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Characterisation of the effects of pulsed radio frequency treatment of the dorsal root ganglion on cerebrospinal fluid cellular and peptide constituents in patients with chronic radicular pain: A randomised, triple-blinded, controlled trial

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SUMMARY

Introduction: Chronic radicular neuropathic pain is a major clinical problem with a life time prevalence of more than 50%. Pulsed radiofrequency (PRF) treatment is a recognised therapy. However, the pathophysiology of chronic neuropathic pain (CNP) and the mechanism of action of PRF remains ill-defined. Improving our knowledge of the mechanisms of CNP and PRF action will enhance our ability to treat patients with this common debilitating problem more effectively. This study aims to characterise the CSF cellular and peptide constituents in patients with CNP and the effect of pulsed radiofrequency (PRF) on these constituents and reported pain.

Materials and methods: Prospective randomised tripled-blinded control trial of patients receiving PRF treatment versus sham for radicular pain. All patients received local anaesthetic to the appropriate dermatome to confirm diagnosis. Clinical assessment using standard clinical assessment tools and examination of CSF using flow cytometry and ELISA for cellular and peptide constituents was carried out before and 3 months after treatment.

Results: Ten patients were randomised to PRF ($n = 5$) or Sham ($n = 5$) treatment. PRF resulted in a significant reduction in pain score (NRS) at 3 months (6.8 to 2.6, $p < .05$). PRF reduced the TNF-α concentration and CD3+ count in CSF. CD4/CD8 ratio of patients with CNP was lower than historical controls (1.4 versus 3.0–4.2). The majority of CD3+ cells in the CNP patients were activated effector memory cells (80%) versus the surveillance central memory cells (85%) seen in healthy controls.

Conclusions: PRF is superior to local anaesthetic administration for the management of radicular pain and is associated with CSF constituent modulation in vivo. Patients with CNP have lymphocyte characteristics which suggest immune activation.

1. Introduction

Radicular pain is a common neuropathic pain in one or more of the cervical, lumbar or sacral dermatomes. It is a major socioeconomic issue and has a lifetime prevalence of more than 50%.(Hartvigsen et al., 2018) It may become a chronic pain, lasting more than 3 months in 30% of cases. The most frequent cause of radicular pain is disc herniation in patients less than 50 years old, and degenerative spinal disease in the over 50's.(Van Boxem et al., 2010; Van Zundert et al., 2010) Significant short-lasting pain relief may be achieved with an injection of local anaesthetic around the nerve's dorsal root ganglion (DRG). Steroids may be added to the local anaesthetic to enhance the effect of the block, but this delivery method of steroids is associated with a number of serious complications including spinal cord ischaemia, paraplegia, and epidural abscess.(Quraishi, 2011) However numerous studies have shown transforaminal epidural steroids to be safe and may produce a therapeutic benefit in radicular pain in both acute and chronic pain states. (Kesikburun et al., 2018; Lee et al., 2018)

Clinical studies support the effectiveness of PRF treatment to the DRG in cervical and lumbar radicular pain with an excellent safety profile.(Van Zundert et al., 2007; Van Boxem et al., 2015) This suggests that the DRG plays an important role in persistent radicular pain and is a potential target for local neuromodulation. Pulsed radiofrequency (PRF) is a high frequency current applied in pulses. The temperature at

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the electrode tip does not exceed 42 °C, and thereby avoiding tissue damage. However, it is worth noting that other studies of PRF to the DRG in radicular pain have resulted in a modest therapeutic effect only. (Shanthanna et al., 2014a) Recruitment difficulties have made achieving powering end points of these studies clinically difficult. Preclinical studies in radicular pain have investigated the molecular changes associated with PRF to the DRG and dorsal horn of the spinal cord and they include increased expression of c-Fos and activating transcription factor 3, enlarged endoplasmic reticulum cisterns and cytoplasm vacuoles in the DRG cells, changes in myelin configuration, reduced glial cell activation, and increased endogenous opioid levels at the dorsal horn.(Shanthanna et al., 2014a; Calvo et al., 2012) Neuropathic pain is strongly associated with neuroimmune changes at the DRG and dorsal horn of the spinal cord.(Calvo et al., 2012; Van Boxem et al., 2014)

The mechanism of action of PRF therapy in vivo remains unclear. Our group previously published a prospective, observational study of PRF treatment to the DRG in patients with chronic radicular pain.(Das et al., 2018) Analysis of cerebrospinal fluid samples from 8 patients who receive PRF treatment for radicular pain revealed a significant reduction in Natural Killer (NK) cell ($CD56^+CD3^-$) frequencies 3 months post treatment (7.5% vs. 0.1%, $p = .03$). In the same study, there was a reduction in IFN-γ levels and an increase in $CD8^+$ T cell frequencies and IL-6 levels post treatment. These results suggest that PRF has a neuromodulatory role at the level of the DRG and this correlates with a reduction in pain intensity. The main limitation of this study was the observational design and lack of a control arm.

This current study is a randomised, triple-blinded, sham controlled study to explore further the results of our observational study. We investigated the neuroimmune changes in the lumbar cerebrospinal fluid following PRF of the DRG in radicular pain. This included analysis of the cellularity of the CSF and the concentration of neuropeptides pre and post treatment implicated in neuropathic pain.

