Viability Testing and Transplantation of Marginal Donor Livers (VITTAL) Trial: Metabolomics of normothermically perfused livers discloses molecular signatures predictive of graft viability and postoperative outcomes

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Introduction

Donor organ shortage has led to increased reliance on high-risk livers for transplantation. Emergence of machine perfusion has led to a paradigm shift in organ preservation from functional suppression in cold storage to full ex vivo metabolic support. The recently completed VITTAL trial demonstrated that normothermic machine perfusion (NMP-L) provides an opportunity to objectively assess graft quality and functional integrity pre-transplantation to safely transplant high-risk livers. Our study investigated the potential of metabolic profiling to elucidate molecular signatures during NMP-L predictive of graft viability, post-reperfusion syndrome (PRS) and early allograft dysfunction (EAD) in these livers.

Summary

This study reveals a metabolic signature of high-risk livers that correlates with VITTAL criteria for graft viability for transplantation during NMP-L. It also demonstrates the potential of NMP-L metabolic profiling for biomarker discovery and applicability as a clinical tool to objectively assess the quality and predict functional integrity of these livers pre-implantation.

What is metabolomics and its potential in donor liver machine perfusion?

- Metabolomics is the identification and quantification of all metabolites in a biological system through the use of high-throughput analytical technologies (e.g. NMR or Mass Spectrometry) and dedicated bioinformatics tools.
- Metabolites are low-molecular weight compounds (<2000 Da) that consist of sugars, amino acids, lipids, organic acids and nucleotides.
- Liver is involved in a myriad of metabolic pathways (glucose balance, fatty acid metabolism, amino acid metabolism, ammonia detoxification and bile acid synthesis amongst others) all perturbed by ischaemia during transplantation.
- Extended criteria donor livers are less tolerant to ischaemia and this may be reflected in metabolic disturbances that can be identified through metabolomics.
- Normothermic machine perfusion is rapidly gaining clinical interest as it enables the functional recovery of cold stored donor organ prior to transplantation and offers a unique platform for transplant viability assessment at near-physiological parameters.
- Viability assessment can be enhanced objectively by metabolic profiling of these livers.

Methodology – Machine perfusion of discarded ECD livers

- The marginal donor liver population investigated was derived from the clinical trial for the Viability Testing and Transplantation of Marginal Livers (VITTAL) using NMP-L (NCT02740608).
- Trial designed to assess feasibility and safety of NMP to increase the number of transplantable livers from those rejected for transplantation by all transplant centres on initial evaluation post-procurement.
- Livers procured with the initial original intention for transplantation and then rejected for transplantation by all UK liver transplant centres due to the perceived marginality of the graft, offered to our centre for inclusion.
- All livers deemed marginal according to established high-risk criteria and underwent NMP-L for a minimum of four hours, at which point a decision was made to transplant based on our unit's established viability criteria.

Viability Criteria for transplantation

Major criterion
Perfusate lactate <2.5mmol

AND two or more of the following
Bile production
Perfusate pH>7.30
Evidence of glucose metabolism
Hepatic artery flow >150ml/min
and portal vein flow >500ml/min
Homogenous perfusion

Methodology – Metabolomics and Statistical analysis

Perfusate aliquots collected during first 4 hours of NMP-L

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Untargeted metabolic profiling of primary metabolites using Ultra-High-Performance Liquid Chromatography

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Data analysed using Metaboanalyst (web-based software for analysis of metabolomics data)

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Multivariate and univariate data analysis to determine degree of variability between metabolic profiles of groups of samples and main metabolites responsible for variability

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Panel of metabolites as markers of transplant viability and clinical outcomes

Results

31 livers high-risk discarded donor livers were received by our centre and subjected to end-ischaemic normothermic machine perfusion for viability testing. Of these, 25 livers fulfilled viability criteria within 4 hours of perfusion. 22 of these livers were transplanted.

