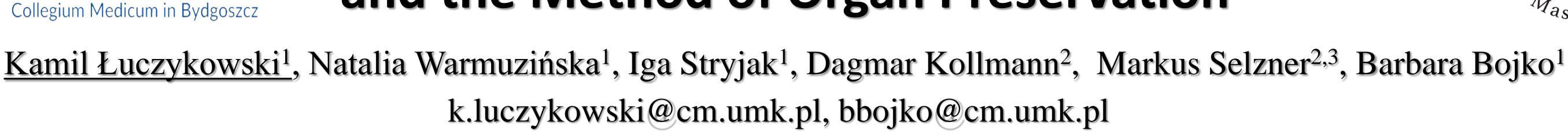
NICOLAUS COPERNICUS **UNIVERSITY** IN TORUŃ Ludwik Rydygier

Analysis of Changes in Bile Acids Concentration in Bile in Response to the Degree of Liver Ischemia and the Method of Organ Preservation



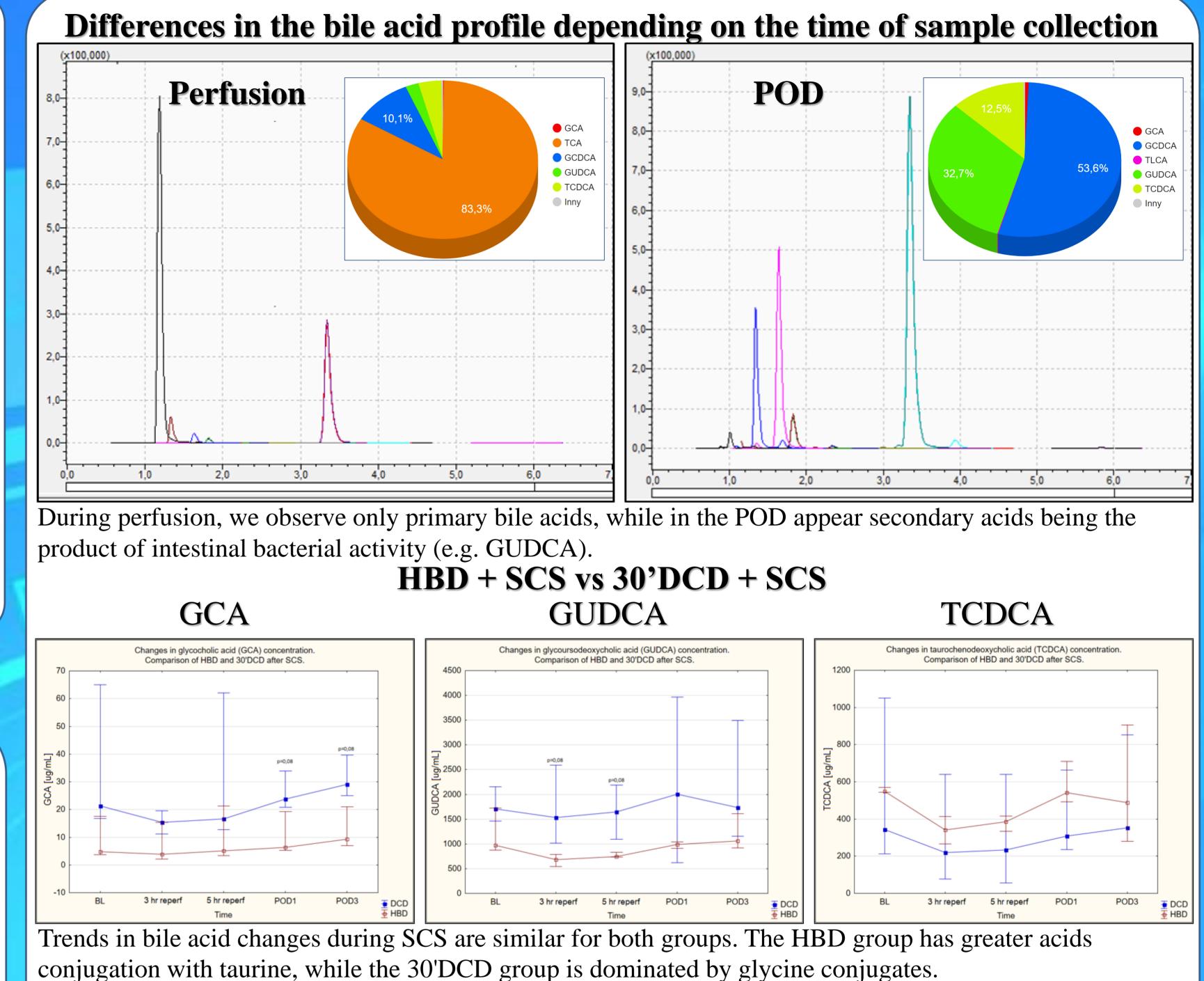
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Introduction