2. Methods

2.1. Patient enrollment

The study received ethical approval from the St James Hospital and Tallaght University Hospital Research Ethics Committee. Patients attending the outpatient pain management clinic at St. James Hospital, Dublin with neuropathic pain in their leg or arm were referred to the research coordinator. Written informed consent was provided by each participant. Randomisation was performed by an independent Clinical Nurse Specialist using a computerised, random number generator. The clinician, the patient and the independent data collectors were all blinded to the randomisation until the study was completed. If the DRG local anaesthetic block procedure failed (failure to stimulate target dermatome or inadequate pain relief 1-h post injection), regardless of whether the patient received PRF or not, details of the failed block were included on the randomisation sheet and the patient was excluded from the study. Another patient was re-allocated to this group.

2.2. Inclusion criteria

Patients suitable for inclusion in the study were aged 18–60 years, described unilateral monosegmental radicular pain in the cervical or lumbosacral nerve roots, symptoms were consistent with MRI findings of a contained herniated disc at one level consistent with the clinical dermatome of complaint, had pain lasting more than 3 months, failed conservative medical management (medication and physical therapy), radicular pain was the primary complaint with minimal axial pain, and NRS score > 3.

2.3. Exclusion criteria

Patients were excluded from the study if they refused consent, described atypical or bilateral radicular pain patterns, primarily back or neck pain, cognitive impairment or language barriers that could impair the patients understanding and reporting of outcomes, malignancy, vertebral fractures, multiple sclerosis, connective tissue diseases, active infection, pregnancy or breast feeding, prior spinal surgery, lumbar spine interventions in the last 6 months (including epidural injections or PRF), coagulation disorder or anti-coagulant treatment, psychiatric disorder, allergy to local anaesthetics or contrast medium, and preoperative NSAID, corticosteroid, methotrexate, immunosuppressant therapy or opioid therapy within the preceding six weeks. In addition, patients were excluded from the study if appropriate nerve stimulation during insertion of the RF needle or adequate pain relief (more than 50%) 1-h post DRG block were not achieved.

2.4. Pulsed radiofrequency procedure

The patients attended the day surgical unit for a local anaesthetic injection of the dorsal root ganglion (DRG). They provided written consent for the procedure. An intravenous cannula was inserted for resuscitation purposes. They did not receive sedation. Oxygen saturation, ECG and blood pressure were monitored during the procedure. PRF probe placement was performed by a single Consultant grade operator. A sterile lumbar puncture was performed prior to PRF probe placement, in the sitting position with a 25 Gauge Whitacre needle following skin infiltration with 2 ml 1% lidocaine. CSF was allowed to drain from the needle until the fluid was clear of visible blood staining. A 2 ml sample of CSF was collected. The DRG side and level for PRF treatment was determined after clinical assessment and review of MRI. The patient lay prone on the theatre table for lumbar PRF and supine for cervical PRF. 2 mls of l% lidocaine was injected in the skin prior to placement of the radiofrequency (RF) needle. A 22-gauge RF needle (5 cm for cervical or 10 cm for lumbar region) with 5 mm exposed tip, was inserted and advanced under fluoroscopic guidance. A Neurotherm model NT2000 PRF generator was used for the procedure. A C-arm fluoroscopy machine was used to guide placement of the needle. The needle tip was placed as close to the relevant DRG as possible as per normal clinical practice. The stylet was replaced with the RF probe. A small volume (0.5-1 ml) of radio-opaque dye was injected to demonstrate spread along the nerve root to the epidural space, and to rule out vascular injection. Sensory stimulation at 50 Hz was sought in a distribution concordant with the usual painful distribution at a voltage ≤ 0.3 V. If stimulation was not elicited in the correct dermatome, the needle position was adjusted. Once stimulation is achieved in the clinical dermatome of interest, motor stimulation (2 Hz) greater than 1.5 times the sensory stimulation voltage was employed to ensure the probe was not applied to the nerve root thereby having its effect on the DRG only. An impedance reading less than 450 Ohms was required. 1 ml of lidocaine 1% was applied to the DRG. Use of lignocaine confirmed that the appropriate dermatome was targeted and prevented sensory appreciation during the treatment whether randomised to either sham or PRF patients' groups. All patients were assessed 1-h post procedure to confirm correct dermatome was targeted during the procedure. Following this, the theatre nurse operating the RF machine opened the randomisation envelope and followed the directions, PRF or Sham treatment - all other personnel in the theatre including the patient and pain specialist performing the procedure were blinded to the treatment delivered. Visual or auditory prompts that could unblind the researchers were removed with the operating screen turned away and the RF machine set on silent mode. The PRF group received one pulsed radiofrequency treatment to the DRG, settings: ≤ 42 °C, 120 s with 2 Hz pulses (20 milliseconds at 500,000 Hz) and output at 45 V. The sham group waited for 120 s with the RF needle in position, but the RF machine was turned off.

Table 1
Demographics of Patients involved

Number of patients (n)	11
Age (years)	48 (range 35–60)
Male:Female	5:6
Side of pain, left:right	6:5
Level of DRG treated (n)	C5 (2) C6 (1) L4 (3) L5 (4) S1 (1)
Duration of pain (months)	29

After 3 months, a repeat lumbar puncture as described above was performed with 2 ml of CSF sampled for analysis.

3. Clinical outcome analysis

A successful clinical outcome was 50% or more improvement in pain at 3 months post-treatment which correlates with a Global Perceived Effect (GPE) score of 6 or 7. The GPE was assessed on a 7-point Likert-like verbal rating scale where: very bad = 1, bad = 2, fairly bad = 3, same as before = 4, fairly good = 5, good = 6, very good = 7. Pain scores were recorded on a 0–10 Numerical Rating Scale (NRS). Quality of life was measured with the RAND-36 questionnaire. (Hays et al., 2001) Functional ability was measured with the Oswestry Disability Index (ODI).(Fairbank et al., 1980) Adverse events and further interventional treatments during the study were recorded.

