**Technical Note**

**A technique to assess perineuronal mediators**

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**Abstract**

Perineural activity of a variety of inflammatory and immune system mediators can activate peripheral nerves leading to the perception of pain. One example of such effects includes the activity of interleukin 1 beta (IL-1β); this inflammatory mediator, upon binding to IL-1R1 neuronal membrane receptors will rapidly induce protein kinases in damage-sensing neurons, consequently altering heat-activated ionic inward currents leading to increased neuronal sensitivity to harmful heat[1](#_ENREF_1).

The ability to detect such mediators in proximity to sensory nerves is therefore crucial to investigating the contributing roles of inflammation in human chronic pain. To date there is no recognized method to assess mediator profiles around human sensory nerve roots *in vivo*.

A novel method is described that can assess these mediators in the human trigeminal system where the nerve leaves the brain stem in its pre-ganglionic portion.

Mediator levels are shown to change between sample locations on the trigeminal nerve root in patients with trigeminal neuralgia.

This methodology may therefore be used to shed insights as to the pathophysiology of trigeminal neuralgia, which may in turn influence clinical decisions concerning the natural history, and treatment options.

Introduction:

Inflammatory mediators in the vicinity of sensory nerves may sensitize, and activate sensory nerve fibres, resulting in heightened sensitivity to painful, and non-painful stimuli, and even in spontaneous pain [1](#_ENREF_1),[2](#_ENREF_2). However to our knowledge, there is no recognized technique to assess inflammatory mediator profiles around sensory nerve roots in humans *in vivo*.

Trigeminal neuralgia (TGN) is a severe facial pain syndrome often associated with neurovascular conflict or compression between the trigeminal nerve and an offending artery or vein. Surgical intervention (‘microvascular decompression’) to resolve nerve-vessel contact is accepted treatment for this condition when refractory to medical treatment either by reason of lack of efficacy or side effects – usually cognitive – of medication.

The aetiology of TGN is not fully established. Although mechanical displacement and contact of vessel with the nerve is important, such “conflict” is also be found in approximately 10% of healthy individuals[3](#_ENREF_3). Moreover some patients have TGN without neurovascular conflict, either primarily, or with returning pain following surgery. In other pain conditions associated with nerve damage, the painfulness of the condition is correlated to the concentration of inflammatory mediators around the damaged nerve, as established by immunohistochemical staining of nerve biopsy samples.[2](#_ENREF_2) It is unknown whether such mediators are also present around the site of neurovascular “conflict” in TGN. , This location is not accessible to traditional sampling methods. A proof of principle study was therefore conducted in five patients, to assess whether it is feasible to measure mediators by obtaining suitable fluid samples from the site of the neurovascular conflict in patients with primary trigeminal neuralgia.

**Methods and Materials:**

The study was conducted following ethics committee review and approval (NRES NW 08/H1002/23), in accordance with the principles of the declaration of Helsinki.

*Patients*: These were patients undergoing microvascular decompression for trigeminal neuralgia. Patients with systemic inflammatory or autoimmune diseases, CVA within 6 month before surgery, or with any other intracranial disease were excluded. Patients were provided information leaflets at least 24 hours before discussing the study, and then written informed consent for the additional albeit minor procedure was obtained.

*Sampling procedure*: The trigeminal nerve was identified without touching it during the dissection process. The sampling procedure was abandoned if there was evidence for bleeding. The following procedure was used only if there was a clear conflict between vessel and nerve; the procedure thus preceded the decompression part of the operation, in which the vessel is dissected free from and mobilized away from the nerve.

An individual piece of cottonoid material was cut to a size of approximately 3x3mm– - and held “gently” in contact at one of four sites - the nerve conflict site, the trigeminal nerve root proximal and distal from the conflict site, and the cerebellum (presumed negative control). This was done without additional aspiration. Macroscopic contact with blood was avoided. Samples were taken from all four sites wherever possible, though the anatomical configuration of the nerve and vessel could make this impractical. The “cottonoid” material is identical to that used routinely as a “neurosurgical pattie”.

