 300-500 ml HTK on the back table. These numbers are for adult donors. Children are perfused with 50 ml/kg. The most important components of HTK are listed in figure 4.

Components		Guiding principles of the HTK solution
NaCl:	15 mmol	Intracellular solution
KCl:	9 mmol	Low potassium
$CaCl_2$:	0.015 mmol	Intracellular solution
$MgCl_2 \times 6\ H_2O$:	4 mmol	Antagonist to calcium
Histidin HCl:	18 mmol	High buffering capacity and free radical scavenger
L-Histidine	180 mmol	High buffering capacity and free radical scavenger
Tryptophane:	2 mmol	Stabilisation of membranes to prevent edema
Mannitol:	30 mmol	Radical scavenger and prevents edema formation
α-Ketoglutarate:	1 mmol	Stimulation of ATP production
Viscosity:	1.0 cP	– viscosity equal to pure water with – flow rate 3x as compared to UW solution – organs cool down more rapidly than with UW

Figure 4: Composition of HTK (Custodiol®) and their effects.

In contrast to the UW solution, HTK contains a low potassium concentration, but a high concentration of buffer. Furthermore, there is no colloid present, and the viscosity is low ("water like"). Therefore, rapid cooling is achieved quite easily.

An Austrian transplant department analyzed the usage of HTK in a prospective multi-center study (Pokorny et al., 2008). There were no differences between HTK and UW regarding post-operative graft function, both patient and graft survival, and biliary complications. No differences were found between HTK and UW regarding acute rejection and the infection rate in the first post-operative year. Both solutions proved to be equally safe and efficient. Yet, HTK is easy to handle because the solution is of low viscosity and there are no substances to add. Another advantage of HTK is that preservation with a total volume of 5 liters for one multiorgan donor shows significant cost reduction.

There was no hint for an effect of the preservation solution on the primary dysfunction (PDF) and early surgical complications. Neither preservation solution nor CIT (cold ischemia time) are independent risk factors for one year patient and graft survival. Neither was there a correlation between CIT and biliary complications. There were no differences in the univariate nor in the multivariate analysis (risk factors for patient survival). Quite the contrary, patient and graft survival are influenced by multiple factors. Major impact comes from (1) the patient's condition at time of transplantation (organ allocation, MELD); (2) graft quality (ECD donor livers).

"The preservation solution does not make a racehorse out of a donkey!"

Ferdinand Mühlbacher (ESOT 2013, Vienna)

In the USA, a randomized controlled trial (RCT) showed again no differences between UW and HTK (Thuluvath et al., 2010). The UNOS data of 2010 analyzed the solutions and their impact on patient respectively graft survival (figure 5).

Center **UW**-user	Patient Survival	Graft Survival
John Hopkins	75.6	69.7
MUSC	87.5	85.0
Univ. Pennsylvania	86.7	84.8
Univ. Wisconsin	90.0	85.2

Center **HTK**-user	Patient Survival	Graft Survival
United States	88.5	84.7
Mothodist -Memphis	92.1	87.4
University of Indiana	90.0	87.4
Clevelnd Clinic	91.6	87.9

Figure 5: UW versus HTK in case of liver graft procurement (UNOS data, 2010)

HTK showed a little bit better survival. However, this is difficult to judge because the centers might have used different organs (elderly, respectively younger patients, different donors), which makes the groups difficult to compare.

In the Eurotransplant region, the use of the UW solution is decreasing over the years 2007 to 2016. At first glance, UW seems to be better in terms of survival (figure 6).