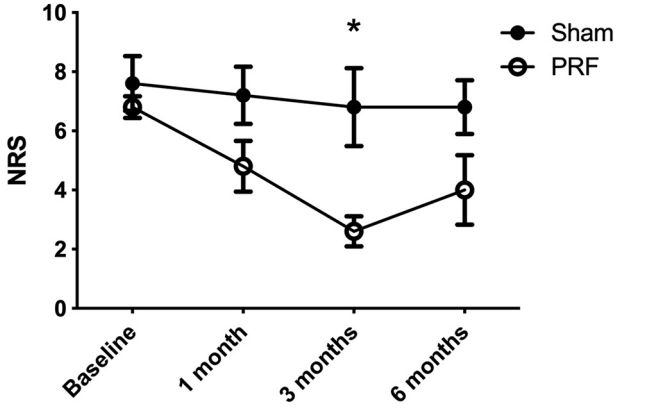

Fig. 2. Pain scores (NRS) at baseline, 1 month, 3 months and 6 months in the PRF and Sham treatment groups. There is a reduction in pain score in the participants receiving PRF, with a significant reduction at 3 months (pre: 6.8 ± 0.37 vs post: 2.6 ± 0.51, $p < .05$). NRS, numerical rating scale 0–10; PRF, pulsed radiofrequency.

Clinical outcome measures were recorded immediately before the procedure, at 1 h post procedure (GPE and NRS only), and at 1, 3 and 6 months post-procedure by independent data collectors. CSF samples

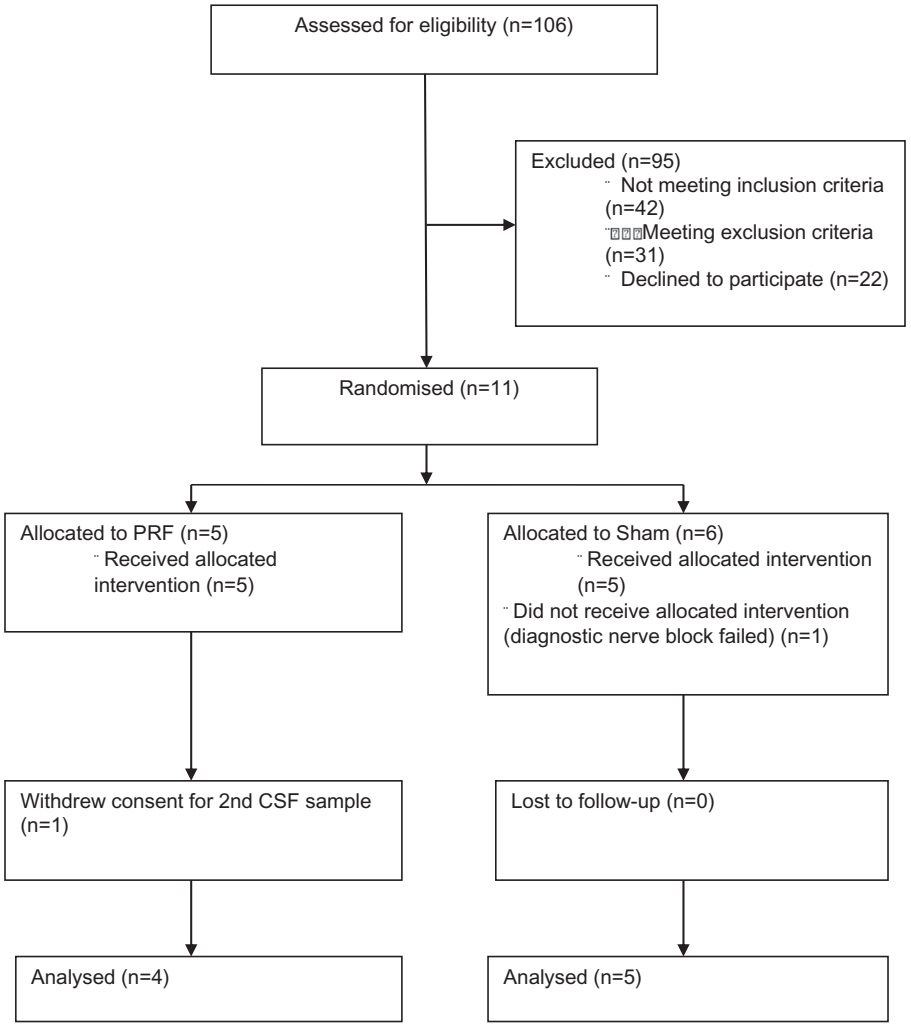

Fig. 1. CONSORT study flow diagram.

