Reflective Report- Summer 1

Introduction

This project was aimed at identifying an interdisciplinary outlook towards the failure of brain energy usage or distribution as one of the possible explanations for delirium; a severe neuropsychiatric disorder causing patients to become highly disoriented. While the disorder remains relatively misinterpreted, this project has allowed me the chance to explore the concept of disrupted brain energy metabolism as a possible cause for cognitive dysfunction. This report details my personal and professional progress exploring this hypothesis by various means and includes my experiences as a researcher and a leader.

Part one: Research Project Experience

Delirium may be defined as an impairment of consciousness, characterized by an inability to sustain, or shift attention[1]. It is known to encompass cognitive dysfunction, altered psychomotor activity and significant emotional distress. These episodes also significantly increase duration of hospitalization. It is a highly prevalent condition but the lack of formal labelling of the syndrome in hospital data, however, has accounted for its widespread under-representation despite its prevalence. While the public awareness of the syndrome has increased of late, its underlying pathophysiology still remains relatively misunderstood, causing a rise in the amount of literature available, exploring all of its possible underlying mechanisms.

Its etiological triggers vary from infection and systemic inflammation to trauma, surgery, and hypoglycemia, as an explanation for disturbed energy supply[2]. As a result, the incidence of delirium has been investigated in various clinical settings such as post-operatively or after an

[1] Wilson et al. (2020). Delirium. Disease Primers, Nature Reviews. 6:90

[2] Engel, G. L., & Romano, J. (2004). Delirium, a syndrome of cerebral insufficiency. 1959. The Journal of neuropsychiatry and clinical neurosciences, 16(4), 526–538.

acute inflammatory insult. The relationship of delirium in the context of impaired glucose supply and uptake is also of note and has been linked to insufficient cerebral glucose levels, further leading to cognitive dysfunction.
This project has aimed at investigating all the possible mechanisms of disrupted cerebral energy by the means of an exhaustive review and the practical assessment of cerebrospinal fluid biomarkers in dementia/delirium post an acute inflammatory insult.

This literature review (while not yet completed) is in progress towards submission of a review article and encompasses many possible explanations for disrupted brain energy/ blood supply. Delirium's varied etiology has caused an interest to investigate a few ways by which it is understood that disrupted brain energy metabolism could come about.

a) **Neuromonitoring-** Cerebral blood flow and cerebral hypoxia is an important possible driver for impaired cerebral metabolism further developing into cognitive dysfunction. Certain popular methods such as 18-Fluorodeoxyglucose positron emission tomography (FDG-PET), transcranial doppler, arterial spin labelling have been vastly used in studies to measure impaired cerebral blood flow and oxygen saturation as precursors to cognitive imbalance.

b) **Glucose availability/uptake-** Some important hypotheses backing the onset of delirium revolve around a few topics. One being defective insulin signaling and insulin resistance. Disrupted insulin signalling can leave brain cells more prone to failure, contributing to cognitive dysfunction. Another important topic discusses the impaired ability of the brain to take up glucose, due to improper functioning of glucose transporters. Glucose is taken up by the brain via two essential transporters: GLUT1 and GLUT3. Impairment of these transporters may limit available brain glucose with consequences for the brain. This has in turn, also caused us to investigate the relationship between diabetes and delirium.

c) **Acute inflammation-** Any type of acute inflammatory insult is likely to contribute to a disrupted energy supply in the brain leading to possible cognitive deficits.

These cognitive variations may reflect in the form of biomarkers in the blood, the brain, and the cerebrospinal fluid. The close monitoring of varied levels of metabolites may be observed to support the above hypotheses and draw useful conclusions.

Noteworthy observations expected to be important while investigating biomarkers for delirium are increased lactate, choline and levels of ketone bodies and lipids in delirious patients. Some of these metabolites point to the employment of an alternative energy pathway, validating the hypotheses.

To investigate these findings practically, cerebrospinal fluid samples of patients that had been exposed to an acute inflammatory insult (such as a hip fracture) were processed by the means of nuclear magnetic resonance (NMR) spectroscopy. NMR spectroscopy is a powerful metabolic tool used to compare the levels of important compounds in order to distinguish the pathophysiology among distinct patient groups. As a part of this, 20 cerebrospinal fluid samples were collected across 2 cohorts of hip fracture patients; patients with dementia/confusion and patients without dementia/confusion. These patient groups were designed to provide insights into the effects of systemic inflammation on dementia, and how it might trigger delirium.

