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Project report for Oxford Transplant Foundation

"Systematic study of effects of duration of brain death on serum markers of cerebral injury in the heart-beating organ donor"

Project background

Kidney transplantation provides the most cost-effective treatment for end-stage renal failure and allows transplant recipients to return to a normal lifestyle. Cadaveric organs from donors after brain death (DBD) or circulatory death (DCD), with the inclusion of extended criteria donors (ECD), remain an important source of organs. The immunological, physiological and systemic changes during the period of donor management in intensive care and their implications on the long-term graft performance and survival have not been systematically characterised yet. Cerebral injury is not only a feature of DBD but due to insufficient (or absent) systemic circulation occurs in DCD. Understanding the pathological processes in the donor following brain death will also form a basis to our understanding how cerebral injury contributes to organ outcomes in the non-heart beating donor. Twelve-month outcome data is available for all selected donors.

This study aimed to provide a first systematic assessment of the changes in serum biomarkers detectable in the donor serum during the period of donor management in hospital. It would allow for a first systematic characterisation of the serum biomarker changes associated with catastrophic cerebral injury and allow us to study how serum biomarkers of cerebral injury change over time. In the context of traumatic brain injury, neuronal and glial proteins can be detected outside of the central nervous system (CNS) early on. This study therefore also studies serum marker changes of such CNS proteins around the time of brain death to complete a neuro-inflammatory time course.

Methods

The Quality in Organ Donation (QUOD) UK biobank was used for sample selection. Serum samples are available across four time points from admission to organ donation. Sample selection was undertaken to identify donors in the biobank with similar aetiology of brain death (isolated intracranial haemorrhage) and exclude patients with other sources of inflammation such as polytrauma or documented infection. Donor samples covering a duration of brain death between 3 and 40 hours were available in the biobank at the time point of donor selection. Samples were selected to allow us to study changes over this duration of brain death. Donors were matched not only for their cause of brain death but also for age, co-morbidities (hypertension, diabetes) as well as body mass index (BMI).

R&D DuoSet ELISA kits were used to the manufacturer's instructions to measure cytokines (Il-6, TNF-alpha), complement C5a, neuronal (NSE) and glial (GFAP) breakdown products changes in duplicates. To create a cumulative average analysis, the average measurement for each time point was log-transformed first. GraphPad Prism v8 and RStudio were used for data analysis and creation of graphs as well as statistical analysis.

Results and discussion

1. Selection of donor cohort

Sample selection was undertaken to identify donors in the biobank with similar aetiology of brain death (isolated intracranial haemorrhage) and exclude patients with other sources of inflammation such as polytrauma or documented infection. Donor samples covering a

duration of brain death between 3 and 40 hours were available in the biorepository at the time point of donor selection. Samples were selected to allow us to study changes over this duration of brain death. Donors were matched not only for their cause of brain death but also for age, co-morbidities (hypertension, diabetes) as well as body mass index (BMI).

2. CNS proteins detected in donor serum

The admission levels measured in our donor cohort were measured and compared to previously published values from traumatic brain injury literature. Table 1 summarises the results and provides a comparison to previously published thresholds associated with adverse outcome. As all patients included in our cohort proceeded to organ donation their initial injury was associated with the adverse outcome of brain death. Of note was the large standard deviation in our cohort, however due to the pragmatic nature of sample collection the duration from admission to brain death diagnosis was between 8 and 220 hours unlike the timed sample collection approach used in most TBI studies (e.g. 6h post injury). The results confirmed that we were able to detect CNS proteins – neuronal and glial breakdown products – in the biobank serum samples and that the levels at admission were comparable to previously published thresholds associated with adverse outcome.