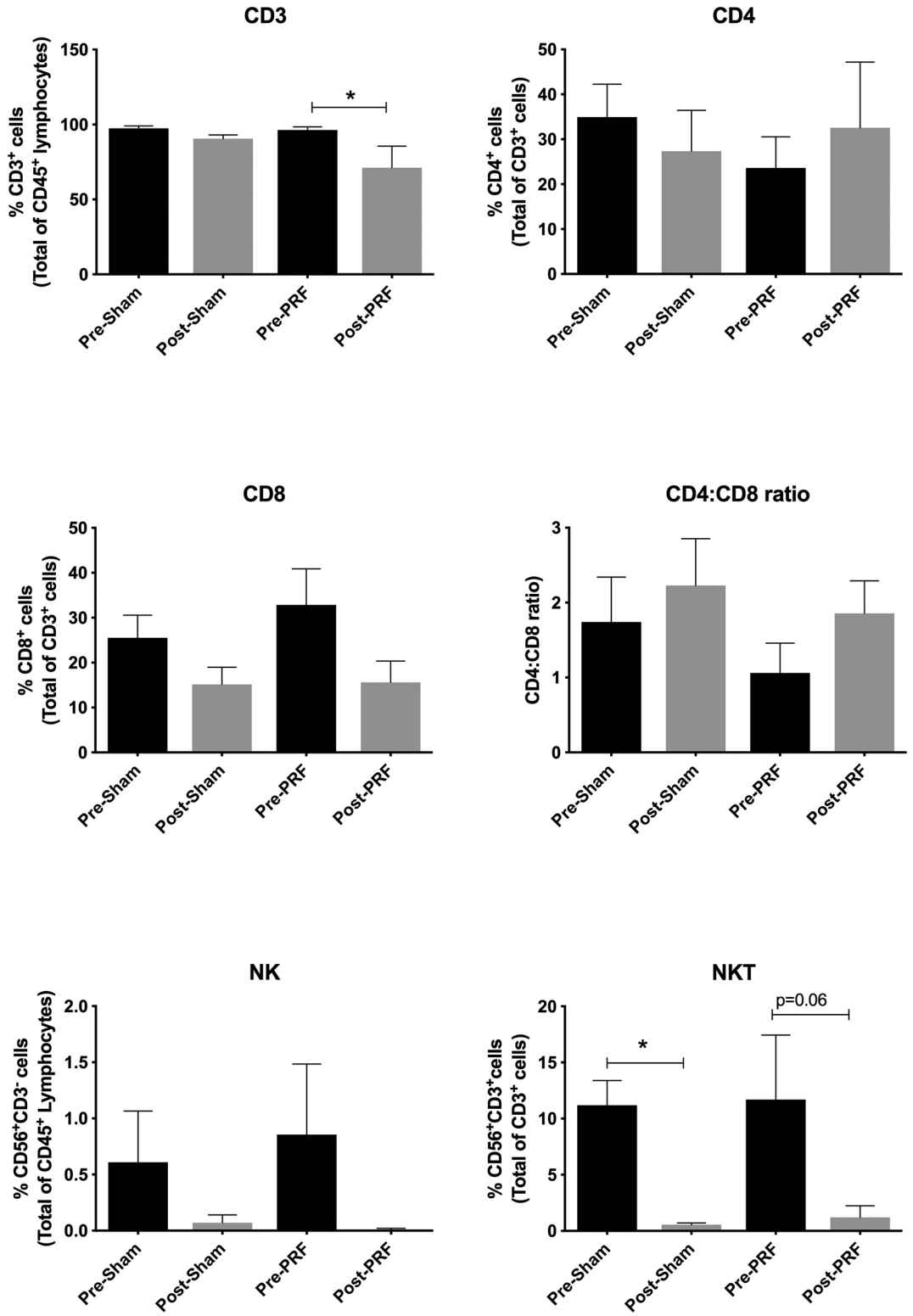

Fig. 3. Bar charts showing percentages of $CD3^+$ T cells and $CD56^+CD3^-$ NK cells (as a percentage of lymphocytes) and percentages of $CD4^+$ T cells, $CD8^+$ T cells and $CD56^+CD3^+$ NK T cells (as a percentage of T cells) pre and 3 months post treatment with either PRF or Sham. There is a significant reduction in $CD3^+$ T cells in the PRF group (pre: 96.33 ± 2.17% vs post: 71.21 ± 14.4%, $p = .0317$). The significant reduction in NK T cells following Sham may represent the active nature of the treatment, lidocaine dorsal root ganglion injection. NK, natural killer; PRF, pulsed radiofrequency.

were taken immediately before the PRF procedure and 3 months post-procedure (Table 1).

3.1. Flow cytometric analysis of lymphocytes in CSF

1 ml of CSF was stabilised in Transfix/EDTA CSF sample storage tubes (Cytomark, UK) at 4 °C for subsequent flow cytometry analysis. CSF was returned to room temperature before 2 ml of sterile filtered

