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Phase I/II parallel double-blind randomized controlled clinical trial of perispinal etanercept for chronic stroke: improved mobility and pain alleviation

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ABSTRACT

Background: Previous open-label studies showed that chronic post-stroke pain could be abated by treatment with perispinal etanercept, although these benefits were questioned. A randomized double-blind placebo controlled clinical trial was conducted to test perispinal etanercept for chronic post-stroke pain.

Research design and methods: Participants received two treatments, either perispinal etanercept (active) or saline (control). Primary outcomes were the differences in daily pain levels between groups analyzed by SPSS.

Results: On the 0–100 points visual analog scale, perispinal etanercept reduced mean levels for worst and average daily pain from baseline after two treatments by 19.5 – 24 points ($p < 0.05$), and pain alleviation was maintained in the etanercept group, with no significant change in the control group. Thirty percent of etanercept participants had near complete pain abatement after first treatment. Goniometry of the paretic arm showed improved mean shoulder rotation by 55 degrees in active forward flexion for the etanercept group ($p = 0.003$) only.

Conclusions: Perispinal etanercept can provide significant and ongoing benefits for the chronic post-stroke management of pain and greater shoulder flexion by the paretic arm. Effects are rapid and highly significant, supporting direct action on brain function.

Trial registration: ACTRN12615001377527 and Universal Trial Number U1111-1174-3242.

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1. Introduction

1.1. Scientific background, clinical relevance, rationale, and objectives

Major impairment after stroke is exacerbated when accompanied by chronic, severely debilitating and intractable long-term pain [1,2]. Strokes in different regions of the brain are often associated with the occurrence of central post-stroke pain (CPSP). CPSP is a neuropathic pain disorder, arising from a combination of both central [3] and peripheral nervous system mechanisms [4]. CPSP is a highly intractable disorder with significant health burdens [1,2], as is stroke disability itself. A recent systematic review of clinical trials for CPSP has shown no beneficial effects for any other experimental treatments [5]. CPSP frequently requires the use of strong analgesics which may result in further significant impairment and reduced quality of daily life. Often, patients also demonstrate clinical features such as depression and have greater risk of suicide [2]. Hence, finding a beneficial therapy is imperative.

The common proinflammatory cytokine, tumor necrosis factor alpha (TNFα) is involved in all phases of stroke, including rehabilitation (reviewed in [6]). Initially, during stroke, TNFα is synthesized and released by astrocytes, microglia, and neurons in response to ischemia and is a major factor in the pathophysiological processes of stroke. TNFα activates the microglia and astrocytes, affects the blood–brain barrier permeability, and can adversely affect synaptic transmission and synaptic plasticity during stroke, including rehabilitation [7]. TNFα has long been implicated as a key factor in the post-stroke neuroinflammatory response with levels in the CSF and plasma [8–10] correlating with severity of symptoms and as a mediator of focal ischemic brain injury [11]. Elevated TNFα levels not only exist in the cerebral spinal fluid in the acute stage [9,12–18] but also in chronic post-stroke patients [19] with increased TNFα expression found in postmortem brain long after the initial stroke episode [20].

TNFα has also been established as an important mediator of neuropathic pain in animal models [21,22] where blocking TNFα alleviated this pain. The role of TNFα in neuropathic pain has recently been extensively reviewed elsewhere [23]. For over two decades, research studies have shown the relief of neuropathic pain, recognized and reported for chronic stroke with the use of the TNFα blocker, etanercept – an agent comprising immunoglobulin fused with the soluble

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TNFα receptor domain (reviewed in [24–26]). Studies with tracers also showed that administering large molecules such as etanercept, via injection into the perispinal space, facilitates their uptake into the cerebrospinal venous plexus, thereby providing an effective method for direct delivery into the brain through the choroid plexus [27,28]; reviewed in detail in [29]. Such treatments using etanercept were also shown to alleviate neuropathic pain in rat models [28,30] and in open-label use for human patients with stroke [31,32]. Thus, previous observational studies on 600 patients reported that perispinal etanercept (PSE) therapy provided rapid improvements (within 30–60 min after treatment) in stroke-related disabilities, including pain [32]. It was concluded that perispinal delivery of etanercept was mediating the rapid actions by a direct effect of blocking TNFα in the central nervous system [27–29,31–33]. These reports were met with controversy such that the American Academy of Neurology published a practice advisory in 2016 noting that the evidence to support or refute a benefit of etanercept for treatment of post-stroke disability was insufficient to determine the treatment's effectiveness [34].

Since its first approval by the FDA in 1998, etanercept has been used to treat a spectrum of rheumatoid disorders as a generally well-tolerated drug with a favorable safety profile, even when administered weekly for many years on a chronic basis in elderly patients or children [35–37]; for review of safety profiles, see [38,39]. Etanercept may provide advantages over other TNF blockers by inhibiting both TNFα and TNFβ; has a higher affinity for TNFα than the monoclonal antibody therapies; and in some studies has shown less adverse effects [40,41]. This trial was designed to determine whether the perispinal etanercept injection procedure developed to treat post-stroke patients for pain and other dysfunction, was in fact successful and worthwhile. Furthermore, the methods followed were exactly those approved and used by Dr Tobinick and have been well documented [32,42–49]. Therefore, we undertook the first randomized double-blind clinical trial to determine the effects of two treatments at day 1 and day 14 of perispinal etanercept therapy with primary outcome measures examining the differences from baseline levels in patients with constant daily post-stroke pain to day 30, 2 weeks after the second treatment on trial.

2. Patients and methods

The CONSORT guidelines were followed in the preparation of this publication.

2.1. Description of trial design including allocation ratio

This was a double-blind randomized controlled parallel trial with 1:1 allocation ratio between individuals receiving either etanercept (active) treatment or saline (placebo) control. Approval of the study was obtained from the Griffith University Human Research Ethics committee (MSC/10/14/HREC). Subject applications were clinically evaluated by a neurologist during the screening process for eligibility. The study protocol was fully explained to participants. Informed and written consent was obtained before participation with allocation based on the numerical value assigned upon enrollment into either the etanercept or control group (Figure 1). Trial clinical investigators including the neurologist were involved in the enrollment of participants.

Specific inclusion criteria used for screening were initially based on the following four requirements:

- Aged between 30 and 80 years old encompassing the most frequent age incidence given stroke is uncommon in young adults [50] and to reduce the possibility of comorbidities in older patients.
- Stroke occurring at least 6 months and not more than 15 years prior to screening for this study.
- Chronic neurological impairment, including hemiparesis, following an ischemic stroke in the territory of the middle cerebral arteries (MCA) (including MCA clot or embolus or carotid occlusion causing MCA territory stroke) or basal ganglia form of intracerebral hemorrhage.
- Constant daily pain post-stroke incorporating one or both contralateral limbs and experiencing intractable chronic post-stroke pain with hemiplegic post-stroke shoulder pain and central post-stroke pain. The post-stroke pain is moderate-to-severe in intensity, with a daily average intensity between 4 and 8 inclusive on an 11-point (0–10) vertical Numerical Pain Rating Scale supplemented with a faces pain scale (vNPRS-FPS) [51].
- The post-stroke pain is in an area of the body affected by the stroke.