Group 1 (n=10)	**Group 2 (n=10)**	**Group 3 (n=10)**	**Group 4 (n=10)**
Hip fracture	Hip fracture	Hip fracture	No hip fracture
dementia	No dementia	No dementia	No dementia
confused	Not confused	confused	confused

Table 1: CSF sample cohorts

The ^{1}H NMR spectra was obtained on an 18.8T NMR spectrometer. The acquired data was then pre-processed by the means of a software known as NMR ProcFlow and further analysed using software MetaboAnalyst.

The metabolic patterns of both patient groups were compared by the means of plots such as a Principal Component Analysis (PCA), Partial least squares discriminant analysis (PLS-DA) (validated by 5-fold cross validation) and volcano plots.

The pairwise comparisons of Group 1 and 2 samples revealed the following results-

- The PCA analysis mostly showed overlap between Group 1 and 2 samples, which may be attributed to the metabolic effects of systemic inflammation experienced by both groups. The few outliers present in the plot may be reflective of slight metabolic differences distinguishing patients with dementia and confusion and those without.

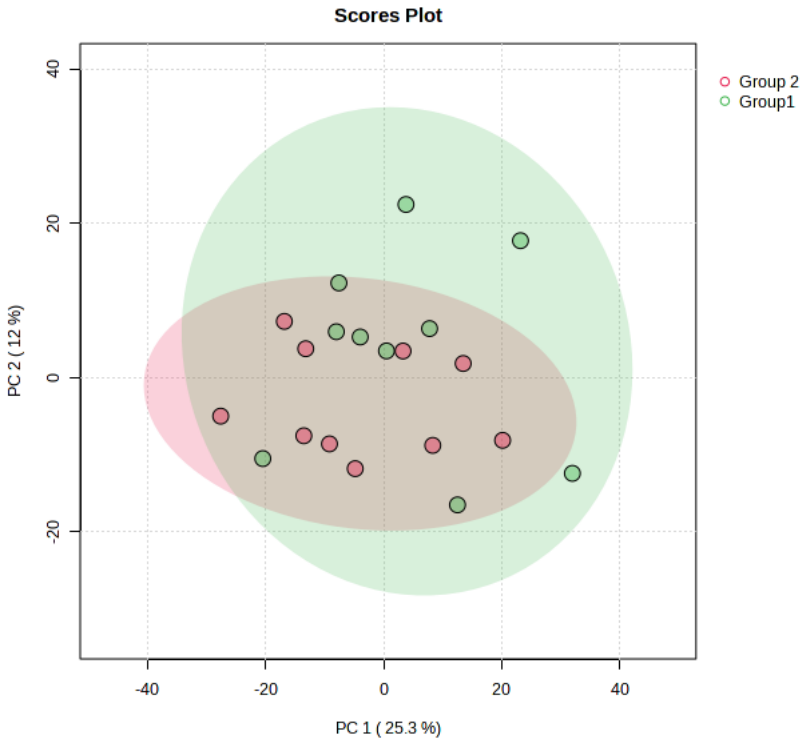

Figure 1: PCA analysis of Group 1 and Group 2 cohorts

- The PLS-DA analysis showed a significant separation between both Group 1 and Group 2 datasets indicating a substantial difference in the metabolic profile of patients with dementia and confusion (group 1) and those without dementia and confusion (group 2).