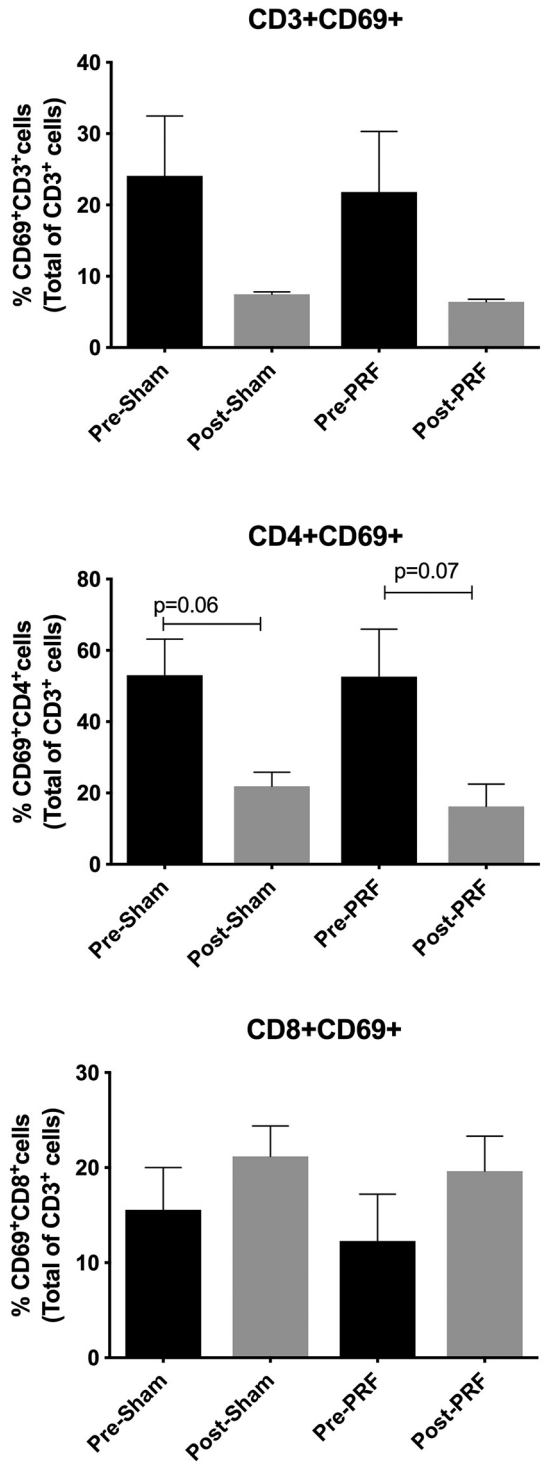

Fig. 4. Bar charts showing percentages of activated ($CD69^+$) $CD3^+$ T cells, $CD4^+$ T cells and $CD8^+$ T cells (as a percentage of total $CD3^+$ cells). CD4+ T cell activation was reduced at the 3 months' time point, irrespective of treatment (Pre vs post sham: 53.02 ± 10.13% vs 21.9 ± 3.91%; p = .06 – Pre vs Post PRF: 52.66 ± 13.3% vs 16.22 ± 6.3%; p = .07). PRF, pulsed radiofrequency.

phosphate buffered saline (PBS) was added to the tube and the sample was mixed gently. Following centrifugation at 540 ×g for 5 min, the supernatant was discarded and the pellet was resuspended in 300 µl of PBS. Each CSF sample was then stained with monoclonal antibodies (mAbs) specific for human surface markers CD45, CD3, CD4, CD8, CD56, CD69, CD45RA, CD27 (Miltenyi Biotec, Germany). Cells were acquired using a CyAn ADP flow cytometer (Beckman Coulter) and analysed with FlowJo software (Tree Star Inc.). The frequencies of $CD3^+$ (T) cells and $CD56^+CD3^-$ (NK) cells in the CSF were quantified as a percentage of $CD45^+$ lymphocytes, while the frequencies of $CD4^+$ T cells, $CD8^+$ T cells and $CD56^+CD3^+$ (Natural T) cells were quantified as a percentage of $CD3^+$ T cells. Lymphocyte activation phenotypes were recorded with quantification of activated $CD69^+$ subsets of $CD3^+$, $CD4^+$ T cell, $CD8^+$ T cell, and NK cell frequencies. Profiling of $CD3^+$ T cell differentiation was performed using the Dieli classification; naive ($CD45RA^+CD27^+$), effector memory (T_{EM}) ($CD45RA^-CD27^-$) and central memory (T_{CM}) ($CD45RA^-CD27^+$) and terminally differentiated (CD45RA + CD27-).(Dieli et al., 2013)

3.2. Quantification of neuropeptide levels in CSF

1 ml of CSF was immediately stored at −20 °C in 2 ml cryovials until use. The following Meso Scale Discovery plates were used to detect the levels of several analytes in the CSF of 9 patients; V-PLEX™ human cytokine plates (VEGF, IL-6, IL-1β, TNF-α, IL-10, IP-10, MCP-1, IL-17 and IFN-γ), 4-Spot Prototype Human bNGF plate and 4-Spot Cytokine Custom Human SP BDNF plate. Plates were used according to the manufacturer's instructions and read using a MesoScale Diagnostics Sector S600.

3.3. Statistical analysis

Statistical analysis was carried out using Prism Graph Pad Version 5.0. Differences between pain severity (NRS), RAND-36 scores and ODI scores at 4 separate time points were assessed using repeated measures ANOVA with Bonferroni post-hoc analysis. Wilcoxon signed-rank tests were used to compare CSF cellular and neuropeptide concentrations pre and post treatment. P values < .05 were considered to be significant.

4. Results

4.1. Patient enrollment

Over a 12-month period, 106 patients with symptoms of radicular pain were screened for eligibility. After applying inclusion and exclusion criteria, 11 patients agreed to take part in the study (Fig. 1). After the diagnostic nerve block, one patient did not report more than 50% pain relief and was therefore removed from the study as per the protocol. In addition, one patient in the PRF group withdrew consent for the second lumbar puncture at three months. Therefore, clinical data was collected for 5 patients in each group at both time points, but CSF data was only available at 3 months on 4 patients in the PRF group and 5 patients in the Sham group.

The mean age of enrolled patients was 48 (range 35–60) with 5 male and 6 females. The radicular pattern of pain was in the following dermatomes (n): C5 (2), C6 (1), L4 (3), L5 (4), S1 (1). The mean duration of the pain under treatment was 29 months and the mean duration of conservative treatment before nerve injection was 27 months. The baseline NRS score was 7.5. The RAND-36 gave scores for physical functioning (40.5), role limitation due to physical health (0), role limitation due to emotional health (33), energy/fatigue (36), social functioning (34), pain (25), and general health (56). The mean Oswestry Disability Index (ODI) score was 41. Comparison of the demographic and baseline scores in both groups (PRF vs. Sham) demonstrated no significant differences.