As aphasia is recognized as less common in patients with right than left sided MCA strokes, this randomized, parallel-group controlled clinical trial was initially aimed at studying the clinical effects of perispinal delivery of etanercept versus saline in a cohort of participants with chronic ischemic stroke in the territory of the right MCA. In addition to pain as a primary outcome measure, shoulder flexion, spasticity, cognition, executive function, hemispatial neglect, and post-stroke depression were examined, some as exploratory outcome measures because it was unknown whether the trial would be adequately powered for these particular endpoints.

Before participating, all participants underwent physical examination and vital signs were recorded. All patients were informed prior to trial that any previous medication used regularly was to be maintained and not altered during the period from 2 weeks before visit 1 on trial until day 30 after visit 1.

2.1.1. Important changes to methods after trial commencement (such as eligibility criteria), with reasons

The initial cohort was based on right MCA stroke but due to the limited numbers of available patients presenting that met the initial criteria, enrollment was widened to expand the age limit to 27 and to include non-aphasic stroke patients with left MCA strokes and right-sided impairment or basal ganglia strokes. Applicants were reviewed on a case by case basis by

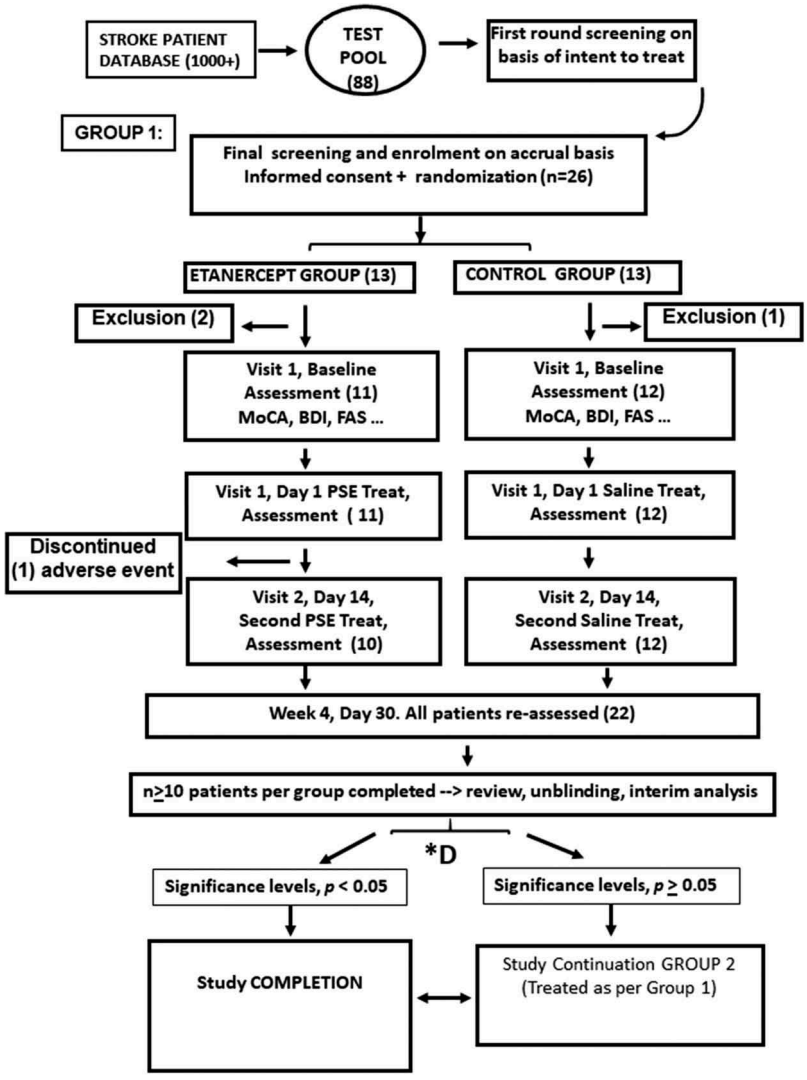

Figure 1. Trial profile. Outline of study protocol and regimen for randomization on enrollment into the etanercept and control group, including numbers of patients in brackets and all dropouts due to exclusion or adverse events. *D: decision point upon trial completion after interim unblinding and primary outcome measures showed significance values for $p < 0.05$.

the neurologist and assessed for their capacity to understand and communicate during verbal testing.

3. Eligibility criteria for participants

3.1. Inclusion criteria

At the time of recruitment, all participants were required to travel to the study site and speak fluent English to facilitate effective communication regarding their pain levels. Participants started a pain diary including listing all medications taken from day −7 prior to visit 1 and were required to score their vNPRS-FPS through to completion at day 30 after visit 1, which were collected. For the complete list of specific inclusion/exclusion criteria, including neurological details of stroke diagnosis, refer to Supplementary Table 1.

3.1.1. Settings and locations where the data were collected

Patient medical histories relating to their stroke and hospital discharge summaries were collected via e-mail or as hard copies for evaluation by the study investigators during enrollment. All data was collected, bound and stored with Case Report Forms for each participating subject at the Clinical Trials Unit, G40 Health Center, Griffith University Gold Coast campus, Southport Queensland.

3.2. Interventions and administering procedure

Two active or control treatments (the first at visit 1/day 1 and the second at visit 2/day 14) on trial were administered with all injections double-blinded to the principal medical investigators and participants. Assessments measuring the responses of participants to treatments were also undertaken in a blinded manner. Treatments were to be halted in the event of an adverse reaction or participant request to discontinue participation. Each etanercept (ENBREL®, Pfizer, USA) single-use injectable dose was prepared by solubilizing with the addition of 1.8 cc of non-bacteriostatic sterile water into a 25 mg lyophilized powdered vial of etanercept (containing 10 mg/mL sucrose, 5.8 mg/mL sodium chloride, 5.3 mg/mL

L-arginine hydrochloride, 2.6 mg/mL sodium phosphate, monobasic, monohydrate, and 0.9 mg/mL sodium phosphate, dibasic, anhydrous). A single dose was administered by injection overlying the spine, as described previously [32,42–49]. Thus, each dose was delivered to the participant in a sitting position with their head bent forward, reaching the chin toward the chest and with the neck horizontal. The injection was given subcutaneously into the posterior cervical interspinous midline (into the interspace midway between C6-C7 or C7-T1 vertebra) using a 27 gauge, half-inch needle, free-hand guided and inserted fully into the skin at an angle near perpendicular to the surface. The injection was quickly followed by Trendelenburg positioning, with participant supine on an inversion table and the head dependent for 4 min at an incline of 45 degrees. This approach is in order to effect entry into the cerebrospinal venous system (CSVS) as a previously validated delivery to the brain via the choroid plexus detected using radioactive or fluorescent-tagged etanercept [27,28]; for an extensive recent review outlining this mechanism of drug delivery to the brain, see [29]).

3.3. Control

The control treatment was sterile saline (suitable for human injection) as a clear colorless solution, with the same appearance as for the etanercept, and prepared in the same type of syringe with the same volume of 1.8 ml as for the test drug. The control was administered using the identical perispinal injection procedure as for the active drug treatment described above.

