Title: A randomised, double-blinded, controlled study with sham and crossover, of Percutaneous Transluminal Angioplasty (PTA) for extracranial vein stenoses in patients with Multiple Sclerosis.

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Objectives: To report on the study design and interim findings of the safety and efficacy of venous PTA in MS patients with extracranial vein abnormalities.

This study requires 160 participants, 80 in the treatment and 80 in the control group to allow for meaningful statistical analysis. We report on our experience and findings of the first 28 enrolled to date.

36 patients were consented, 4 (9%) were found not to have treatable abnormalities, 4 were not within the eligibility criteria and 28 were consequently enrolled.

Participants were randomised into the treatment or control arm and followed up for 24 months. At the start of the study participants in the control arm had the sham procedure where the balloon was not inflated but they crossed over to the treatment arm and had the ballooning at 12 months. The participants, the consulting neurologists and radiologists reading the images were blinded to the time of the PTA procedure.

To test the effectiveness of the procedure participants had three types of vein imaging, ultrasound, Magnetic Resonance Venography (MRV) and Digital Subtraction Angiography (DSA) at the start of the study and at 6,12,18,and 24 months. They underwent neurological testing of disability (EDSS), cognition/memory (CogState, PASAT), fatigue (FSS) and Quality of Life at the start of the study and at 1,3,6,12, 13, 15, 18 and 24 months. Three types of imaging were used because there are limitations with each type.

The procedure was found to be safe. There were 10 procedure related adverse events, bruising, groin and cannulation (needle) pain and headache were reported. Two participants relapsed; one was in the control arm and required treatment, the second had increased tingling at the base of the head soon after the balloon procedure but did not undergo treatment.

The blinding, recruitment and follow-up were not compromised over the duration of the study. Only 4 participants were not able to complete the study up to 24 months.

No conclusions can be drawn from the interim analysis because the number of participants is small. All that can be reported are trends. For the majority of the follow-up tests performed no difference was found between the two groups. The tests for which a trend to a difference was found were the neurological assessment EDSS and two out of the seven cognitive test (CogState). A difference was also found with the participants-self-reporting on Quality of Life. Improvement was found in the treatment arm but not the control arm. Imaging analysis has not been completed to date.

Laymen's abstract
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Maria Marcella Laganà

Title: Combined study of neurodegeneration, cerebrovascular reactivity and venousdrainage impairments in Parkinson's Disease and Multiple Sclerosis

Multiple sclerosis (MS) and Parkinson's disease (PD) are two central nervous system disorders whose exact causes remain poorly understood and for which no cure is available. While the diseases manifest themselves in considerably different ways, both are characterized by progressive, irreversible structural/functional loss of brain neurons, termed neurodegeneration. The available treatment options for MS and PD have little to no effect on this component of the disease. This is particularly unfortunate as it is the neurodegenerative aspect which in large part drives disability over the long term. The exact mechanisms that are involved in MS- and PD-related neurodegeneration remain poorly understood. However, it has been proposed that neurovascular changes may be in part responsible for neurodegeneration in MS and PD. Moreover, alterations of the veins draining the blood from the brain have also been noted in these patient populations. Although the consequence of such findings is still a matter of debate, it is plausible that they may result in upstream changes with respect to brain tissue alterations. It is possible that extra-cranial vasculature pathology may also lead to intra-cranial vasculature changes in the form of altered perfusion characteristics and dilatory capacity of blood vessels (i.e. cerebrovascular reactivity (CVR)).

We have been studying a cohort of MS patients, PD patients and healthy controls using magnetic resonance imaging (MRI) for estimating the brain perfusion rate (cerebral blood flow, CBF) and the brain drainage flow rate. Our aim is to test the association of extra-cranial drainage impairment and perfusion/CVR. CVR was estimated with magnetic resonance imaging, acquiring the brain perfusion at rest compared with that obtained while breathing a gas mixture with 5% of carbon dioxide, referred to as a hypercapnic stimulus. The latter tends to increase CBF, which can subsequently be compared to CBF under normal conditions as a means to evaluate CVR.

During the first year of the project we recruited 49 of the 85 planned subjects. The MS group is almost completed, while the recruitment of PD group will be finished during the second year.

The results of our preliminary analyses on 20 MS patients compared to 20 age- and sex-matched healthy controls (HC) showed no differences in the total venous flow rate. A lower CBF in MS patients was found in anterior cingulate and paracingulate gyri. The correlation between the total venous flow rate and CBF was widespread in HCs but drastically reduced in MS patients. The hypercapnic stimulus produced a global and significant increase of CBF in both the groups, but the perfusion changes expressed by the CVR index were not significantly different between the two groups.