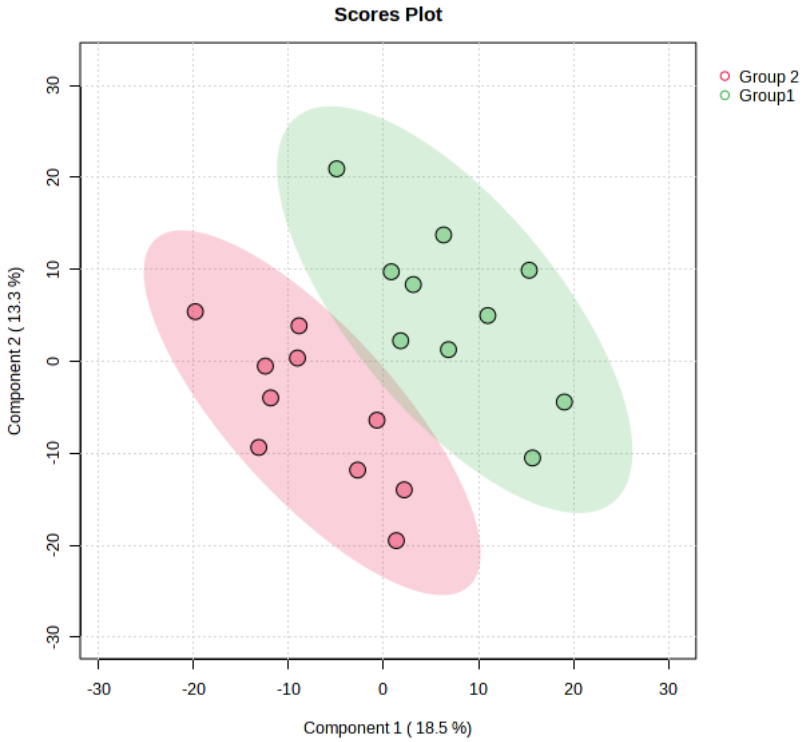

Figure 2: PLS-DA analysis of Group 1 and Group 2 cohorts

Additionally, the data was validated by 5- fold cross validation. The Q^2 values for this comparison were found to be quite low (Q^2= 0.054) indicating that this sample size does not appear to be significant enough to produce a predictive model. Using an increased number of samples may possibly yield better results.

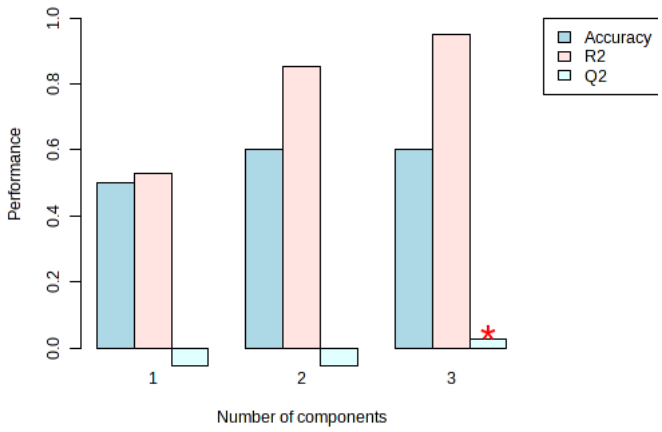

Figure 3: 5- fold cross validation of PLS-DA for Group 1 and Group 2 cohorts

Finally, a volcano plot also revealed the significant alterations in some metabolites in the comparison of Group 1 and Group 2 samples, in the form of metabolic bins.

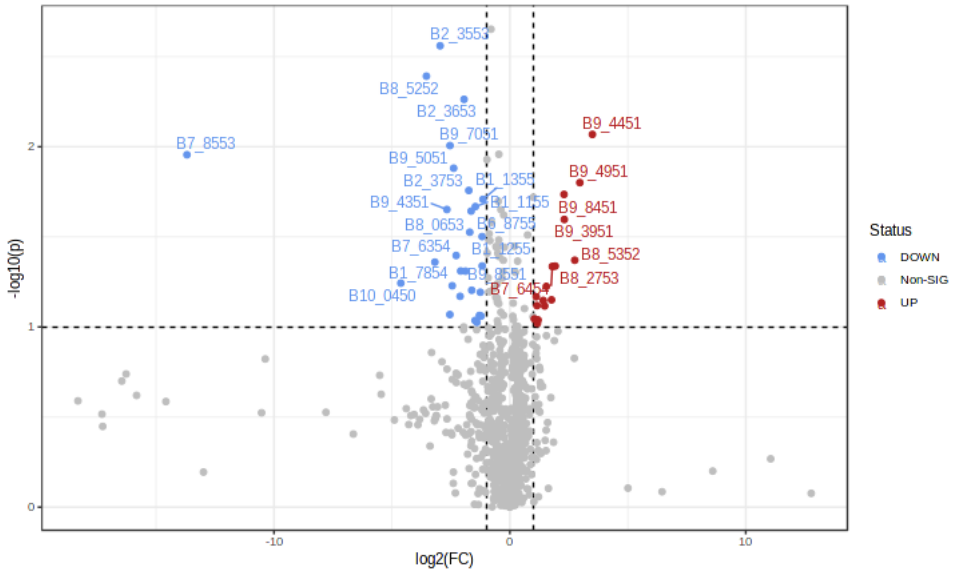

Figure 4: Volcano plot analysis Group 1 and Group 2 cohorts

Statistically significant points in this plot would be those that serve as outliers on the plot. These points can further be processed via the human metabolome database (HMDB) to draw further conclusions about changing metabolomic concentrations in patients with dementia and confusion in responses to systemic inflammation.

