Monomeric C-reactive protein and inflammation in vascular dementia

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Research Question: Does monomeric C-reactive protein drive inflammation and cognitive decline in vascular dementia?

Outline: Vascular dementia (VaD) represents the second leading cause of dementia after Alzheimer's disease, but, despite this, there are no symptomatic treatments for the disease. As such, there is an urgent need to identify the pathological mechanisms underlying VaD, in order to develop effective therapeutic strategies. Multi-infarct dementia, defined as cognitive impairment resulting from multiple infarcts in both the white and grey matter following occlusions in cerebral arteries and arterioles[1], is considered the most common form of VaD[2,3]. Current evidence suggests that inflammation plays a critical role in the pathogenesis of multi-infarct dementia[4], and, consequently, identifying and blocking relevant inflammatory mediators represents an attractive therapeutic strategy.

C-reactive protein (CRP) is an acute phase protein produced in the liver, and circulating levels are significantly elevated in VaD patients[5,6]. However, it is currently unknown whether CRP expression purely correlates with VaD progression, or actively contributes towards its pathophysiology. There are two distinct isoforms of CRP. Native CRP is a soluble pentameric oligoprotein found in circulating plasma and upregulated during active inflammatory disease. Native CRP can irreversibly dissociate to form non-soluble, tissue-bound monomeric CRP at sites of vascular damage/inflammation[7]. Native and monomeric CRP signal through different receptors and therefore differ in their biological effects. Native CRP promotes an anti-inflammatory phenotype in endothelia, whereas monomeric CRP promotes a pro-inflammatory phenotype[8]. Importantly, we (Slevin) have recently shown that pro-inflammatory monomeric CRP accumulates at sites of cerebral infarction in human VaD samples[9,10] **(Fig 1)**, indicating that monomeric CRP is an important regulator of ischaemia-associated neuroinflammation and a relevant therapeutic target in multi-infarct dementia.

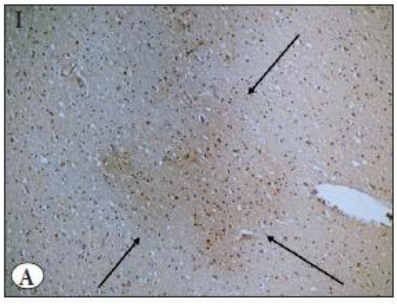

Figure 1: *Immunohistochemical staining for monomeric CRP (DAB, brown) showing increased expression within a micro-infarcted area (arrows) of a mixed-dementia brain tissue sample. Image reproduced from Slevin M et al 2017[10].*

Methodology: In order to interrogate the contribution of monomeric CRP to cognitive decline in multi-infarct dementia we (Strangward & Allan) are developing a novel murine model of the disease. In the aforementioned experimental model, the common carotid of C57BL/6 mice is exposed to a strip of filter paper saturated with ferric chloride ($FeCl_3$, 30% m/v) for 3 minutes, followed by extensive washing in saline. This results in the formation of a thrombus that occludes the carotid. The carotid is then subsequently manipulated mechanically in order to promote rupture of the clot, recanalization of the carotid and embolization of the subsequent micro-thrombi within the deeper brain microvasculature **(Fig 2)**. This results in multiple cortical and subcortical infarcts/lesions within the relevant hemisphere, consistent with multi-infarct dementia **(Fig 2)**. Infarction is induced in both hemispheres in two surgical windows separated by a two week interval, resulting in multiple, bilateral

infarcts, again consistent with the clinical picture. Moreover, unlike existing rodent models of VaD, this model mimics the pathophysiology of human disease i.e. atherosclerotic plaque rupture followed by microthrombi embolization, arteriolar occlusion and cerebral infarction.

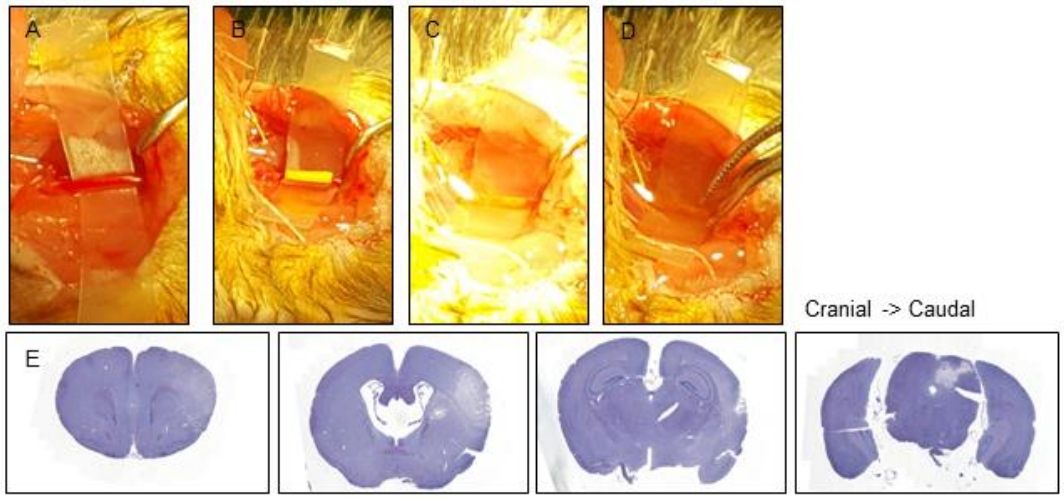

Figure 2: (A-D) *Representative images of the different surgical steps in the multi-infarct dementia model.* ***(E)*** *Representative H&E images 24 hours post-surgery in a C57BL/6 mouse, demonstrating multiple cortical and subcortical infarcts of restricted size.*

MICRA/Seedcorn funding will be utilised to cover salary costs for the lead applicant and early career researcher Strangward to characterise the cognitive deficits present in this novel murine model of multi-infarct dementia. Specifically, two weeks after the second surgical procedure, cognition in multi-infarct and sham mice (n=8 per group) will be assessed utilising the radial arm maze, Morris water maze and Barnes maze.

Expected Output: This pump-prime funding will enable completion of critical preliminary work required to acquire external grant funding (MRC and BHF October 2018 deadlines) and an ARUK junior fellowship (July 2018 deadline) researching the role of monomeric CRP and other novel therapeutic targets in multi-infarct dementia.

Summary of costs: Costs are requested for: 7 weeks salary = **£5880**

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