Although our results should still be considered preliminary, they warrant further investigations. As such, we will complete the acquisitions in the second year as planned. We also wish to recruit another group of MS with greater disability as well as to investigate the relationship with neuropsychological measures. This analysis may help shed further light on the mechanisms involved in these diseases as well as their relationships to patient outcomes.

Clarifying the mechanisms between neurodegeneration and intra-/extra-cranial vasculature changes will help construct a more complete picture of MS- and PD-related pathological processes. This is likely to help in the development of novel therapeutic strategies.

Diagnostic and prognostic use of neurolymphatic biomarkers in Multiple Sclerosis

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Multiple Sclerosis (MS), the leading cause of permanent neurologic disability in young adults (Ramagopalan & Sadovnick, 2011), is increasingly being viewed and studied not only as a group of immune-mediated central nervous system (CNS) demyelinating diseases, but as *vascular* neuroinflammatory conditions. The complex pathogenesis of MS can only be appreciated when and if vascular contributions are recognized as significant features of all forms of MS etiology (**Yun et al., 2016**). Based on our previous report "***Inflammation induces neuro-lymphatic protein expression in multiple sclerosis brain neurovasculature***" (Ganta et al., J. Neuroinflammation, 10, 2013:125) we proposed that disturbances in the expression and organization of 'neurolymphatic' / 'glymphatic' features of blood brain barrier (BBB) vessels represented important and under-recognized characteristics of the MS inflamed cerebrum that contributed to progression and intensification of the MS with the brain endothelial cells as the 'failing gatekeeper'. Although the presence of serum/plasma borne biomarkers have now been used to diagnose and discriminate different forms of MS, the assumption has been that the presence of neurolymphatic markers in blood reflected: 1) a brain endothelial origin because of the intimate endothelial association of the vascular surface and the blood, 2) segregation of these markers in 'microparticles' ('***Blood circulating microparticle species in relapsing-remitting and secondary progressive multiple sclerosis. A case-control, cross sectional study with conventional MRI and advanced iron content imaging outcomes'.*** Alexander et al., J Neurol Sci. 2015;355(1-2):84-9.) Because of the perception that the CNS lacks lymphatics, studies on lymphatic involvement in MS pathogenesis have largely neglected roles brain lymphatics play in eliminating CNS fluid and mediators. CNS interstitial fluid and solutes can drain along 150-200nm wide '*lacunae*' in the basement membranes of arteries and capillary walls (Carare et al., 2008; Kida, Pantazis, & Weller, 1993; Weller, Djuanda, Yow, & Carare, 2009; Weller, Engelhardt, & Phillips, 1996; Weller, Galea, Carare, & Minagar, 2010; Weller, Kida, & Zhang, 1992), representing a conduit for elimination of lymphatic contents. More recently Nedergaard defined brain-wide pathways that facilitate the clearance of interstitial components from the brain, termed the 'glymphatic' system (Iliff et al., 2013; Iliff et al., 2012; Yang et al., 2013). So far molecular features or cellular origins of these networks remain uncharacterized but represent important diagnostic, staging and therapeutic targets. To further investigate this paradigm we have now validated the expression of a large panel of conventional neurolymphatic structural proteins (podoplanin, lymphatic vascular endothelial hyaluronic acid receptor (LYVE-1)), signaling modules (vascular endothelial growth factor-3 (VEGFR-3), vascular endothelial growth factor-D (VEGF-D)) and transcription factors (forkhead transcription factor C2 (Fox-C2), and Prospero homeobox-1 (Prox-1)) in both mouse and human brain endothelial cells consistent with the presence of these markers in both brain endothelial cells and in plasma/serum from clinical and experimental MS. We further proposed and were funded intramurally for investigation of an additional set of neurolymphatic biomarkers including FOXC2, integrin a9, Cdk5 and reelin). We proposed 4 aims over 2 years. Aim 1: Demonstrate that blood-borne microparticles are the major source of brain blood vessel-derived neurolymphatic biomarkers released during clinical and experimental forms of neurovascular stress. Aim 1a. Determine which 'NEBuLA' are constitutively expressed in human and mouse BECs. Aim 1b. Determine how brain endothelial expression of NEBuLA expression changes in experimental MS (exposure to inflammatory cytokines or MS serum). We have now completed Aim 1a 1b and will investigate how MS plasma and serum influence BEC NEBuLA release. In Aim 2. Determine the expression patterns/levels and vectorial release of neurolymphatic markers in microparticles from healthy individual and compare with a collection of clinically defined specimens and experimental sera. We have completed half of Aim 2a and 2b (Measure levels of apically released microparticles (AMPs) released by control, cytokine-treated and MS plasma / serum treated BEC; Aim 2b. Measure and compare NEBULA+ MPs release in response to human and experimental MS plasma/sera and