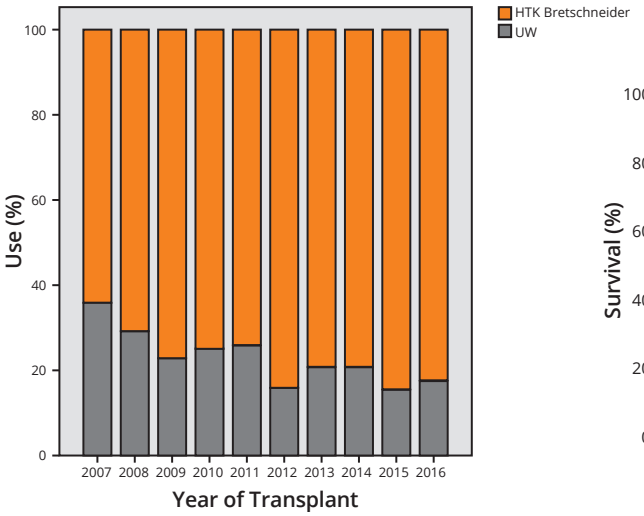
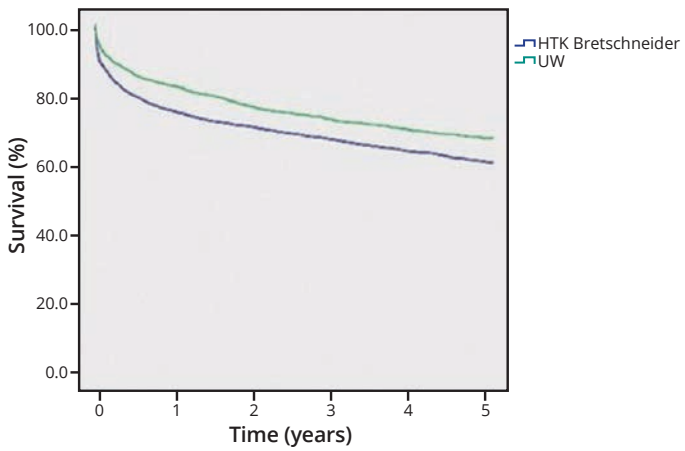


Figure 6: The use of HTK and UW in the Eurotransplant region.



Kaplan-Meier survival analysis by preservation fluid (n = 10628), graft survival.

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However, one must be careful to where the patients came from. The patients came from Germany, where patients have a much lower survival compared to non-German patients.

Within Germany, 6174 transplantations were performed with HTK and 463 with UW. In non-German countries, 2029 and 1989 transplantations were performed with HTK- and UW-preserved livers, respectively. Outcome stratified for transplantation region (Germany/non-Germany) and preservation fluid (HTK/UW) showed significantly lower overall graft survival in Germany (figure 7).

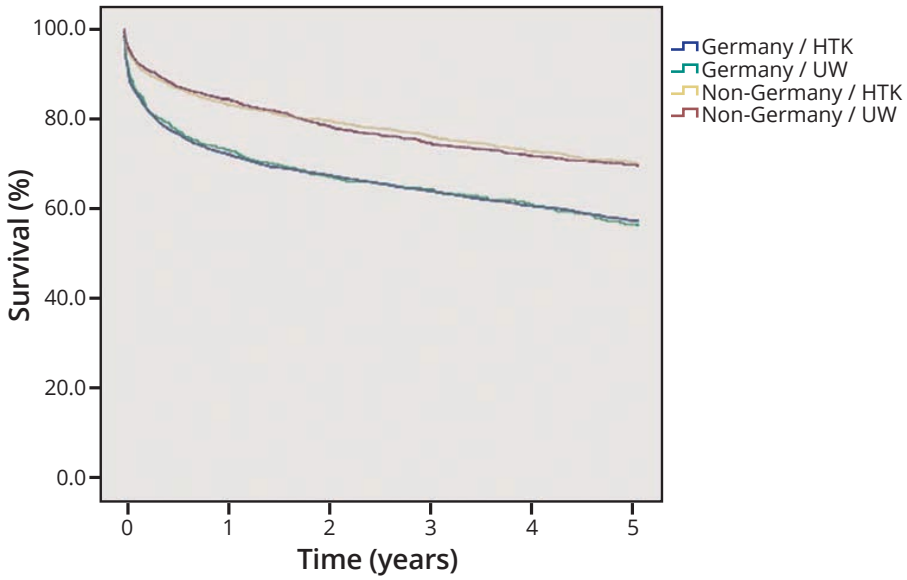

Figure 7: Kaplan Meier survival analysis of graft survival by preservation fluid and transplant region (Germany vs non-Germany), n = 10628).

This is reflected by the patient data of the HTK versus the UW group. All the following parameters were higher in the HTK group (donors and recipients): Donor age and GGT (gamma-glutamyl-transpeptidase), recipient BMI, ischemic time, rescue allocation time, MELD score of the recipient was even much higher than in the UW group (figure 8).