3.4. Outcome measures

Measures are underlined below.

3.4.1. Primary outcome measures

3.4.1.1. Pain. On the vertical Numerical Pain Rating Scale (vNPRS), patients were asked where they would mark the number between 0 and 10, or 0 and 100 that fits best to their pain intensity. Zero represented 'no pain at all' whereas the upper limit represented 'the worst pain imaginable'. The 11-point vertical Numeric Pain Rating Scale supplemented with a Faces Pain Scale (vNPRS-FPS) [51] was provided to patients in the weeks preceding trial visit 1. This was used to assess their levels of pain and familiarize prospective trial participants with the pain test as a guide for self-reporting daily pain intensities recorded on their pain diary from day −7 before participation on trial, until day 30. Participant pain levels were also assessed by interview using an expanded 0–100 point (1 cm/5 point numeric interval on the vertical visual analog scale with two emoticon face indicators: one for 'no pain' at 0 and one for 'worst pain imaginable' at 100) vNPRS-FPS placed before patients during trial visits 1 and 2, recording both pre- and post-treatment scores, as well as recorded on day 30 by phone interview. Average and Worst levels of Pain: Changes in mean vNPRS-FPS (change in pain intensities on the 0–100 point scale) for values recorded from visit 1 before treatment (the baseline (PRE) values) compared with on the final day of participation on trial (day 30 after visit 1; D30). Participants were asked at visits 1 and 2 on trial before and after treatment to rate their 'average' and 'worst' pain levels considering the last 8 h and the previous week.

3.4.2. Secondary outcome measures

Average level of Pain: Change in mean vNPRS-FPS scores (0–100 point scale) from baseline compared with day 1 (visit 1 after treatment) and day 14 (visit 2 after treatment), where the subject was rated for his or her 'average pain' over the last 8 h and considering the previous week. Worst level of Pain: Change in mean vNPRS-FPS score (0–100 point scale) from baseline compared with visit 1 and 2 after treatment, where the subject was rated for his or her 'worst pain' over the last 8 h and considering the previous week. Instant Change in Worst Level of Pain: between 30 and 60 minutes before treatment on day 1 (visit 1) to 30–60 min after treatment at visit 1. Shoulder Flexion/Spasticity: mean change in paretic arm by angle of shoulder flexion (active and passive) (measured in degrees of rotation by goniometry) before and after treatment at each visit on day 1 and day 14. Cognition: mean change in Montreal Cognitive Assessment (MOCA) [52] score from baseline on day 1 compared to day 14 (visit 2, after treatment). The Albert's Line Bisection Test is used to detect unilateral visuospatial neglect [53]. Hemispatial Neglect: mean change in Albert's Line Bisection Test from baseline on day 1 to day 14 (visit 2, after treatment). Fatigue Assessment Scale (FAS): mean change in FAS level (out of 50) from baseline to day 30. The FAS has 10-items as statements about different aspects of fatigue, each rated from 1 to 5 (1, never; 2, sometimes; 3, regularly; 4, often; and 5, always) and is a valid and reliable test in stroke with higher scores indicating greater fatigue [54].

3.4.3. Exploratory

The Clock Drawing Test (CDT) is used as a psychometric measure for mild to moderate cognitive impairment [55]. Cognition: mean change in CDT score from day 1 of treatment to day 14 (visit 2, after treatment). Motor Function/Balance: mean change in time to complete the five times Sit-To-Stand test from day 1 to day 14 (visit 2, after treatment). Psychological/behavioral function: mean change in Beck's Depression Inventory (BDI) scores from day 1 to day 14 (visit 2, after treatment). Hemispatial Neglect (Instant change, in participants with hemispatial neglect on day 1): mean change in Albert's Line Bisection Test score from 30 to 60 minutes before treatment on day 1 to 30–60 min after treatment on day 1. Thermosensory analysis: mean change in pain detection and pain thresholds using the TSA II thermosensory device (Medoc Advanced Medical Systems) from day 1 (visit 1, prior to treatment) to day 14 (visit 2 after treatment).

3.4.3.1. Changes to trial outcomes after the trial commenced, with reasons. A Fatigue Assessment Scale (FAS) test, shoulder flexion as well as algometry (ALG) were added as secondary outcome measures to assess changes in patient fatigue levels and sensitivity to pressure. A Force 10 FDX-25 force gauge (Wagner Instruments) in peak mode was used for algometry over the medial area of the lower anterior arm regions, repeated three times on each arm and averaged over triplicate measures in Newtons for analysis. The Medoc

TSA II Quantitative Sensory Thermoanalyser was also included to evaluate the mean of triplicate tests for patient thermal detection and pain sensitivity over the medial forearm [56,57].

3.4.4. Sample size and power estimation

The sample size of 20 patients on trial with 10 completed in each study group (at least 10 control and 10 etanercept participants) was based on the published data from observational studies reported previously [32,43]. The assumption was that the population of participants and their outcomes were normally distributed. Dr David Schoenfeld's Harvard website http://hedwig.mgh.harvard.edu/sample_size/js/js_parallel_quant.html was used for sample size determination. This was validated by the trial biostatistician. For the outcomes used in this study, power estimations were as follows:

3.4.4.1. Power calculation for sample size based on previous reports of analyses by pain test. The values used for this power calculation were taken from those reported [32] (Table 10), obtained after one injection of perispinal etanercept treatment. From this study, the group baseline for the vertical assessment scale (VAS) mean score (±S.D.) was 7.1 (± 2.09) on the 11-point scale [32]. After treatment, the mean score (± S.D.) was 2.3 (± 2.81). Based on this data, a minimum total of 14 patients (7 in each group) were required for enrollment with power to detect a treatment difference = 83% at a two-sided 0.05 significance level. Hence, given the predicted size effect, the study was considered suitably powered with a minimum number of patients at 20 to reach levels of significance.

3.4.5. Interim analyses and stopping guideline

After the first randomized group reached 10 patients on both the control and etanercept groups (total of n = 26 enrolled patients with n = 22 participants completing week 4/day 30 on trial), interim unblinding and analysis of outcomes was triggered.

3.4.6. Method used to generate the random allocation sequence

A computer-based random number generator in blocks of five was used by the pharmacist to establish the trial unblinding code for random assignment of enrolled patients into either group (allowing up to a total of at least 40 patients in each group, if required).

3.4.7. Randomization, blinding and patient replacement procedures

Patients were assigned based on the randomization code if they met all the inclusion criteria and none of the exclusion criteria. Every subject who passed initial screening based on inclusion/exclusion testing was included as intent to treat as they were assessed and entered numerically into the test pool. A final round of screening occurred as patients attended the first treatment, visit 1 at the clinic for validation as suitable for enrollment. A numbered ID was assigned to each participant by the trial pharmacist. Any patient on trial who discontinued or failed to complete the study was replaced and the replacing patient denoted with the next numbered ID in the order of the randomization number code. Allocation was concealed from clinical investigators, assessors, and participants during the trial to ensure double-blinding.