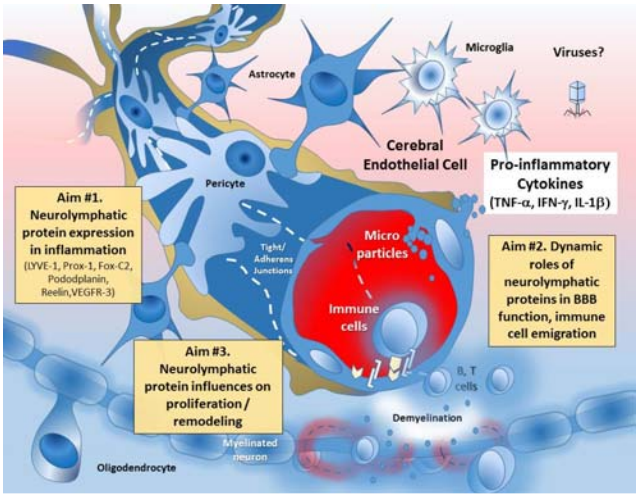

Fig. 1. General description of Aims 1, 2 and 3. BBB associated lymphatic proteins are reorganized in neuroinflammation with effects on transvascular barrier, immune cell migration and vessel remodeling.

inflammatory cytokine-primed human and mouse brain endothelial cells in culture). We have largely finished Aim 2c. (Measure the amounts of basolateral microparticles ('BMPs') released by 3D cultured human and mouse endothelial cell using transwell models of brain microvasculature) and have validated basolateral release of microparticles as an important feature of neurovascular stress. We have now determined lymphatic protein expression levels and spatial distribution patterns in brain endothelial cells (BECs) under basal and inflammatory cytokine activated states. We have now also demonstrated that the exposure of human and mouse brain endothelial cells to inflammatory cytokines provokes changes in the expression of neurolymphatic biomarkers and alters the distribution of these proteins within microparticles released by brain endothelial cells (**Aims 1, 2**). Under a material transfer agreement with Buffalo Neuroimaging Analysis Center (BNAC, Drs. Murali Ramanathan, Robert Zivadinov) >300 serum samples from RRMS, SPMS and matched controls have been obtained by flow cytometry analysis. Drs. Heinz Weindl and Luisa Klotz (University of Münster, Münster, Germany) have similarly agreed to provide a cohort of >100 clinical MS and control samples (**Aims 1, 2**). Importantly additional clinical and MR imaging studies for these samples is available and may be applicable in creating more comprehensive patient profiles. In Aim 3 (Demonstrate the roles of NEBuLA in brain endothelial barrier function) we have obtained all reagents for Aim 3a. (Study specific NEBuLA in brain endothelium using directed siRNAs to evaluate their contributions to blood brain barrier) and have begun Aim 3b. (Use VEGFR-3 kinase blockers to examine how VEGFR-3 signaling influences brain endothelial barrier.) In year 2 we will make progress for Aim 4. (Evaluate the ability of statins to diminish release of NEbuLA expressing microparticles and rescue barrier disturbances produced by cytokines and MS plasma / sera.)

CEG Study (Cardiovascular, Environmental & Genetics) risk factor study – Multiple Sclerosis

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Multiple Sclerosis (MS) is a disease affecting the nervous system (brain and/or spinal cord). More than 80% of the patients present with bouts of exacerbation and remissions and a minority have a slow progressive decline in neurologic function from onset. It is known to be an immune mediated process where the myelin, a covering of the nerve cells, can be destroyed by the person's own immune system. In the last decade, various studies have attempted to study an alternative cause of MS due to the presence of abnormal neck veins. These neck veins are responsible for draining blood out of the brain.

An ongoing follow-up study conducted at our institution aims to study a group of MS patients over 5 years. It is investigating the influence of these venous abnormalities on worsening clinical outcome in MS patients. We are reporting the results of an interim analysis of this study. 83 MS patients were enrolled of which 47 patients were relapsing remitting subtype and 36 patients had progressive MS. The average age of the patients was 53 years. The clinical outcomes were measured using a widely accepted disability scale (Expanded Disability Status Scale). The difference in the scores from the first visit (baseline) to the 5 year follow-up was calculated. Using a set of preformed guidelines, an ultrasound of the neck was done to determine the presence of any abnormalities in the veins, both at baseline and follow-up. Our study did not find any associations between these venous abnormalities and development of clinical disability in MS patients over 5 years.

The results presented here are preliminary and thus, should be interpreted with caution. To the best of our knowledge, this is the first and longest follow-up study investigating these associations and their implications on the managing MS patients. Over the course of this study, we hope to better understand the complex relationship between the abnormal neck vessels and the clinical characteristics of MS patients.