Protocol Amendment # 006

Proposed by: *RPC Essen / Univ. Münster* **Date:** *24-04-2005*

Title: *Kidneys of the older donor – Histology, immunohistochemistry and Western blotting for evaluation of kidney tissue damage during CS and MP*

Background and Aim

The aim of this amendment is to examine the tissue damages set by CS and by MP and to demonstrate if MP is able to reduce tissue damage or causes different problems.
Tissue damage as a result of storage in cold ischemia can only in part be demonstrated by conventional light microscopy. Expression of stress proteins such as HO-1 and HSP 70 as well as activated, phosphorylated signal transduction kinases (p-erk, p-act, c-jun) is increased by hypoxia. Such modified expression levels can be demonstrated quantitatively by Western blotting, while immunohistochemistry is capable of revealing their topochemical distribution. Preliminary research showed that correlations with clinical and prognostic parameters can be established. Additionally, apoptosis in resident renal cells shall be studied (TUNEL, X-IAP, activated caspases).

Method

From each kidney graft of donors older than 55 years two needle biopsies will be taken: one prior to the start of MP and one biopsy one hour following reperfusion, or immediately prior to closure (time should be noted). The responsible surgeon will obtain the biopsies and the perfusionist will store the biopsy and transport it to the respective RPC. Biopsies will be examined at the Institute of Pathology of the University of Münster at the end of the trial.

To be analyzed:

- *Expression of stress proteins such as HO-1 and HSP 70 as well as activated, phosphorylated signal transduction kinases (p-erk, p-act, c-jun): Western blotting and immunohistochemistry*
- *Apoptosis in resident renal cells (TUNEL, X-IAP, activated caspases)*

Tissue damage markers in MP kidneys and in a control group of CS kidneys will be compared and correlation to outcome will be analyzed .

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