Table 1 Comparison of measured serum levels at admission with published thresholds for adverse outcome after TBI/CVA. (n=27 donors, measured in duplicates)

	Mean +/- SD	Levels associated with adverse outcome thresholds in TBI/CVA
NSE	15.58 +/- 21 ng/ml	20 +/- 14 ng/ml (El-Maraghi et al, 2013)
GFAP	8.38 +/- 25 ng/ml	1.69 ng/ml (Lei et al, 2015)
Il-6	344.7 +/- 699 ng/ml	21.9 ng/ml (Antunes et al 2010) 6h post injury
TNF-alpha	352.4 +/- 860 ng/ml	?
NB admission duration before BD 8-220 hours		

3. Time course analysis

To understand serum changes over time, we measured n=3 time points for each donor (admission, after confirmation of brain death, before procurement). The pragmatic nature of sample collection for QUOD meant that each sample had a different relative time point. The only time point that was comparable between individuals was the confirmation of brain death (DB2). We therefore set DB2 as time = 0 and plotted all values in relation to this time point. Fig 1 shows the serum levels of all n=27 individual donors, each donor shown in a different colour. The individual serum levels were then combined to create a cumulative average analysis to study the overall trend of serum changes over time, as shown in Fig. 2. For each of the measured serum proteins, this analysis allowed us to study how serum levels change over time and particularly how brain death and the subsequent donor management period are characterised. NSE, a marker of neuronal damage (Fig 2A) showed constant levels from admission to procurement, whilst GFAP which shows damage to glial cells (Fig 2B) demonstrated a rise around the diagnosis of brain death with a slight increase in serum levels throughout donor management. As glial cells outnumber neurons in the central nervous system this can reflect the effect of the initial injury in the CNS with spreading of oedema and damage to glial cells surrounding the initial injury. This swelling leads to the eventual brain stem damage and coning process, represented by the increase in glial damage around brain death and subsequently. Fig 2C and 2D show how the pro-inflammatory cytokines TNF-alpha and Il-6 change over the same period. TNF-alpha shows a steady decline from admission onwards, whilst IL-6 mirrors the changes seen with glial damage around brain death. Of note, there does not appear to be a further rise in serum IL-

with prolonged duration of brain death. Complement C5a serum levels (not shown) mirror the step change around brain death seen in GFAP and the further rise after prolonged donor management. In summary, our data shows very distinct patterns of change in serum levels indicating that rather than a widespread and progressive pro-inflammatory activation, intracerebral haemorrhage leading to brain death is characterised by distinct inflammatory pathways which are activated. In addition, whilst the time surrounding brain death appears to show a peak in pro-inflammatory IL-6 levels, the subsequent donor management period does not show a progressive increase in this proinflammatory cytokine. To understand this further, it will be important to study how anti-inflammatory molecules change over this period.

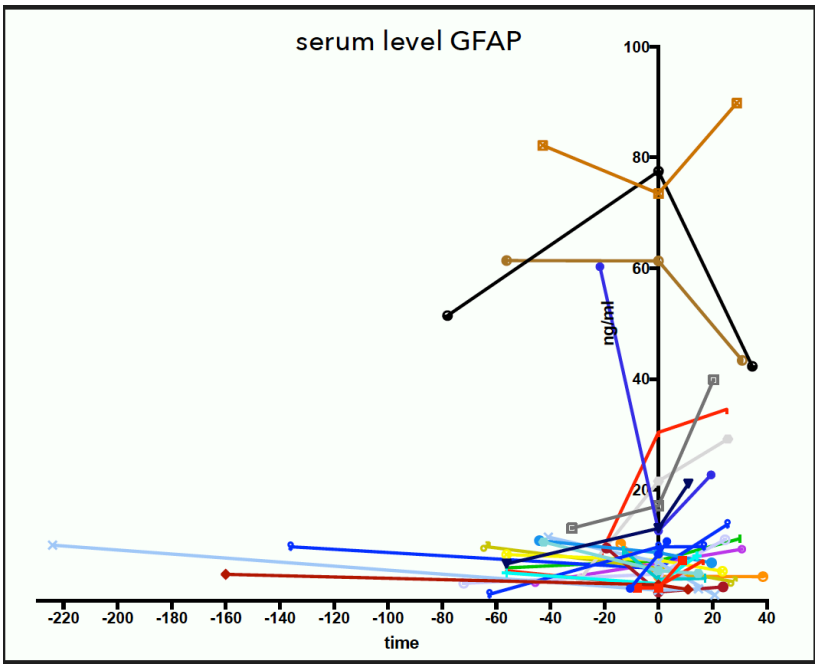

Figure 1 Individual donor time courses of serum levels of GFAP (n=27, three time points per donor, each time point measured in duplicates)