Interventions were prepared by the pharmacist to be identical in appearance and placed inside containers labeled only with patient ID and number on enrollment, with the identification of the intervention sealed inside an unblinding envelope at the bottom of the containers and to be opened only in the event of emergency.

4. Statistical methods used to compare groups for primary and secondary outcomes

For statistical analyses, Statistical Package for Social Sciences (SPSS; Vn25) software program was used, available at Griffith University. The data from all analyses of each individual participant were recorded on their Case Report Form and data from this entered onto spreadsheets using Microsoft Excel and then processed via SPSS for generalized estimating equations with first-order autoregressive relationship as the working correlation matrix. Other analyses included two-tailed paired or independent-samples t-tests, ANCOVA, logistic regression using the general linear model with repeated measures or non-parametric and other suitable tests as required. Univariate analysis of variances (UNIANOVA) was used to provide an estimation of effect size (η^2; eta-squared value) and observed power. Analyses included comparison of outcomes within and between the etanercept and the control groups for differences in median scores using the Wilcoxon signed-rank test for pain scores out of 100 points or mean scores from cognitive function, sensory, motorneuron or other tests, as well as differences from baseline. The data analyses included changes from baseline scores with standard error about the mean or interquartile ranges about the changes in median levels. Assumptions of normality were satisfied using Shapiro–Wilks test, normal Q–Q plots of differences and box-plot outlier analysis. For Mann–Whitney U exact tests, the treatment effects on pain levels (difference between groups) were quantified using the Hodges-Lehmann (HL) estimator from SPSS. This estimator (HLΔ) was used to determine the median of all possible differences in outcomes between each subject in the etanercept group versus each subject in the control group. A non-parametric 95% confidence interval for HLΔ accompanied these estimates and determined the median of differences between the two groups or the location shift in the median. For analysis of thermal perception and pain thresholds, multilevel modeling with the linear-mixed models (LMM) function in SPSS was also used. Bonferroni corrections were applied for multiple comparisons. Where relevant for all the above analyses, p-values <0.05 were considered significant and <0.01 highly significant.

Four participants initially enrolled into the trial were excluded. From the etanercept group, one developed shingles after visit 1, one had complications of severe lower back pain from spinal stenosis considered unrelated to stroke, and one demonstrated delusional features and hence, was unreliable for assessment. One patient was excluded from the control group after starting oxycodone medication the week of visit 1 and on Day 1, no longer experienced any pain. Patients were

recruited over the period from November 2016 through to March 2019.

4.1. Decision for study completion

After interim review of the first cohort (n = 22 participants) showed significance ($p < 0.05$) across both primary (baseline to day 30 change in vNPRS-FPS pain measures on 0–100 scale) and secondary outcomes (pre-post visit 1 change in vNPRS-FPS and shoulder flexion in arc degrees by goniometry), the completion phase was triggered. The study was then stopped early due to the significance of the positive results. Of the completing participants, for each individual, all four data points (baseline; visit 1 after treatment 1; visit 2 after treatment 2; and day 30) were included in the vNPRS analysis and by original assigned groups with n = 10 in the active and n = 12 in the control group used for comparison.

4.2. All important harms or unintended effects in each group

No serious adverse events were recorded on trial. However, a single adverse event occurred with one patient developing shingles after visit treatment 1. A clinical review of etanercept usage reported herpes viral opportunistic infections as a treatment-emergent adverse event of special interest (AESI) [39], as specified by the Federal Drug Administration, USA and hence is not a serious adverse event. The risk of shingles was previously noted only after long-term therapy with anti-TNFα medication [58] and in Strangfeld et al. (2009), the risk of developing shingles was not increased for those treated with etanercept [41]. Hence, the present case may have resulted from a compromised immune status due to the chronic pain. Consequently, this patient was excluded from further participating on-trial as the results would be complicated by the pain from shingles and potential for post-herpetic neuralgia.

5. Results

Of the 26 CPSP patients enrolled on the trial, 22 completed the protocol. Only the one adverse event of special interest (AESI) [39], due to a case of shingles occurred as a possible treatment-emergent risk. The distribution of the study group and participant baseline characteristics are shown in Table 1. All participants initially demonstrated significant intractable and constant daily CPSP with pain scores at baseline entry between 40 and 80 inclusive on the 0–100 point vNPRS-FPS, with their pain refractory to analgesic medications (including oxycodone or pregabalin). Seven participants in the etanercept and five in the control group had limited shoulder flexion of their paretic arm with active motion ≤75 degrees at baseline. No significant baseline differences between the groups were noted for any of the trial measures by independent samples t-test ($p > 0.05$; Table 1) and no unequal variances by Welch's t-test.

5.1. Primary outcome measures

5.1.1. Significant decline in pain severity of patients receiving perispinal etanercept compared to control

The descriptive statistics for the pain scores out of 100 for each group at baseline, visit 1 (V1) after treatment and at day 30 are reported in Table 2, including the medians and means for the two groups and the interquartile ranges (IQR). The change from baseline in pain intensities (based on the 0–100 point score on the vNPRS-FPS) for the mean values over the four repeated measures of average pain (including baseline (PRE); visit 1 (V1) after treatment 1; visit 2 (V2) after treatment 2; and at day 30 (D30 after treatment 1) scores) for each group were compared (Figure 2(a)).

Analysis by original assigned group of the repeated measures using generalized estimating equations showed a significant group*treatment interaction for worst pain (Wald $\chi^2 = 4.58$; df 1; $p = 0.032$) and for average pain (Wald $\chi^2 = 4.161$; df 1; $p = 0.041$). Post-hoc analysis demonstrated a greater reduction in pain levels for the etanercept compared

Table 1. Baseline characteristics of patients on the stroke trial.

Characteristic mean (±S.E.)	Etanercept 25 mg (n = 10)	Saline control (n = 12)	Mean difference (95%CI), p value (2-tailed)
Age, y	57.3 (4.95)	61.65 (8.66)	−4.35 (−15.3 to 6.6), $p = 0.42$
Weight, kg	77.74 (5.73)	85.85 (4.86)	7.09 (−22.9 to 6.7); $p = 0.266$
Gender M:F	5:5	7:5	
Years since stroke	4.18 (0.72)	4.98 (1.15)	−0.8 (−3.43 to 1.82), $p = 0.52$
Average daily pain (vNPRS-FPS)	68 (3.35)	60.42 (485)	7.58 (−5.18 to 20.35), $p = 0.23$
Worst daily pain	82.5 (3.96)	74.58 (3.45)	7.92 (−3.0 to 18.83), $p = 0.15$
Active Shoulder Flexion/ROM[a]	55 (15.69)	84.11 (13.26)	−29.11 (−73 to 14.8), $p = 0.179$
Passive Shoulder Flexion/ROM[a]	103 (10.25)	116.11 (12.35)	−13.11 (−46.73 to 20.51), $p = 0.42$
MOCA	21.8 (2.33)	25.5 (0.77)	−3.66 (−8.6 to 1.29), $p = 0.138$
BDI	17.6 (3.32)	20.8 (3.36)	−3.23 (−13.18 to 6.72), $p = 0.51$
FAS	31.4 (1.54)	34.8 (1.62)	−3.43 (−8.16 to 1.29), $p = 0.145$
STS	19.5 (2.7)	24.4 (3.5)	−4.88 (−14.4 to 4.6); $p = 0.298$
CDT	N/A	N/A	N/A
Albert's Line Bisection Test/	N/A	N/A	N/A

S.E, standard error; y, years; vNPRS-FPS, vertical Numeric Pain Rating Scale with a Faces Pain Scale (0–100); MOCA, Montreal Cognitive Assessment test; BDI, Beck's Depression Inventory; STS, 5 times Sit-to-Stand/seconds; FAS, fatigue assessment score/50. CDT, Clock Drawing Test; N/A, not applicable. [a]Values shown for patients with restricted (<180 degree) shoulder flexion/rotation of movement (ROM) in arc degrees at baseline only for the paretic/hemiplegic arm (n = 10 in etanercept group, n = 9 in control group).