Sampling microcentrifuge tubes, which have a small removable net inserted on their top (Microcon 30kDa Centrifugal Filter Unit with Ultracel 30 membrane), were pre-filled with 40ul DH20, and then before the start of the operation were placed on ice in a container in the operation room, with lids left open, and net placed on top. The cottonoids were then removed, and each placed into the net of an individual sampling tube. As sampling was completed, the tubes were transported on ice to the research laboratory (3minutes), and spun at 10000rpm for 5 minutes at 4C, to sample the fluid soaked up in the cottonoids on the bottom of the tube. The net was then removed. Samples were stored frozen at -80C for later analysis. This procedure yielded between 10ul and 15ul of fluid per site. Once all samples had been obtained, they were simultaneously analyzed as per manufacturers instructions on a Luminex ® reader system using a multi bead cytokine kit (Luminex Human Ultrasensitive Cytokine Ten-Plex, Invitrogen).

**Results**

Five patients were included. All patients happened to be male, and had primary, classical trigeminal neuralgia. Samples from conflict-, and cerebellum control-sites were taken from all five patients; distal zone samples were available from four of these patients, and proximal zone samples from two patients. In three cases the position of vascular contact was too close to the origin of the nerve from the brain stem to permit a more proximal sample, and the converse in the case from which no distal sample was feasible. Most mediator concentrations were below detectable range, or were very low (not shown). Interferon-gamma was raised in the distal zone; each of the distal zone samples had returned higher values than any of the samples from the other zones. Some samples also showed raised concentrations for tumor necrosis factor alpha (Table, sensitivity for both mediators <1pg/ml).

**Discussion**

A novel method for collecting mediator-*solute* from the pre-ganglionic trigeminal nerve root in humans in vivo for ex vivo analysis is described.

In that the results show some clear differences between areas of the nerve for certain mediators, the results suggest that the method does appear to genuinely give information concerning these mediators.

The trigeminal nerve was chosen, because it is exposed on a regular basis for the treatment of various conditions, but in particular for trigeminal neuralgia in which one element at least of the pathology is situated at the point of exit of the nerve root from the brain stem – at the so called root entry zone, the point at which central myelination transitions for peripheral myelin. This seemed therefore an ideal site at which to develop the methodology for assessing mediator levels, as an immune activation in response to the vessel in conflict with the nerve might be expected.

Although most mediators remained below test-threshold, the methods picked up increases in interferon gamma and tumor necrosis factor alpha in some samples. We show that mediator-concentrations for Interferon-gamma, but not other mediators are increased differentially between sample locations along the trigeminal nerve root in patients, suggesting that the method may be sensitive to location-specific factors.

Although the method does seem to give real information and to show promise, there are a number of areas in which the methodology needs to be improved so that reliable and meaningful data can be acquired.

It is difficult to establish reliable quantification of the mediators, in part as the nerve is bathed in CSF, which may variably dilute or cross-contaminate the samples. The numbers are small; neither the size nor the absorption characteristics of the cottonoid were standardized in this work, but approximate only. In that the cottonoid is held in position manually it is possible that differential pressure occurred between samples. No attempt was made in this work to control for these variables as the main aim was to see if molecules could be recovered and subsequently measured at all; the use of a pipette-based aspiration method might improve test-accuracy. A further limitation was that any effects from dissection to display the nerve cannot be controlled for. As the nerve itself was not manipulated, we consider the risk for such confounding low.

If confirmed, then this technique may be useful for future translational research aiming to correlate the symptomatology in chronic pain conditions with local immune activation, including in trigeminal neuralgia and radiculopathies.

Further work would be methodological – in making sure that results were reproducible and quantifiable – for example to control the size of the cottonoid Additionally inclusion of samples from patients undergoing posterior fossa surgery with normal trigeminal nerve and no trigeminal pain may be informative to control for the effects of unspecific factors on the mediator concentration.

It would then be necessary to correlate results with the clinical and radiological features such as length of history, the classification of the TGN (typical or atypical [4](#_ENREF_4)), radiological features derived from MRI imaging, possible neurophysiological correlates such as nerve conduction, trigeminal evoked potentials[5](#_ENREF_5), activity of the ganglion [6](#_ENREF_6), quantitative sensory testing[7](#_ENREF_7), and medication use[8](#_ENREF_8). This should all be considered alongside theories of the pathophysiology of TGN.[9](#_ENREF_9) Finally a better understanding of the role of local inflammation may support surgical decision-making.

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