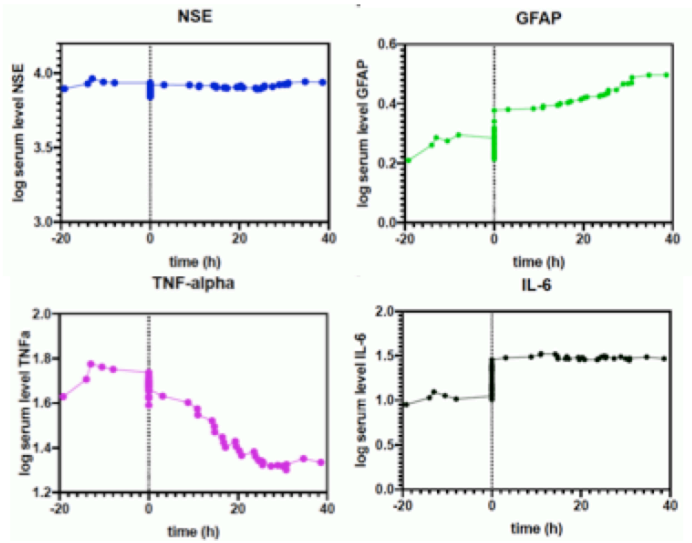

Figure 2 Cumulative average analysis to study serum changes over time, shown for NSE, GFAP, TNF-alpha and Il-6 (n=27 donors in total with 3 time points per donor, each time point measured in duplicates)

4. Development of modeling approach

In parallel to the analysis of measured serum samples, we have worked on creating models that can demonstrate different serum changes over time and whether using 3 samples per donor in a cumulative average can allow to infer serum changes. We assumed that possible time courses over the study period can theoretically can take one of the following four shapes: (1) either a constant level, (2) a step change (around time of brain death), (3) a peak or trough (at time of brain death, Fig 3C) or a (4) steady increase following brain death and created models that included 27 individual time courses. Each of the models was centred around the time point of brain death confirmation, however there was an off-set 'jitter' used to reflect that the confirmation of brain death by clinicians does not represent a defined pathophysiological 'time point' and can therefore not be use as the the exact point of irreversible brain injury and brain death. For each hypothetical time course, three random time points were selected to mimic the sampling process used to obtain the donor serum samples (Fig 3D). Finally, the cumulative average of those three time points was calculated (Fig 3E). This allowed to compare the experimentally obtained data and cumulative average analysis (Fig 3B) with the modelled approach (Fig 3E). In the next steps we are working on developing an approach to quantify the degree of correlation between experimental and modelled data.