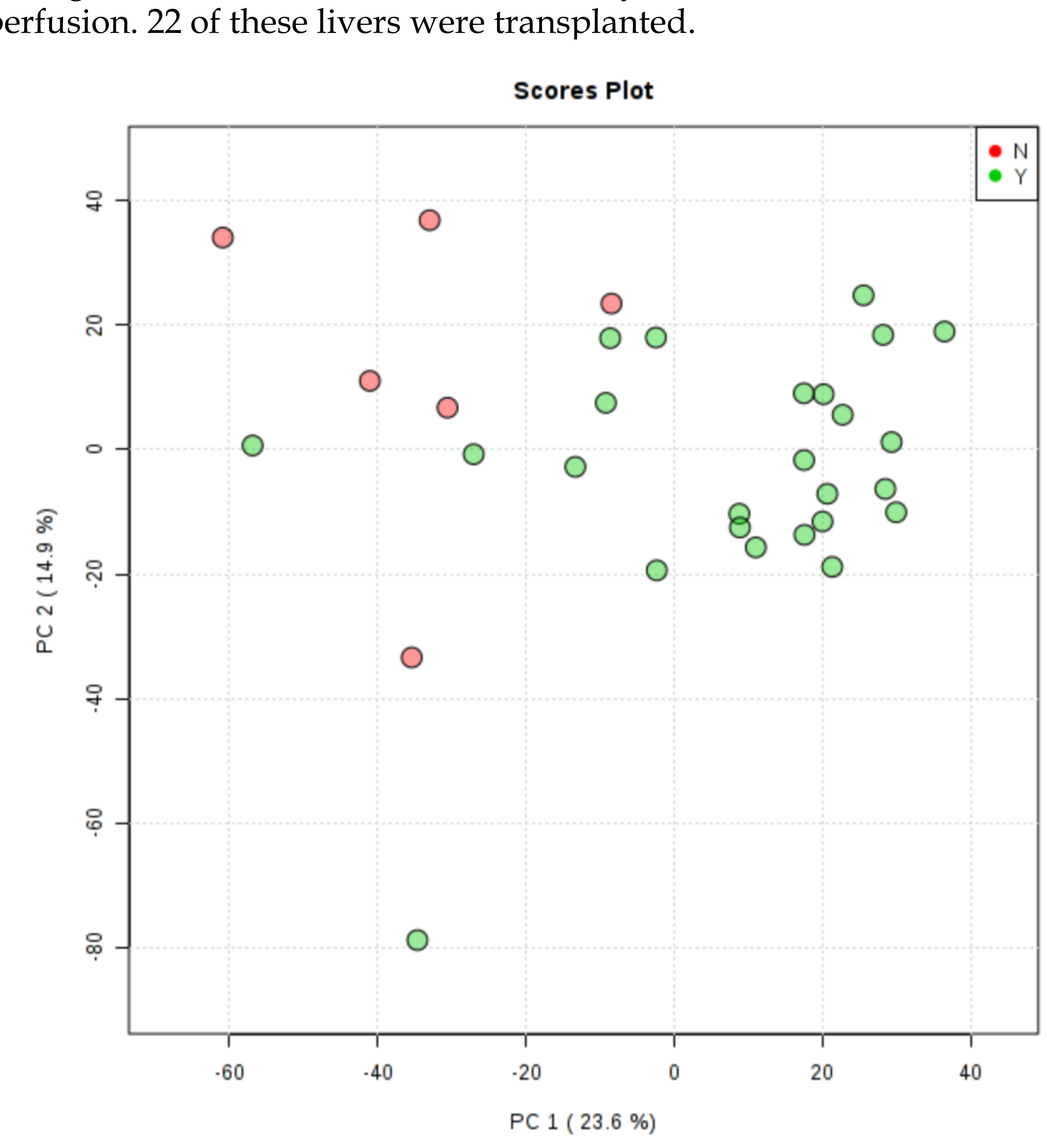

Figure 1

Figure 1 is a plot of the multivariate data analysis or Principal Components Analysis of donor liver metabolic profiles following 4 hours of NMP-L. This describes the variance in the metabolite dataset according to the main variables or principal components (PCs). Each point is a donor liver metabolic profile. The plot shows separation of viable (n=25, green) versus non-viable (n=6, red) donor liver metabolic profiles, illustrating metabolic dissimilarities separating the two groups.

Univariate analyses revealed key changes in metabolites relating to lipid and phospholipid metabolism, notably upregulated in the non-viable group ($q<0.05$) indicating significantly different liver metabolic profiles according to viability criteria fulfilment following 4 hours of perfusion. In the transplanted cohort; changes in 52 metabolites distinguished EAD (n=7) from non-EAD livers (n=15) after 4 hours of perfusion. The PRS group (n=10) revealed changes in 36 metabolites after 4 hours of perfusion compared to non-PRS group (n=12).

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