4.2. PRF results in a significant decrease in pain scores at 3 months

Pain scores (NRS) in our groups were as follows: patients in the sham group had a mean NRS of 7.6 at baseline vs 6.8 in the PRF group (0.8 difference with a p value > .50). At one-month post procedure, NRS difference between the groups had increased to 2.4, with a lower

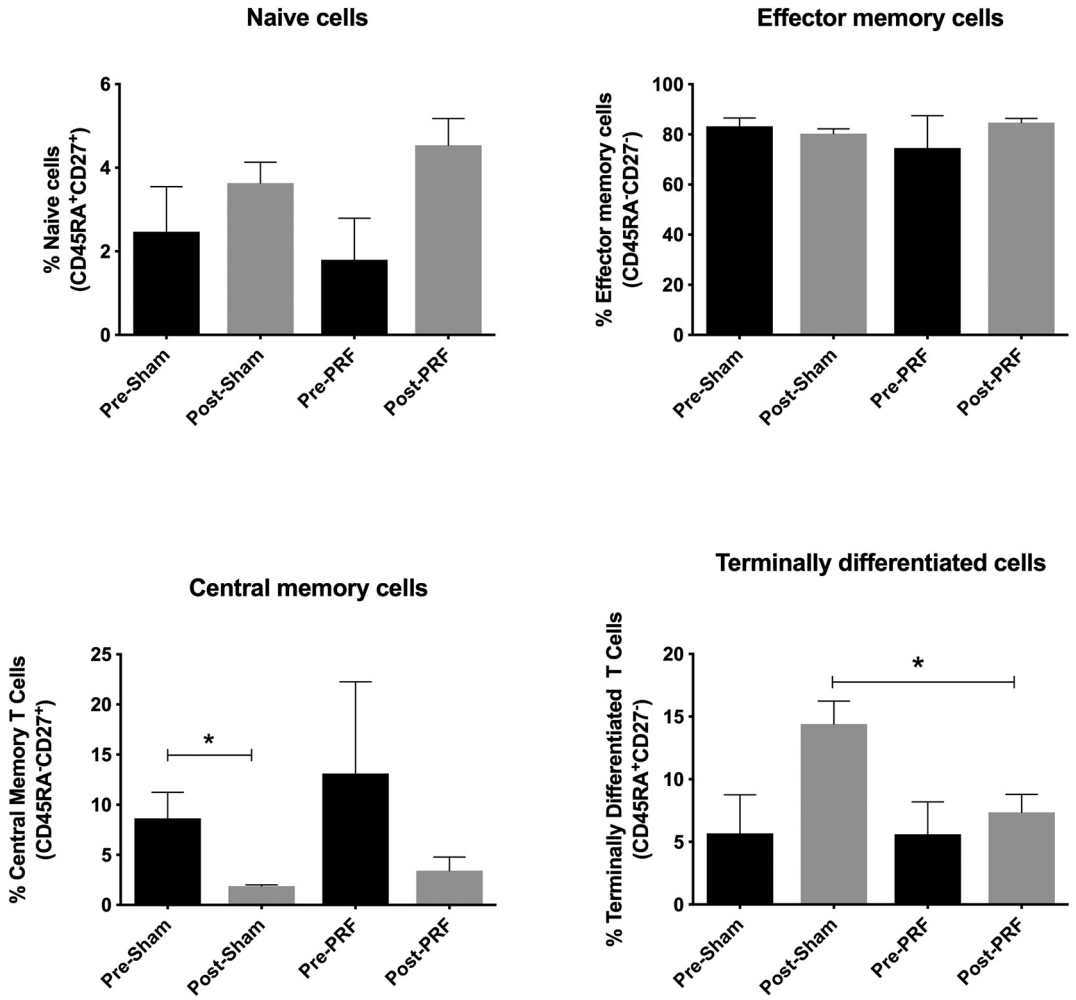

Fig. 5. Bar charts showing the percentages of naive ($CD45RA^+CD27^+$), effector memory (T_{EM}) ($CD45RA^-CD27^-$), central memory (T_{CM}) ($CD45RA^-CD27^+$) and terminally differentiated (CD45RA + CD27-) T cells (as a percentage of total $CD3^+$ cells). No changes were observed in the frequencies of naive T cells or effector memory T cells between pre and post treated CSF samples. Significant changes were observed in T_{CM} cells 3-months post sham treatment (8.64 ± 2.6 v 1.88 ± 0.14%; $p = .0159$) and in terminally differentiated T cell in the post-PRF group compared with the post-sham group (14.4 ± 1.84% v 7.36 ± 1.44%; $p = .0286$). A high percentage of the T cells in the pre-sham group (83.2 ± 3.33%) and the pre-PRF group (74.58 ± 12.87%) were effector memory cells. This did not change in either group post treatment. PRF, pulsed radiofrequency.

mean NRS in PRF group 4.8 versus sham 7.2 however this was not statistically significant with a p-value of > 0.05. At three months post–procedure, the NRS difference between the groups had increased further to 4.2, PRF 2.6 vs Sham 6.8 which was both clinically and statistically significant $p < .05$). At 6 months post procedure, an NRS difference of 2.8 between the groups was observed but this was not statistically significant with a p-value $> .05$.

There was a persistent but non-significant reduction in pain at 6 months (Fig. 2). The GPE scores at 3 months reflect patient satisfaction with PRF treatment (GPE in PRF vs Sham groups, 6 vs 4, $p = .008$). This positive GPE was sustained at 6 months (GPE in PRF vs Sham groups, 6 vs 4, $p = .04$). There was no significant change in the other quality of life and disability scores between the two groups.