	HTK (n=8,176)	UW(n=2,452)	p-value
Donor age	56 (45-67)	55 (43-65)	<0.001
BMI	26 (24-28)	25 (23-28)	<0.001
Last GGT	43 (22-99)	31 (17-62)	<0.001
Ischemic time	8.6 (6.3-11.0)	7.3 (5.0-9.6)	<0.001
Rescue*	2,613 (32%)	389 (16%)	<0.001
MELD 25+	2,215 (27%)	482 (19%)	
DRM**	2.85 (2.31-3.51)	2.56 (2.09-3.08)	<0.001

Figure 8: Risk factors of liver transplantation within the HTK and UW groups.

*Rescue allocation = To increase the number of transplanted organs, the Eurotransplant foundation uses a so-called „rescue-organ-allocation" procedure for organs that had been rejected by at least three consecutive transplant centers for medical reasons (EDC, bad function). The transplant center that finally accepts such an organ can then freely choose a patient from its own waiting list, without being bound to regular allocation criteria.

**DRM = donor-recipient model calculated from risk indices (including MELD).

This explains the difference found in figure 6 (right), but not the type of preservation solution is responsible for this. In conclusion, it is a factor of the patient characteristics and not of the preservation solution.

When adjusted for all risk factors associated with 5-years graft survival, no difference could be detected between both preservation fluids in transplantations performed in Germany (P = 0.572) (Figure 9) or non-Germany (P = 0.522). In all transplantations, also no difference in long-term outcome could be shown.

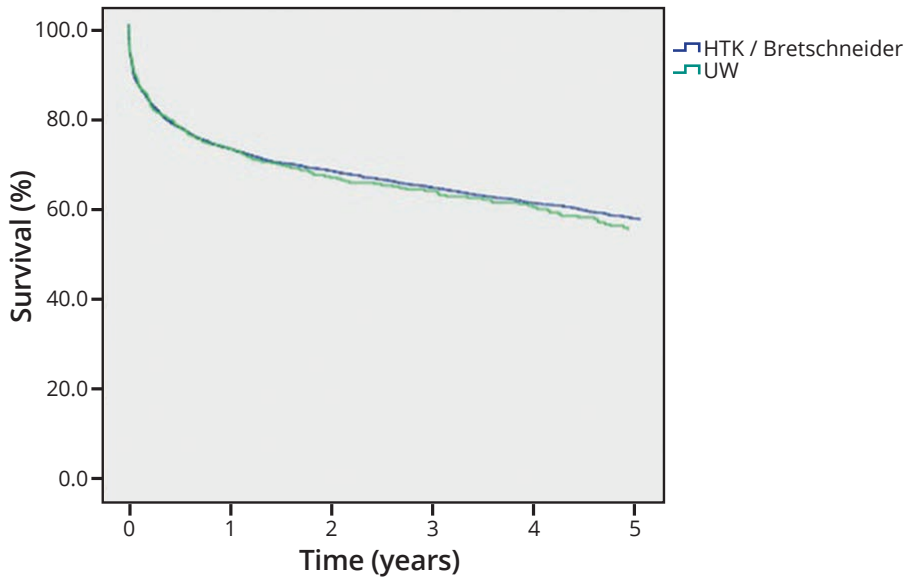

Figure 9: Risk adjusted graft survival. Germany adjusted for all separate risk factors.

It is difficult to compare countries, because each one uses specific preservation solutions like Marshall and UW in the UK, IGL-1 in France and HTK in Germany.

Adam et al. (2015) compared a HTK cohort predominantly derived from Germany with European centers predominantly using UW solution. In general, there is a selection bias in registry data because you cannot simply compare the solutions from centers which are quite different (see data from Germany shown above). There is no difference between UW and HTK solution in treatment success after liver transplantation according to RCTs (Nashan et al., 2015).

In a rather new study from the United States, HTK was compared to UW solution in DCD (n = 5956) and DBD patients (n = 82,679) (Cotter et al., 2022). For DCD patients, there is a great benefit for using HTK as the primary perfusion and preservation solution. In the DBD group HTK and UW are comparable.

Summary and Conclusion

Custodiol (HTK) solution

- Demonstrates notable clinical efficacy and safety
- Provides robust protection against biliary complications
- is recognized as the standard preservation solution across numerous countries globally

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