Franziska Denk - Cerebrospinal fluid – a window into how our central nervous system processes pain?

Summary: This project is collecting cerebrospinal fluid from patients who are undergoing implantation of high frequency stimulators as a treatment for neuropathic pain. We will analyse both the cells that are in the fluid, as well as the proteins. The Pain Relief Foundation has agreed to support examination of particular immune cell types, so that we can maximise the information we receive from every one of our participants who are so generously donating their fluids for research. Other aspects of this study are supported by the BRC and the Rosetrees Trust.

Rationale: Chronic pain can be caused by abnormal activity in the nervous system at various locations. Sensory nerves which transmit information from our body to the spinal cord can become hypersensitive in a pain state. Equally, in the spinal cord, there can be abnormal activity in the complex networks of nerves that integrate the information that comes in from our body. And finally, in disease states, our brain is no longer able to effectively fine-tune the sensory information that comes in from our bodies – making it more likely that we will experience pain.

Not all this dysfunction is due to nerves alone. From animal work, there is good evidence that immune cells play an important role as well, especially so-called microglia in the spinal cord. These specialised immune cells are normally a cross between cleaners and security guards in the central nervous system. They mop up the occasional dying cell and make sure that no pathogens or viruses enter. However, in a pain state, they become unusually active, and we know that this is not a good thing: it is thought that activated microglia will start harming healthy neurons and worsen the abnormal activity in spinal nerves that is already present in disease. Soothing or deactivating microglia is therefore one treatment option that many scientists have considered over the years. However, one issue with this idea is that we do not have a very good way of looking at these cells in humans. There are imaging techniques, but they are somewhat limited by the lack of microglial-specific markers. Another potential option is to examine cerebrospinal fluid.

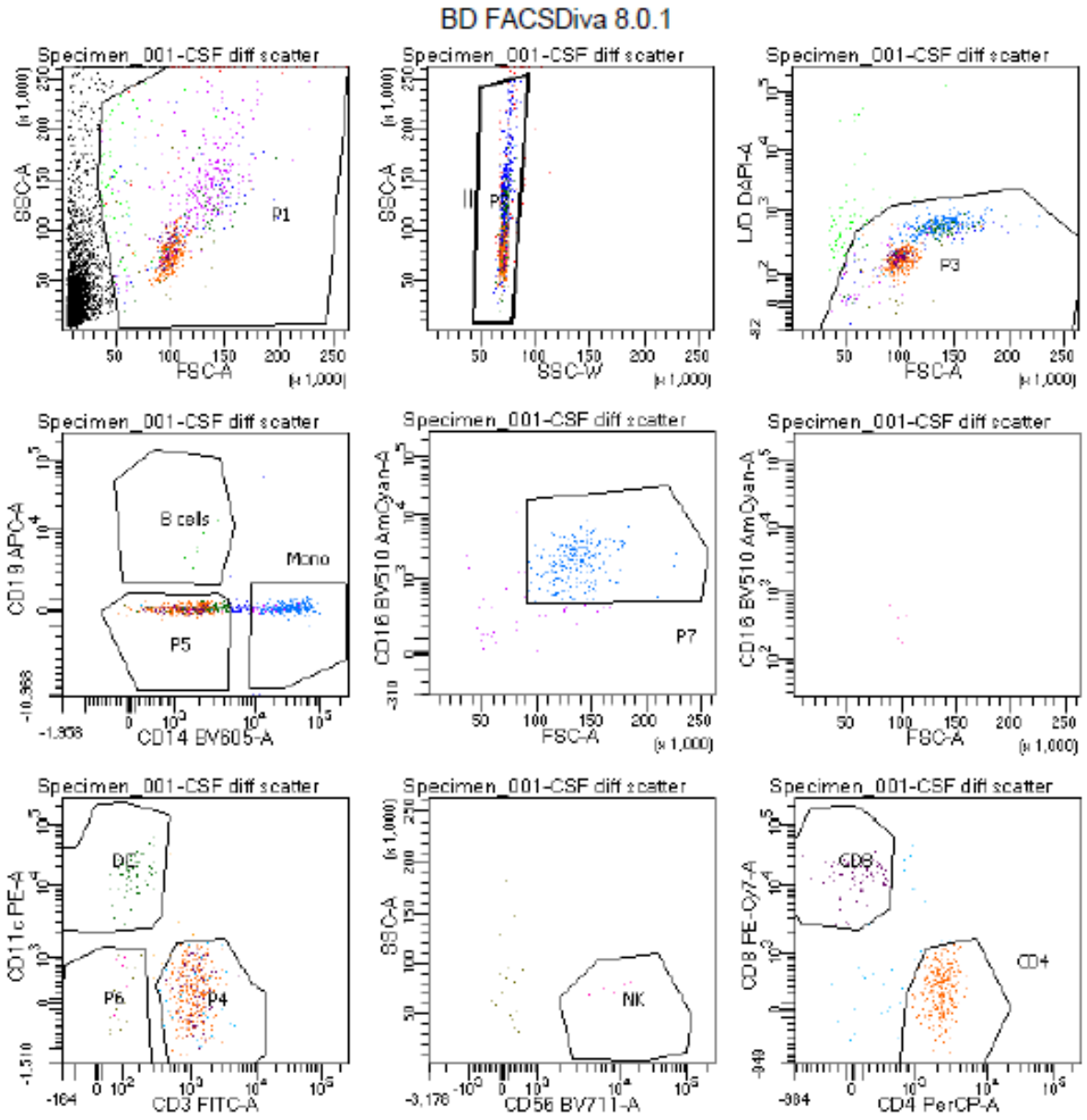
Cerebrospinal fluid (CSF) is the liquid that our brain and spinal cord is bathed in. It supplies our nervous system with nutrients and clears away its waste products. It also contains immune cell types. No cells can cross between CSF and spinal cord, but substances can – which makes it possible that we can use the CSF to capture cross talk between spinal cord microglia and CSF immune cells (mostly monocytes and T cells).

Approach: To test this, we are studying individuals who are undergoing spinal cord stimulation surgery for chronic neuropathic pain. In neuropathic pain, there is a lot of microglial activation in animals, and we expect this to decrease in patients in which the treatment is successful and the pain decreases. By examining CSF, we hope to capture this change. We will also test whether we can measure microglial activation proteins in CSF and whether CSF immune cells are in a resting or an activated state. Our study has received all the relevant ethical approvals, and patients who agree to take part will fill out questionnaires about their quality of life as well as their pain, both before and after implantation of the stimulator when we will collect CSF.

Results to date: Our study commenced in summer 2019. We first had to set up the protocols that allow us to sort CSF immune cells. This is not a trivial task: CSF has very few cells which are sensitive and likely to die if not processed within two hours of harvesting. We are very lucky that at King's

College London we have a wonderful team of clinicians on site, as well as the facilities to sort our patient samples within the hour. An example of what a CSF sort looks like is given below. We have confirmed that the cell types we collected are pure and of good quality using a technique called qRT-PCR and a machine called a Bioanalyser.

With this in place we have begun patient recruitment. To date, four individuals have donated CSF samples for our study. We thank them for their trust in us, and will do our utmost to gain as much information from their CSF as possible. We are securely storing batches of their sorted cells and CSF proteins until recruitment is completed and will then analyse all the samples in parallel. This is very important to avoid technical bias being introduced into our various tests by processing them at different times.



Fluorescence-Activated Cell Sorting (FACS) of Human CSF. Different immune cell types can be distinguished in human CSF using antibody staining in a FACS machine. We sorted monocytes, CD4+ and CD8 T cells, as well as B cells.