Table 2. Nonparametric related-samples tests and descriptive statistics for pain scores comparing within-group responses.

A. Descriptive Statistics

GROUP /(Numbers)		Mean	S. D.	Min.	Max.	Percentiles 25th	50th (Median)	75th
1. Etanercept (10)	V1_PRE_Av_Pain	68.00	10.593	50	80	58.75	70.00	76.25
	V1_POS_Av_Pain	44.00	31.429	0	90	15.00	50.00	71.25
	D30_Av_Pain	44.10	28.862	0	80	22.25	50.00	65.00
	V1_PRE_Worst_Pain	82.50	12.528	60	100	72.50	87.50	90.00
	V1_POS_Worst_Pain	49.00	35.182	0	90	3.75	60.00	78.75
	D30_Worst_Pain	63.00	24.967	0	90	57.50	70.00	80.00
2. Control (12)	V1_PRE_Av_Pain	60.42	16.714	30	85	50.00	62.50	70.00
	V1_POS_Av_Pain	57.08	24.258	0	95	50.00	57.50	72.50
	D30_Av_Pain	57.50	25.540	0	95	42.50	60.00	77.50
	V1_PRE_Worst_Pain	74.58	11.958	50	90	66.25	77.50	85.00
	V1_POS_Worst_Pain	61.25	25.595	0	100	52.50	60.00	75.00
	D30_Worst_Pain	72.08	16.440	40	95	60.00	75.00	87.50

B. Related-Samples Wilcoxon Signed-Rank Test

Related-Samples:	V1PRE-D30 Average Pain		V1PRE-D30 Worst Pain		V1PRE-V1POST Average Pain		V1PRE-V1POST Worst Pain	
Group:	Etanercept	Control	Etanercept	Control	Etanercept	Control	Etanercept	Control
Total Numbers	10	12	10	12	10	12	10	12
Test Statistic	2.000	12.500	3.500	23.000	3.000	26.500	0.000	13.000
Standard Error	7.133	5.809	9.747	9.740	7.141	11.164	7.133	11.186
Standardized Test Statistic	-2.243	-0.258	-2.462	-0.462	-2.100	-0.582	-2.524	-1.788
Asymptotic Sig.(2-sided test)	0.025	0.796	0.014	0.644	0.036	0.560	0.012	0.074
Exact Sig. (2-sided test)	0.023	0.859	0.012	0.687	0.039	0.584	0.008	0.079

S.D., Standard Deviation; V1, visit 1; PRE – before treatment, POS – after treatment; D30, day 30. Min., minimum; Max., Maximum. Percentile scores are shown out of 0–100 on the vNPRS-FPS to indicate interquartile ranges; Sig., significance.

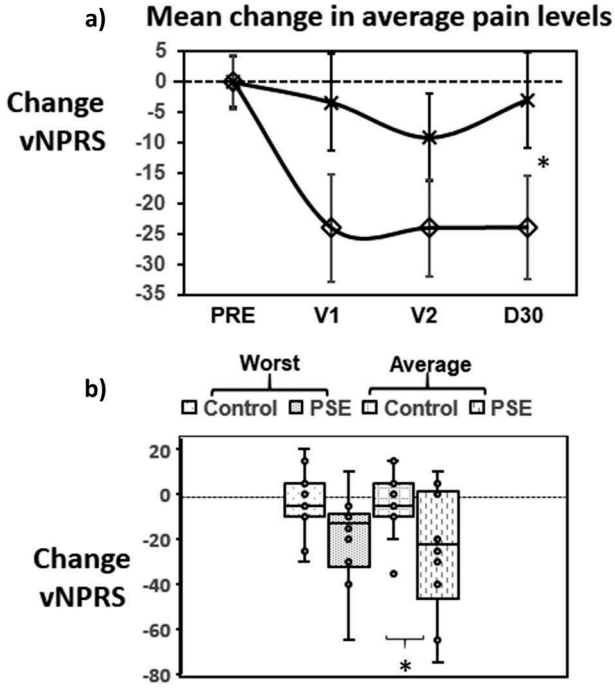

Figure 2. (a) Pain levels are rapidly decreased following PSE treatment. Mean changes in score levels of average pain on the vNPRS-FPS (0–100 points scale) comparing between the saline control (×) and etanercept (◊)-treated groups ± S.E. PRE: baseline; V1: Visit 1 after treatment 1; V2: Visit 2 after treatment 2; D30: day 30 after Visit 1. (b) Box-plot analysis of differences in median of worst and average pain levels comparing between baseline and day 30. Control group compared to perispinal etanercept (PSE) treatment group with medians, quartile ranges, and outliers for changes in pain levels as shown on 0-100 point scale. * $p < 0.05$.

to the control group over the trial period with a mean decline (change on 0–100 point scale ± S.E.) in average and worst daily pain levels from baseline to day 30 within the etanercept group of 24 ± 9 and 19.5 ± 6 points, respectively (Figure 2(a, b)). The decline in pain levels from baseline to day 30 within the etanercept group for both the average and worst daily pain levels were significant (two-tailed paired t-test: $t_{(9)} = 2.63$; $p = 0.027$; $t_{(9)} = 2.94$; $p = 0.017$, respectively).

Mann–Whitney U tests showed a significant reduction in both average and worst pain scores comparing the differences in vNPRS-FPS (on the 0–100 point score) from baseline to day 30 after the two perispinal etanercept treatments. From Figure 2(b), the values from the Mann–Whitney U test were for average pain: U = 29.5; $p = 0.04$, HLΔ between group medians = 15; 95% CI = 0–40; and for worst pain: U = 26; $p = 0.023$, HLΔ between group medians = 15; 95% CI = 5–30. Table 2 also shows the results from the related-samples Wilcoxon Signed-Rank test summary with the levels of significance for the median of differences within groups from baseline. Again, the median of differences (two-sided test) between baseline and day 30 for worst ($p = 0.014$) and average ($p = 0.025$) pain scores from the etanercept group were significantly different, whereas the pain values for the control group (p > 0.5) were not significantly altered.