While there is still a lot to look in to with respect to delirium in terms of how it comes about and how it may be predicted better, I have achieved significant progress in attempting to understand the various mechanisms that cause delirium and am in the process of writing a review that covers the same. Based on the NMR analysis as well as my own research, a disrupted energy supply can be considered to provide impetus for subsequent cognitive decline and delirium. With this research, I also aim to be one step closer to increasing the awareness about this disorder and improve prognostic outcomes.

Part two: Leadership Attributes and Personal Development

This project has been an opportunity for immense learning and research skills but also, a chance to develop myself as a person and a leader.
In terms of final outcomes for this project, I have successfully been able to statistically analyze cerebrospinal fluid samples and draw important conclusions regarding disturbed brain energy metabolism via an extensive literature review.

I did encounter a few roadblocks as a part of this project. However, I made sure to persevere and not let them hinder my progress. Firstly, the NMR analysis did not fit the time period planned. It started much later than expected due to increased temperatures in the NMR room. Due to these unprecedented changes, the spectral peaks obtained with the data were not as sharp. While this did cause quite a significant delay in my project plan, I was able to continue processing samples after two weeks, allowing me to gather useful data at a later point.
I was also unable to analyze pre-clinical model data (mouse datasets) as a part of this project (as mentioned in my project proposal). However, I have made significant contributions analyzing the available clinical data making this experiment more fruitful than I would've imagined it to be. While these logistical issues did cause a delay with respect to time constraints and data analysis, I learnt the value of patience and resilience. I tried to focus on what was in my control, which proved to be very beneficial.

Being an independent self-led project, I was fortunate enough to get plenty of opportunities to be independent at various points throughout the course of my project. This further helped me reflect on my leadership style and what I think my attributes as a leader are. I was able to take responsibility of writing the review independently, pushing me out of my comfort zone. I was made to think analytically and attempt to derive valuable conclusions from my readings. I was also in charge of running my own experiments on the NMR spectrometer and was responsible for optimizing the machinery to fit the requirements of each sample. This experience strongly reflected development in terms of the PDP goals I had set for myself before this project. One of these goals was to be able to make decisions more

effectively and quicker. Being responsible for my CSF samples made me a lot more independent in my thought process, allowing me exercise full control over my data and its analysis.

While reflecting on my research experience over the summer, I am reminded of the project management workshop we were lucky enough to attend as a part of the LEAD1 training day in May. That workshop resonated with me in terms of organizational leadership. I sometimes struggle with project organization and time management, especially with respect to lengthy projects like this one. This workshop however was very valuable as I was able to respect the importance of thorough planning before and during a project, allowing me to optimize my results and handle time more efficiently. Luckily, I was able to apply these insights to my project allowing me to organize all my tasks in a systematic manner and yield the best possible outcomes.

Going into this project, I had a very different perception to research and how I would work as an independent researcher. I often assumed I would have the answers to everything and instinctively know what to do every step of the way. This project helped me realize that this was not the case. It's often challenging and intimidating starting out with research at an early stage. However, it is normal to ask for help and support through the process. Towards the beginning of my project, I felt like I was more dependent on guidance from my supervisor and was reliant on help to structure my project and its components. With time, I got more confident in my abilities, both with respect to reviewing scientific articles and being able to independently run the NMR spectrometer. Eventually, I was also able to carry out my statistical analysis for the NMR data completely independently giving me a broad insight into my leadership style.

Conclusion: Areas for Expansion

This project has been a valuable and unforgettable experience in terms of independence, leadership, and research skills. I think I've been able to achieve significant progress with regards to understanding delirium and the mechanisms that surround it. I am now able to back the possible hypothesis that a disruption of brain energy metabolism can be considered as a possible driver for delirium. Having said that, there is much we don't know about the syndrome and its biomarkers. The NMR analysis has yielded some useful results but as with all scientific experiments, it is not free of its fair share of limitations. While there is still a lot to explore, (the brain itself being relatively unexplored), I'm looking forward to continuing research in delirium.

In terms of personal growth, I have achieved significant progress in terms of leadership qualities I think I've built up over time such as communication skills, project management and thinking outside the box. However, as many things in life, this too is an ongoing process, one that I hope to nurture further with my Leadership in Action experience next summer.