Feasibility of Neurapheresis™ Therapy for Multidrug Resistant Gram-negative Bacterial Meningitis

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Background

The World Health Organization has identified *Pseudomonas, Acinetobacter* and *Klebsiella* (PAK) as three multidrug resistant (MDR) gram-negative pathogens that pose a threat to human health. The greatest threat lies in hospitals, nursing homes, and patients with devices such as intravenous catheters and ventilators. Gram-negative bacterial meningitis (GBM) manifests when these bacteria invade the central nervous system. Due to increasing antibiotic resistance and the high mortality associated with MDR GBM, we have tested a closed-loop, extracorporeal cerebrospinal fluid (CSF) filtration system (Neurapheresis™ Therapy) for its applicability in this context. ***Here we demonstrate feasibility of Neurapheresis Therapy for MDR GBM and characterize system parameters for bacterial, endotoxin, and cytokine clearance.***

Methods

- Bacterial cultures grown separately, diluted to 1x10^7 cells/mL in 150 mL Luria-Miller broth or artificial CSF
- Solution passed through tangential flow or dead-end filters in a single-pass or closed loop paradigm (Fig. 1)
- Sampling: immediately post filter in single pass experiments, after each cycle in closed loop experiments
- Bacterial load quantified via CFU counts, endotoxin via Limulus Amebocyte Lysate (LAL) assay, cytokines via Luminex assay

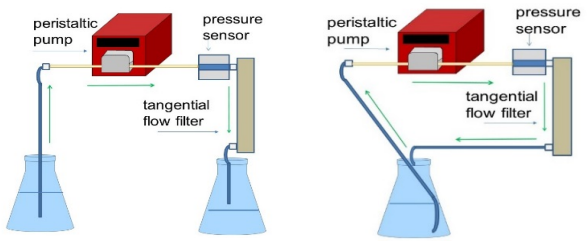

Figure 1: Experimental setups. Schematic of single pass experiments (Left). Schematic of close loop paradigm (Right).

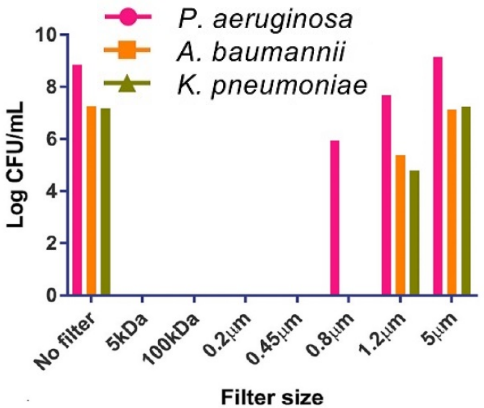

Figure 2: Size Exclusion. PAK was passed through TFF and dead-end filter paradigms (single-pass) to evaluate the ability of each to completely eliminate PAK.

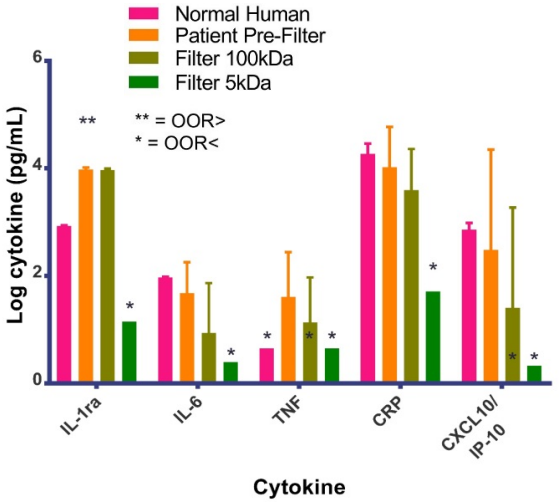

Figure 4: Cytokine Filtration. Preliminary data demonstrating the ability of the 5 kDa and 100 kDa filters to remove key neuroinflammatory agents.

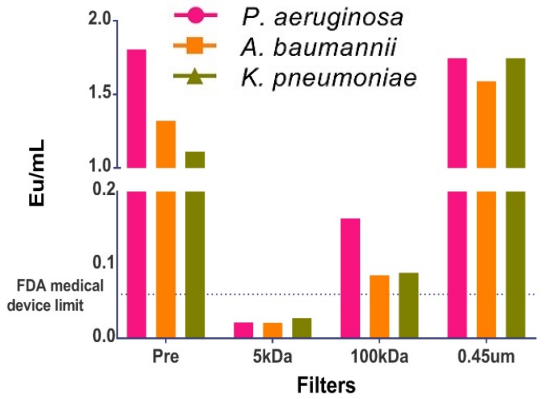

Figure 3: Endotoxin Filtration. The LAL assay was used to quantify the reduction of endotoxin after a single pass through 5kDa TFF, 100 kDa TFF, and 0.45 um dead-end filters.

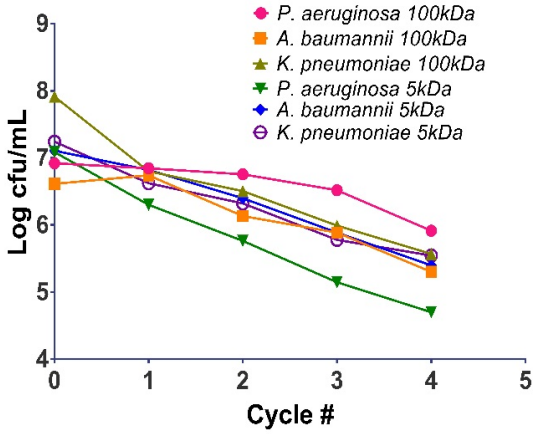

Figure 5: CFU Reduction. Using a closed loop system, both 5 kDa and 100 kDa filters were capable of reducing PAK CFUs during a 5 hour time course.

Results

- Complete removal of bacteria with filters 0.45µm or smaller (Fig. 2)
- >99% reduction of endotoxin with 5kDa filter, >95% with 100kDa filter, single-pass (Fig. 3)
- 5kDa filters reduced cytokine load 2 log (>99%) (Fig. 4)
- 1-2 Log CFU (90-99%) reduction of all bacteria over 4 filtration cycles (Fig. 5)

Conclusions

Neurapheresis shows potential to be an efficient multi-modal tool for controlling and treating MDR GBM in this *in vitro* model. Extending closed loop filtration over time demonstrates capability for rapid sterilization of the CSF. Future studies will include *In vivo* experiments to assist in the development of a human Neurapheresis system tailored to MDR GBM removal (Fig. 6). Future iterations may include adjunctive intrathecal drug delivery to further accelerate elimination of bacteria. ***Reduction of bacteria, endotoxin and cytokines by Neurapheresis may have significant implications for controlling the damaging neuro-inflammatory response during MDR GBM.***

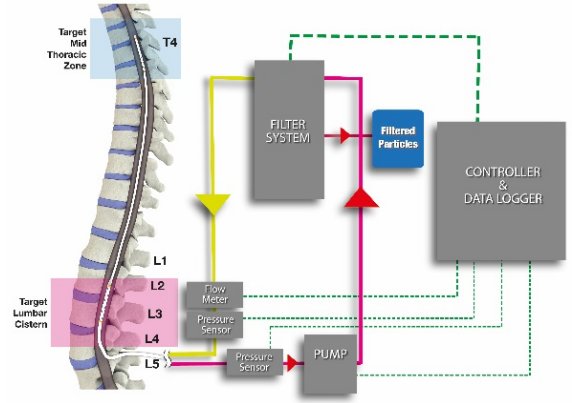

Figure 6: Human system schematic.

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