In order to obtain an estimate of the power of the study for detecting the change in pain levels, UNIANOVA was applied and the comparison between groups for changes in mean vNPRS-FPS scores out of 100 points from baseline to day 30 after treatment were significant (for worst pain: $p = 0.037$, $\eta^2 = 0.20$, observed power of 56% and for average pain, $p = 0.045$, $\eta^2 = 0.19$, observed power of 53%). At the individual level, 4 of the 10 patients in the etanercept group showed no or limited effects on pain, whereas 3 others had rapid and complete or almost complete resolution of their pain levels directly (by 30 min) post-treatment during visit 1.

Within the control group, there was no significant decrease in the median values for the average or worst daily pain levels (which each decreased by only 2.5 points on the 0–100 point scale) comparing baseline to day 30 (D30; by related-samples Wilcoxon Signed Rank two-sided tests, p > 0.5 and by paired t-tests: average pain, $t_{(11)} = 0.52$; $p > 0.5$ and worst pain, $t_{(11)} = 0.59$; p > 0.5). Categorically, one participant in the control group demonstrated a response for average pain during the trial (Figure 2(b); outlier).

5.2. Secondary outcome measures

1) Instant differences in worst and average pain.

For the secondary outcome measures, within the etanercept group a significant difference was detected in the immediate effects from baseline at visit 1 compared to directly after treatment (within 30–60 min) on the 0–100 point scale for the change in worst and average pain levels (paired t-test: $t_{(9)} = 3.24$; $p = 0.01$ and $t = 2.59_{(9)}$; p = 0.029, respectively). Post-hoc nonparametric tests demonstrated that for this instant pain reduction, the median decrease (on the 0–100 point vNPRS-FPS) for worst pain was by 27.5 points (related-samples Wilcoxon signed-rank test; p = 0.012) and the mean ± S.E. decreased by 33.5 ± 10.4 points (out of 100) within the etanercept group (Table 2; Figure 2). By comparison, within the control group, the median of the differences in worst pain was 17.5 points (related-samples Wilcoxon signed-rank test; p > 0.05) and the mean change was by 13.33 ± 6.7 points, which was non-significant (paired t-test: $t_{(11)} = 1.98$; $p > 0.05$). Pain scores in the control group also returned to baseline levels by day 30, indicating no significant effects overall on the pain levels within the saline control group (Figure 2).

2) Improvements in functional mobility.

At baseline, the limited extent of shoulder flexion by the paretic arm by all participants (comparing arc degrees of ROM across the entire cohort) was highly correlated comparing the active and passive movements (Pearson's correlation: r = 0.898; $p < 0.01$). Analysis of repeated measures using generalized estimating equations for changes in mean active shoulder flexion range of motion (ROM/degrees of arc) by the paretic arm demonstrated a highly significant group*treatment interaction over the course of the trial (Wald $\chi^2 = 8.625$, df 1; $p = 0.003$; Figure 3(a)).

Post-hoc analysis showed successive improvements occurred within the etanercept-treated group for their active ROM when compared to baseline, initially shoulder flexion improving by a change in mean (± S.E.) of 30 ± 7.3 arc degrees after treatment 1 within 30–60 min (paired t-test: $t_{(9)} = 4.07$; p = 0.003), which increased to 55 ± 12 arc degrees after treatment 2 (paired t-test: $t_{(9)} = 4.54$; p = 0.001) (Figure 3(a)). UNIANOVA demonstrated a significant difference between

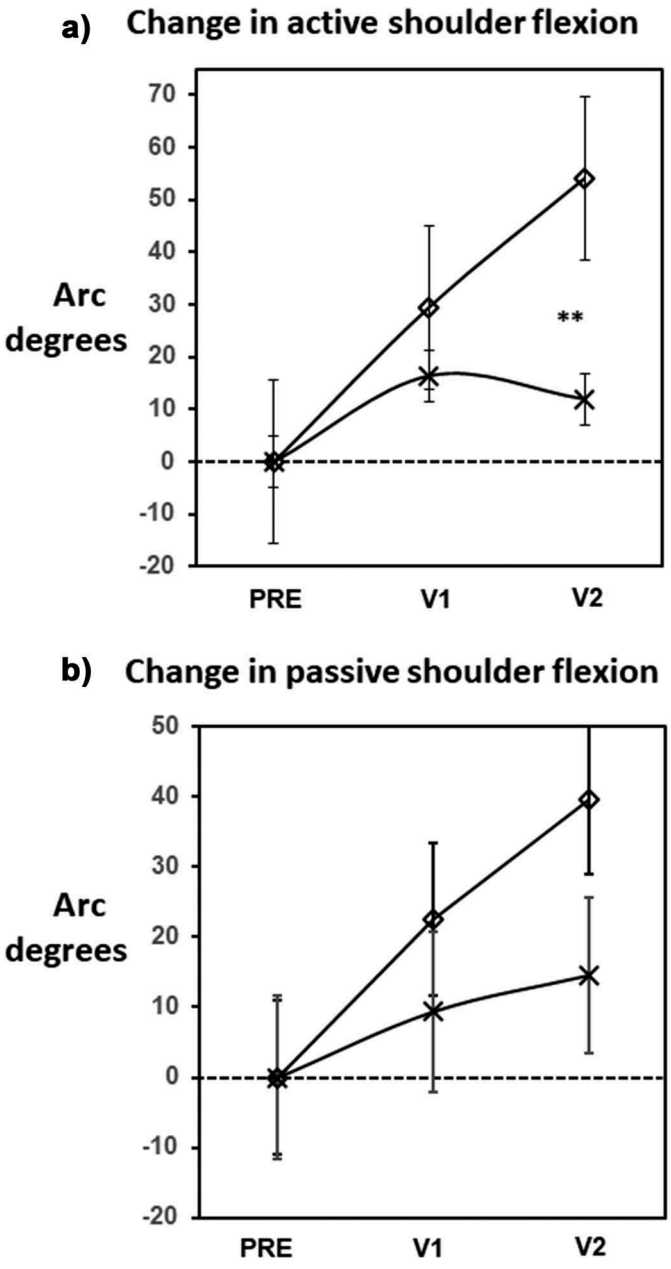

Figure 3. Rapid and marked improvement in active shoulder flexion by paretic arm after PSE treatment. Goniometry for mean changes in rotated angle (arc degrees) of (a) active and (b) passive shoulder flexion from baseline, comparing between the control (×) and etanercept (◊)-treated groups (± S.E.). PRE: baseline; V1: Visit 1 after treatment 1; V2: Visit 2 after treatment. ** $p < 0.01$.

groups in arc degrees of shoulder flexion from baseline to after treatment at visit 2 ($p = 0.011$, $\eta^2 = 0.28$, observed power of 76%). Categorically, 9 out of the 10 patients from the etanercept group with restricted paretic arm mobility had improved shoulder flexion of their paretic arm, such that 6 of these 10 showed marked improvements in active shoulder flexion by an increase ≥60 arc degrees, 3 of the 10 fully regaining 180 degrees of flexion. Again, as with the changes seen with the reduced pain levels, this improvement was noted to begin immediately (by 30 min) after the first treatment during visit 1 (Figure 3(a)).