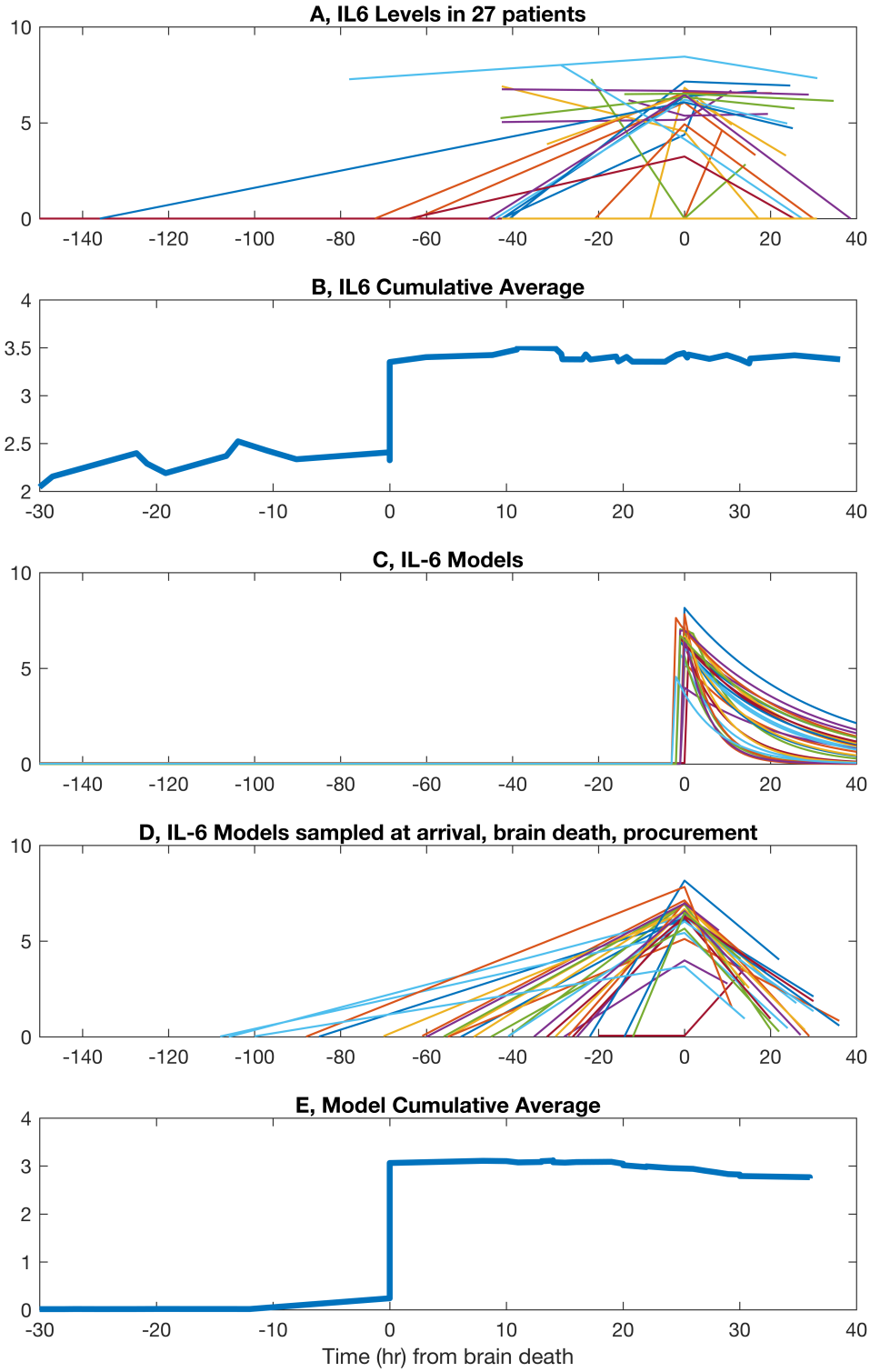

Figure 3 Development of modelling approach to infer chronological serum changes from n=3 samples per donor and comparison to measured serum levels, shown for IL-6. Experimental data is shown in 3A and B (individual donor serum levels and calculatated cumulative average) and modelled data is shown in 3C-E (Fig created by Dr J Tabak)

Future directions

The results of this pilot project have revealed interesting findings about the changes in neuroinflammatory proteins in the serum of donors. It was possible to detect neuronal and glial protein breakdown products.

Future work will include the study of serum marker changes in a wider variety of heart-beating donors to understand if there are specific changes that reflect different injury mechanism. The combined approach of modeling with measured serum levels allowed to infer chronological changes – future work will further validate this combined approach and allow to use it for more complex measurements such as –omics or similar to create a comprehensive catalogue of serum changes surrounding brain death.

We aim to create more detailed models of time course analysis of the serum and plasma changes to identify pathways that can be targeted. The understanding of changes over time will allow us to correlate those with recipient outcome data to identify which pathways might play a role for example with regards to delayed graft function and also long term organ function and survival.

Project output

1. Presentations and publications

2018

- Oral presentation at American Transplant Congress (ATC) 2018 in Seattle, USA
- Oral presentation at 5th National QUOD meeting in Cambridge, UK
- Local presentation at OUCAGS Forum in Oxford, UK

2019

- Oral presentation at British Transplant Society 2019 Congress in Harrogate, UK
    - shortlisted for Herrick Society Prize
    - selected as one of Top 6 abstracts
- Poster presentation at American Transplant Congress 2019 in Boston, USA
- Abstract submitted to ESOT 2019 conference

- Project write up for publication – ongoing

2. New collaborations and projects resulting from current project

- Collaboration with Dr Joel Tabak Sznajder of University of Exeter for complex data analysis and creation of data models
- Collaboration with Professor James Kennedy to collect and study serum samples from Amici study to compare serum neuroinflammatory marker changes in patients with stroke who survive – future work