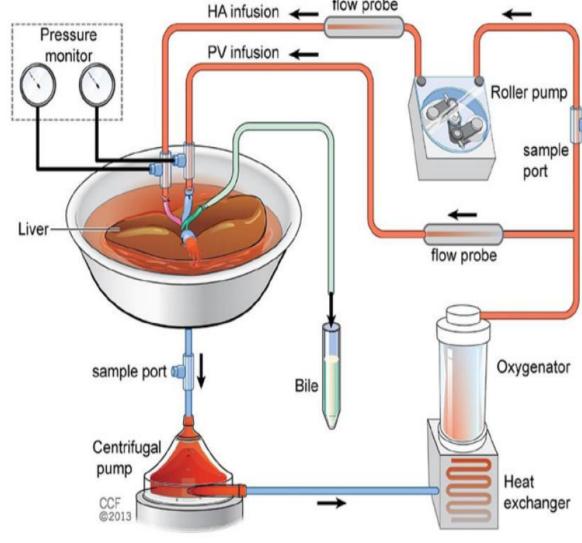
Liver transplant surgery is currently the standard of treatment in patients with end-stage organ failure. Nowadays, the dominant method of organ preservation used by most transplantation centers is static cold storage better method (SCS). However, of preservation a organ is sought, which would allow extending the storage time of the graft while maintaining its proper quality The proposed method is normothermic ex-vivo liver perfusion (NEVLP), based on maintaining normal metabolic activity, which gives the opportunity of better assessment of liver viability before implantation. One of the possibilities is to assess the production of bile by the liver perfused in these conditions. It is considered that the production of bile alone is not sufficient evidence for the proper functioning of the liver and directs the research to assess the composition of bile. Therefore, it is assumed that changes in the concentration of bile acids, which are the main component of bile, may correlate with changes occurring in the transplanted organ.

Results



Methods

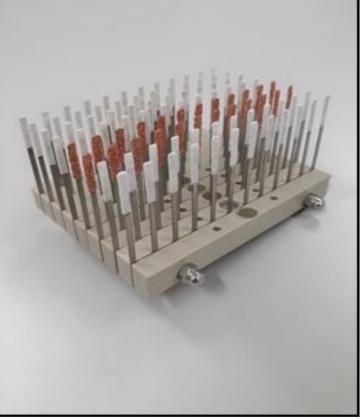
1. Collection of bile during SCS or NEVLP [1]



The study was performed on bile samples obtained from two types of porcine model donors: heart beating donor (HBD) and donor after cardiac death (DCD). Samples were collected during SCS and NEVLP at specific time points: before organ harvest, during perfusion (for NEVLP), reperfusion and the first few days after transplantation. The DCD group was divided due to the time of organ ischemia: 30' for SCS and 30', 60', 90' for NEVLP (n=3 in each group).

2. Sample preparation

Sample preparation was performed according to the thin-film solid phase microextraction (TF-SPME), using C18 sorbent as the extraction phase.



A. Extraction:

• 10 μ L bile:990 μ L PBS + 10 μ L IS • TF-SPME (5 mm C18 coating) • 60 min, 25°C, 1000 rpm agitation

B. Desorption:

• 1 mL MeOH • 60 min, 25°C, 1000 rpm agitation

*For glycochenodeoxycholic, glycoursodeoxycholic and taurochenodeoxycholic acid, the extracts were diluted 200x.