A significant group*treatment interaction was also demonstrated for changes in mean passive flexion ROM for the paretic arm (Figure 3(b); Wald $\chi^2 = 4.861$, df 1; $p = 0.027$). Changes from baseline comparing the active to passive flexion ROM within the etanercept-treated group were strongly correlated (Pearson's correlation, $r = 0.805$; $p < 0.01$), indicating that the etanercept effect was improving both aspects of mobility for the paretic arm. No significant effect on either active or passive shoulder flexion by the paretic arm was detected within the 9 out of 10 in the control group with restricted mobility (Figure 3; paired t-test, $t_{(8)} = 0.828$; $p > 0.5$ for active; $t_{(8)} = -1.076$; $p = 0.313$ for passive). It should be noted that one of the patients in the control group (the outlier in Figure 1(b)), regained complete 180-degree flexion during the trial.

No significant relationship was detected when comparing the decreased pain levels and increased shoulder flexion of the paretic arm in the etanercept group ($r = 0.001$, $p > 0.05$). Categorically, although the majority of patients in the etanercept group showed improvements in shoulder flexion for their paretic arm, one patient in this group had significant pain reduction, but without any accompanying changes in arm mobility, whereas another four from this group, whilst showing limited changes in their pain levels, had greatly improved shoulder flexion.

5.3. Pressure pain sensitivity

Analysis by algometry (ALG) measured in Newtons across all participants for baseline responses of the forearms to applied pressure showed significantly lower levels detected as painful by the paretic versus the unaffected arm (paired t-test: $t_{(21)} = 2.3$; $p = 0.03$). Repeated measures from baseline (PRE), visit 1 (V1) and visit 2 (V2) after treatment were compared for changes between groups (Figure 4(a,b) for the paretic and unaffected arm, respectively).

During the trial, a mean increase in the applied pressure (Newtons, N) was required to induce pain from the paretic arm of the control group, whereas a decrease was demonstrated for the etanercept group (Figure 4(a)) and analysis by generalized estimating equations demonstrated that the group*treatment interaction was significant ($p = 0.02$). Post hoc analysis demonstrated that the mean pressure pain threshold (in Newtons) for the paretic arm within the control group at visit 2 after treatment was significantly greater than the mean level at baseline (paired t-test: $t_{(11)} = 2.53$; $p = 0.028$). Within the etanercept group, analysis of the unaffected arm also showed a decrease in the pressure pain threshold at visit 2 after treatment compared to baseline, although this was not significant (paired t-test: $t_{(9)} = 1.58$; $p = 0.147$; Figure 4(b)).

5.4. Other secondary measures

No significant effects were detected for the between-group differences in fatigue (FAS), depression (BDI) or sit-to-stand (STS) measures over the course of the trial (Table 3).

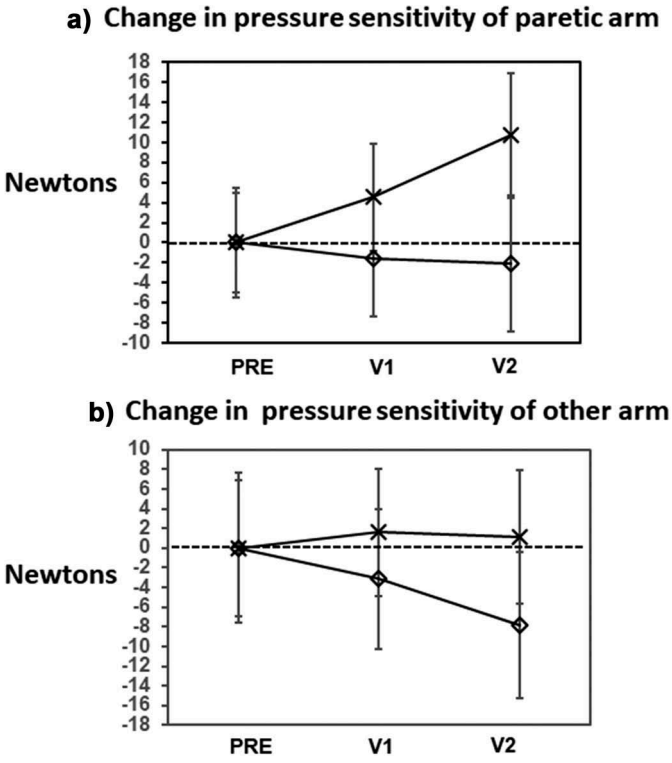

Figure 4. Change in pressure sensitivity by algometry. Comparison of etanercept versus control treatment groups for sensitivity to applied pressure by algometry on the (a) paretic/hemiplegic arm versus (b) unaffected arm. Mean change shown for the control (×) and etanercept (◊)-treated groups (± S.E.). PRE: baseline; V1: Visit 1 after treatment 1; V2: Visit 2 after treatment. $p > 0.05$. Scale for applied pressure measured in Newtons (N).

Impairment from stroke in parameters including MOCA, STS and BDI were noted to be insufficient in our study groups in order to enable detection of any changes and hence, these parameters were not further assessed. The change in fatigue levels (FAS; out of 50) from baseline for the two patient groups showed a similar trending improvement based on the slopes of the graphs (Figure 5).

5.5. Thermosensory analysis

Approximately 40% of patients with CPSP reportedly experience hypoalgesia [59]. Consistent with this observation, 7 of the 10 etanercept and 5 of the 12 control group (totaling n = 12 out of the 22 participants on trial) showed thermal pain insensitivity at baseline with extensive thermal hypoalgesia displayed by the paretic arm (Supplementary Table 2). Using the thermosensory analyzer, reduced sensitivity to perceive stimuli as either hot ($p < 0.001$) or cold ($p < 0.001$), as well as lower hot or cold pain thresholds (both $p < 0.001$) were detected when comparing the paretic to the unaffected arm across all the participants at baseline.

Apart from a trend toward an increase in cold pain threshold over time in the etanercept group (Figure 6; generalized estimating equations: group*treatment interaction, p = 0.053), no other significant differences were apparent in thermal detection or pain thresholds between the active and control groups. Etanercept within-group bivariate analysis showed a significant correlation (Pearson's r = 0.730; $p = 0.016$) in the magnitude of change from baseline to visit 2 (after treatment) comparing the decrease in % pain levels (by the 0–100 point vNPRS-FPS) with the increase in cold pain detection. No significant correlation was detected within the control group (r = 0.045, $p > 0.5$).

In summary, these results demonstrate that there was a rapid (by 30–60-min post-treatment) pain alleviation which was maintained over time, together with marked improvement in both active and passive mobility of the paretic arm following two doses of etanercept treatment.

6. Discussion

This is the first double-blinded randomized controlled trial in CPSP demonstrating the significant effects of two doses of perispinal etanercept in reducing pain and improving mobility. The significant reduction in pain remained evident 30 days after trial enrollment and was not only statistically significant but exceeded the minimal clinically important difference or MCID [60] indicating the clinical relevance of such findings. The effects were demonstrated to occur rapidly (starting within 30–60 min) after the first treatment and were also complemented by improvements in mobility indicating a role for perispinal etanercept in improving the overall quality of life in CPSP.