4.3. PRF significantly reduces the frequency of $CD3^+$ T cells in CSF

The frequency of $CD3^+$ cells was significantly lower in the post-PRF versus the pre-PRF group (Pre: 96.33 ± 2.17% vs Post: 71.21 ± 14.4%, $p = .0317$) (Fig. 3). No difference in the frequency of $CD3^+$ cells was observed in the sham treated group. While there appeared to be a non-significant decrease in $CD8^+$ T cells in the CSF post-PRF treatment, this was not specific to the PRF group as CD8+ T cells were also decreased in the post-Sham treated CSF. No differences were observed in $CD4^+$ T cell frequencies. Both NK and NKT cells were reduced 3 months post treatment, irrespective of the treatment they received. The CD4:CD8 ratio in both groups' pre-intervention was 1.4 ± 0.36. This increased to 2.06 ± 0.38 post-intervention.

Further analysis of the activation status of T cells within the CSF revealed that while CD4+ T cell numbers were not altered by treatment, their activation, as measured by CD69 expression was greatly reduced at the 3 months' time point, irrespective of treatment (Pre vs post sham: 53.02 ± 10.13% vs 21.9 ± 3.91%; $p = .06$: Pre vs Post PRF: 52.66 ± 13.3% vs 16.22 ± 6.3%; $p = .07$). No changes in CD69 expression by $CD3^+$ or $CD8^+$ T cells was observed (Fig. 4).

Based on CD45RA and CD27 expression T cells can be grouped into naïve, effector memory, central memory or terminally differentiated. No changes were observed in the frequencies of naive T cells or effector memory T cells between pre and post treated CSF samples (Fig. 5). There was a significant decrease in the frequency of central memory T cells 3-months post sham treatment (8.64 ± 2.6 v 1.88 ± 0.14%; $p = .0159$), a similar but non-significant decrease was observed in the post-PRF sample. No differences were observed in terminally differentiated T cells between pre and post treatments but there was a significant reduction in the frequency of terminally differentiated T cell in the post-PRF group compared with the post-sham group (14.4 ± 1.84% v 7.36 ± 1.44%; $p = .0286$). A high percentage of the