3. LC-MS/MS analysis

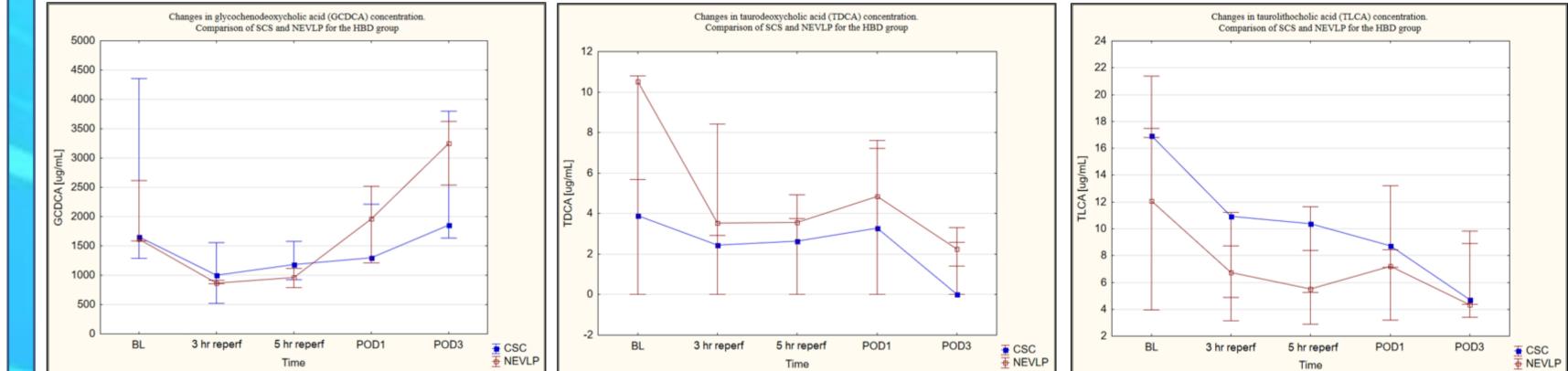


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Analyte	Rt [min]	M [g/mol]	MRM transition (m/z)	CE
GCA	1,66	465.62	464,2→73,85	39
(Glycocholic acid)			464,2→402,15	36
			464,2→410,1	46
TCA	1,17	515.70	514,15→123,8	54
(Taurocholic acid)			514,15→106,8	55
			514,15→280,9	30
GCDCA	3,29	449.62	448,15→73,85	37
(Glycochenodeoxycholic acid)			448,15→386,4	35
			448,15→330,05	47
TLCA	4,22	483.71	482,15→123,8	51
(Taurolithocholic acid)			482,15→106,8	54



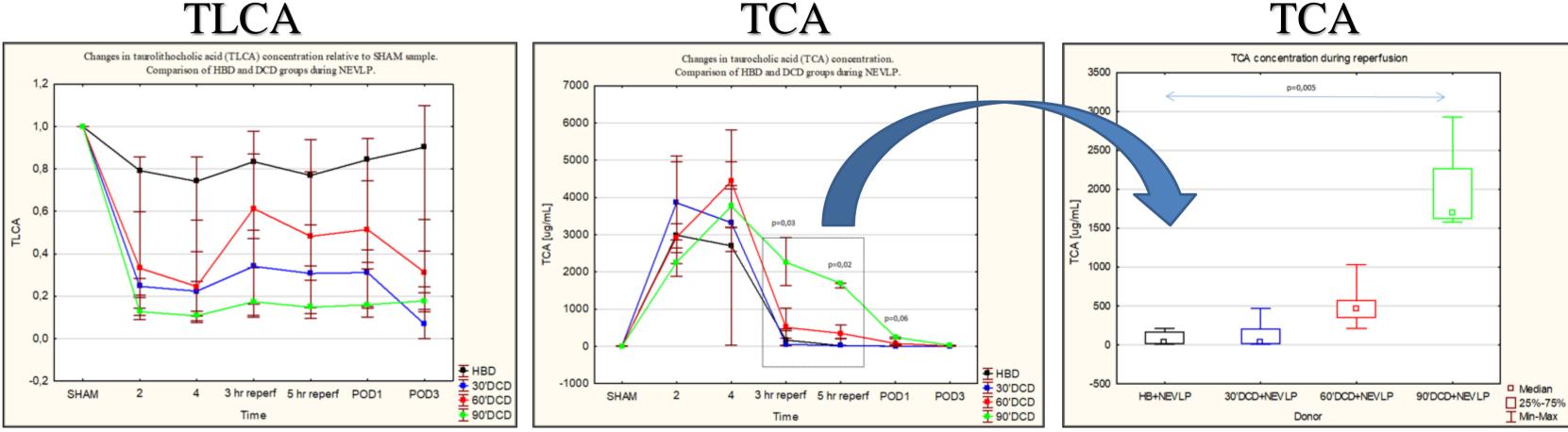
TLCA

Clinic



After transplantation, we observe an increase in glycine conjugated acids and a decrease in taurine conjugated acids in both groups. GCDCA levels increase more rapidly in the first postoperative days after NEVLP compared to SCS.

HBD + NEVLP vs 30'DCD,60'DCD,90'DCD + NEVLP



In groups with ischemia, we note a lower concentration of TLCA and its significant decrease during



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24

- Only trace amounts of free bile acids were found in bile. There is a change in the concentration of conjugated bile acids during transplantation.
- Changes in bile acid concentrations in bile samples may correlate with the metabolic processes occurring in the transplanted organ.
- Further research of bile composition extended to other bile acids and their metabolites may allow to find biomarkers of liver function.

This research has been made possible with the support of a grant for young researchers CM UMK. Ð SHIMADZU

COURAGE LIVES HERE

[1] A. Nassar et al., "Surgical Innovation," no. April, 2014

Excellence in Science