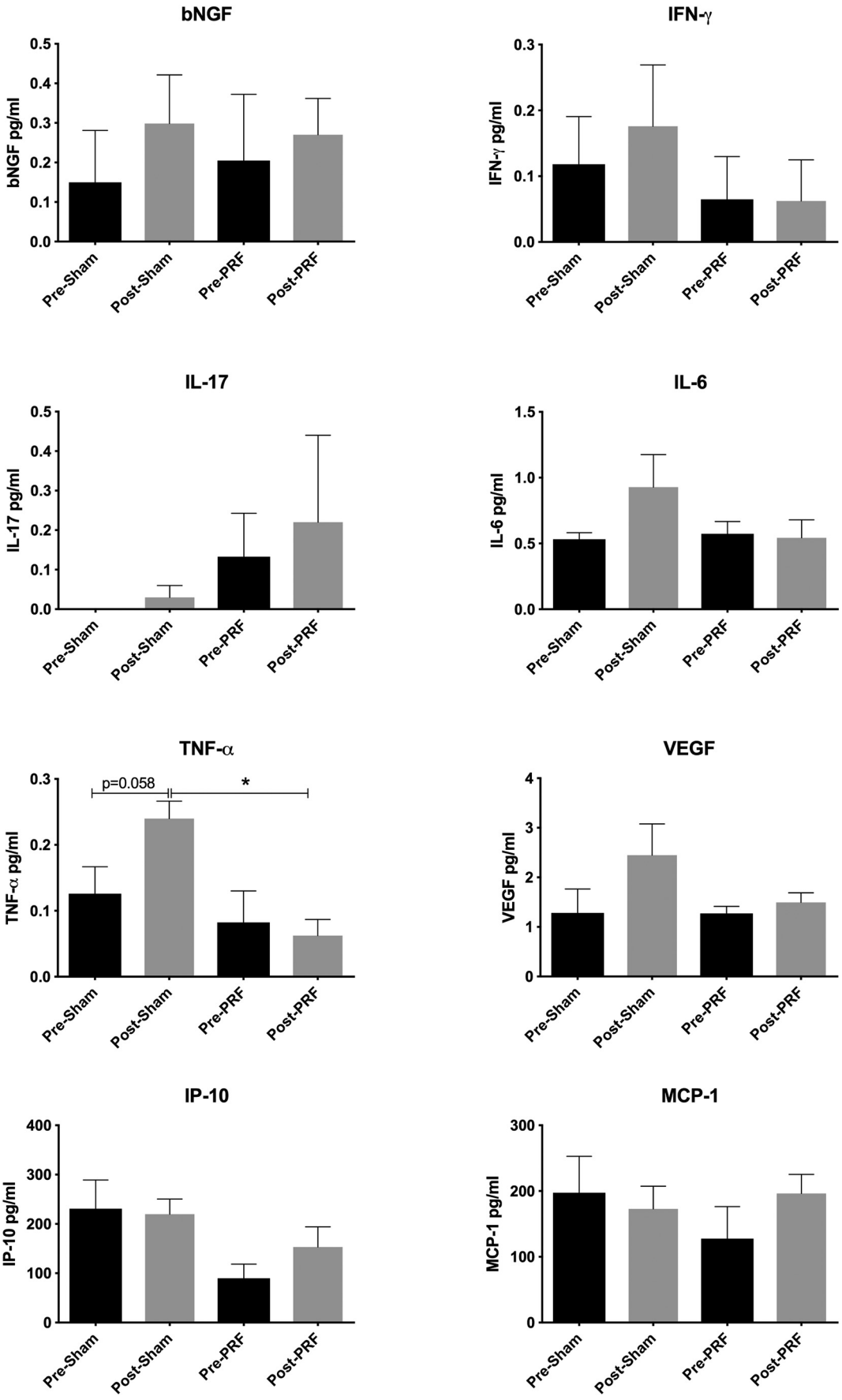

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Fig. 6. Bar charts showing the ELISA concentrations of bNGF, IFN-γ, IL-17, IL-6, TNF-α, VEGF, IP-10 and MCP-1 17 in the CSF pre and 3 months post treatment (PRF or sham). Concentrations of cytokines and chemokines are shown in units of pg/ml. TNF-α was significantly lower in the post-PRF CSF sample compared with the post-sham sample (0.24 ± 0.026 pg/ml v 0.0625 ± 0.024 pg/ml; p = .0159). A trend in reduced CSF concentrations of IFN-γ, IL-6 and VEGF post-PRF was noted but did not achieve significance. PRF, pulsed radiofrequency.

T cells in the pre-sham group (83.2 ± 3.33%) and the pre-PRF group (74.58 ± 12.87%) were effector memory cells.

4.4. CSF TNFα concentrations were reduced in the PRF group versus sham

Multiplex ELISAs were run to determine if any changes in inflammatory cytokines or neuropeptides were induced as a result of PRF treatment. No changes in bNGF, IFN-γ, IL-17, IL-6, VEGF, IP-10 or MCP-1 were observed between sham and PRF treated CSF samples. TNF-α was significantly lower in the post-PRF CSF sample compared with the post-sham sample (0.24 ± 0.026 pg/ml v 0.0625 ± 0.024 pg/ml; $p = .0159$) (Fig. 6). A trend in reduced CSF concentrations of IFN-γ, IL-6 and VEGF post-PRF was noted but did not achieve significance.

5. Discussion

All participants in this study received the same dose of lignocaine to the clinically problematic dermatome as a recognised treatment for radiculopathy which was confirmed by clinical response one hour post intervention. The participants were then randomised to a treatment group that received PRF, and a sham group who did not. All study investigators and participants were blinded to this.

Previous studies with PRF in lumbosacral pain have led to contrasting results, likely due to variable inclusion criteria as well as heterogeneous populations, devices and stimulation protocols. We have aimed to reduce this bias by standardising our inclusion criteria with extensive exclusion criteria, use of a “sham” group and also an internationally accepted PRF stimulation protocol using standard equipment.

A recent paper by Vigneri et al. showed high-voltage PRF of the DRG delivered through a multifunctional electrode provided significant pain relief and may be considered a valuable treatment in chronic lumbosacral radicular pain with neuropathic features.(Vigneri et al., 2020)

The participants in this study were of working age and reported high levels of persistent pain (NRS > 7) with associated physical disability and reduced global function prior to treatment. Their ODI score (41) reflected moderate to severe disability levels. Their RAND-36 quality of life scores demonstrated worse function across all domains compared to the general population scores from the Medical Outcomes Study.(Kravitz et al., 1992) The PRF group had a significant reduction in pain scores at 3 months. Pain scores remained lower at 6 months but were no longer significant. The sham group did not experience any pain score reduction at 3 or 6 months. This is consistent with other PRF studies in radicular pain cohorts that show a peak effect at 3 months that begins to reduce at 6 months). (Kesikburun et al., 2018; Lee et al., 2018; Abejón et al., 2007; Shanthanna et al., 2014b; Van Boxem et al., 2016) The combination of a reduced score on the NRS coupled with a high GPE score (6 or 7) is a good predictor of a positive treatment outcome, and this was the case in our PRF group.(Shanthanna et al., 2014b)

The mechanism of action of PRF treatment in vivo is poorly understood. However, in this study TNF-α concentration in CSF was reduced following PRF treatment and there was a reduction in CD3+ lymphocytes. There is growing evidence supporting the concept that neuropathic pain chronicity is driven from the neuroimmune interface at the DRG and dorsal horn.(Van Boxem et al., 2015; Grace et al., 2014)

Participants in this study, prior to intervention, demonstrated a CD4/CD8 ratio of 1.4 with a high concentration of effector memory cells ($CD45RA^{-}CD27^{-}$) which is quite different from that reported in a historical healthy volunteer study. The study by de Graaf and colleagues analysed CSF samples from 84 healthy volunteers to record the lymphocyte frequencies and phenotypes.(de Graaf et al., 2011) They demonstrated that $CD4^{+}$ T cells made up 70% of the lymphocytes in the CSF. There were much fewer $CD8^{+}$ T cells with a mean CD4/CD8 ratio of 3.0. The majority of the $CD3^{+}$ lymphocytes (85%) were central memory cells (TCM). These are inactive surveillance cells. The majority of the $CD3^{+}$ lymphocytes in our neuropathic pain patients were the activated effector memory cells (TEM) (80.0%). Another study of human CSF in patients with a broad range of neurological diseases revealed a mean CD4/CD8 ratio of 4.2. Patients with lower CD4/CD8 ratios similar to our patient cohort were those with HIV where painful neuropathy was a prominent complaint.(Kowarik et al., 2014)

6. Conclusion

This study confirms PRF is an effective treatment for chronic neuropathic radicular pain and superior to local anaesthetic administration on its own. Patients with chronic radicular pain have a lower CD4/CD8 lymphocyte ratio in their CSF compared to historical controls and also a phenotypically different CD3 population (effector memory) suggesting immune activation. PRF treatment reduced CD3 counts and TNF alpha concentration. This study provides further information supporting the neuroimmune concept of neuropathic pain chronicity and tentatively suggests that a PRF mechanism of action involves modulation of CSF peptides and T cells.

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Declaration of Competing Interest

None.

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