Improvements in mechanical and thermal pain sensitivities also showed some interesting trends. Changes in functional sit-to-stand times or psychological measures were not significantly different between the two groups. Caution should be observed when interpreting the results of this trial. Although the overall responses for the etanercept group were clearly apparent with the observed lowering in mean pain levels reaching significance, universal improvement in the primary outcome was not achieved. Four of the 10 etanercept group showed no or limited effects on their

Table 3. Secondary Outcomes: differences in test scores at Visit 2 after treatment compared with baseline.

	Group Statistics		Independent samples t-test for equality of the means	
Assessment/Out of total score	Etanercept (n = 10) (SE)	Saline control (n = 12) (SE)	Mean Difference (95% CI)	p value (two-tailed)
BDI/63	−3 .8 (1.23)	−6.67 (2.46)	2.87 (−2.96 to 8.7)	0.31
MOCA/30	−0.6 (1.79)	0.55 (0.9) (n = 11)	0·56 (−5.22 to 2.93)	0.56
FAS/50[a]	−4.3 (1.35)	−5.8 (1.82)	0.78 (−4.12 to 5.69)	0.74
STS/seconds	−9.2 (2.37)	−9.04 (2.64)	0.16 (−7.71 to 7.4)	0.97
ALG AFF ARM/N	−2.1 (3.9)	10.7 (4.23)	−12.8 (−25 to −0.6)	0.04
ALG GOOD ARM/N	−7.81 (4.91)	1.12 (5.89)	−8.93 (−25.35 to 7.5)	0.27

[a]At day 30 compared to baseline. Abbreviations: ALG: Algometry of paretic/hemiplegic (affected; AFF) versus good arms measured in Newtons (N); BDI: Beck's Depression Inventory; MOCA: Montreal Cognitive Assessment; FAS: Fatigue Assessment Score; STS: Five times Sit-To-Stand.

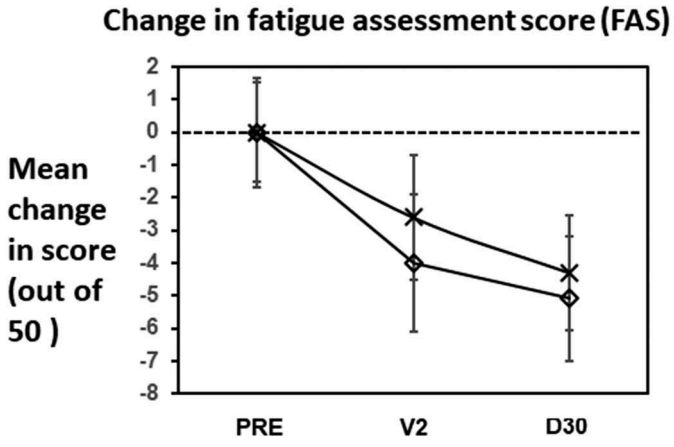

Figure 5. Similar change in Fatigue Assessment Scores (FAS). Both control (×) and etanercept (◊) groups (± S.E.) showed improvement in mean fatigue levels (lower values out of total 50) over the trial period. PRE: baseline; V2: Visit 2 after treatment 2; D30: day 30 after Visit 1. $p > 0.05$.

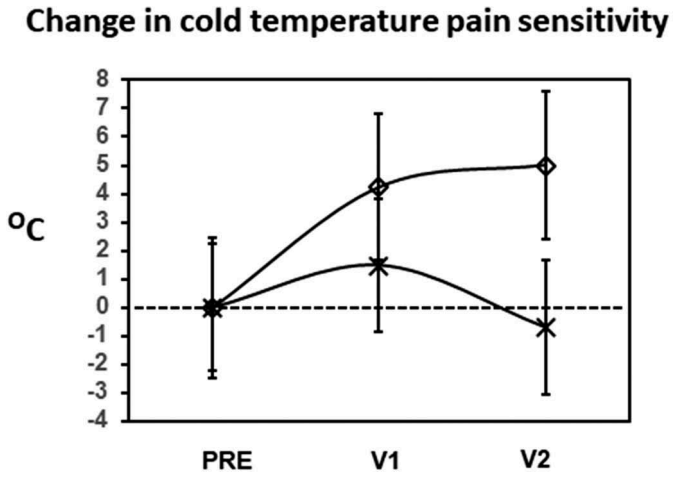

Figure 6. Quantitative thermosensory analysis. Change in recorded temperature (^{0}C) for cold pain sensitivity of control (×) versus etanercept (◊) groups (± S.E.) during trial. PRE: baseline; V1: Visit 1 after treatment; V2: Visit 2 after treatment. $p > 0.05$.

pain levels. However, in contrast, 3 in 10 of the etanercept group showed complete or almost complete resolution of CPSP after either the first or second of the two perispinal treatments. These results indicate that etanercept demonstrates variable responses on CPSP at the individual level. At present, the possible reasons for the significant reduction in pain levels for several of the patients from the perispinal etanercept group, but not by all patients in this group, is not clear. Possible reasons may relate to the extent and outcome of the post-stroke reorganization in the central nervous system and factors including lesion size, extent and severity of the stroke damage, and time elapsed since stroke. Further studies will be required to resolve these points.

Although the processes associated with the reduction in constant levels of pain in the stroke patients are not readily apparent, trends toward a normalization of mechanical and thermal sensitivities were also demonstrated. A previous study of CPSP treatment using deep brain stimulation reported similar improvements in pain associated with normalization of deficits in somatosensory perception, including thermal sensitivity [61]. The results for algometry and thermosensory analysis of CPSP patients obtained in the present study suggest that the changes in the etanercept group were trending toward normalization of somatosensory function occurring with increased thermal and pressure pain sensitivity, in line with the other trial outcomes of improved neuromuscular function.

Typically, normal pain thresholds are approximately 45ºC and 10ºC for hot and cold (Medoc Advanced Medical Systems, Ltd) respectively. Based on the Medoc thresholds and from comparing thermal responses of the paretic to the unaffected arm, almost half of the patients in each group demonstrated thermal hypoalgesia at baseline. Notably, the two patients with the strongest pain reduction in the etanercept group also showed the greatest normalization in their thermal and mechanical pain sensitivity, consistent with the increasing sensitivity associated with returning or restoration of neurosensory function. This was further confirmed by bivariate correlation when comparing the magnitude of the reduction in CPSP levels with the increase in thermal sensitivity to cold temperature-related pain responses.

6.1. The role of TNFα in central post-stroke pain

The data from this trial further adds to the mounting evidence supporting a direct role within the central nervous system for TNFα involvement in pain modulation [62] and specifically for neuropathic pain conditions [23] such as CPSP. The present outcomes from this randomized clinical trial also support the previously reported observational data that showed positive clinical responses to TNFα inhibition in patients with chronic stroke and associated improvements in moderate to severe disability [32,33,43]. Elevated TNFα levels have not only been demonstrated in the cerebral spinal fluid of acute stage [9,12–18] but also chronic post-stroke patients [19], as well as in patients with clinical depression [63], traumatic brain injury [19,64], multiple sclerosis, dementia and probably a host of other neurological disorders, as reviewed in [7,42,45]. This raises the specter of TNFα having a major role not only in the stroke penumbra and acute phase of damage, but also impacting on the ensuing global inflammatory aspects affecting the wider range of normal brain function [65]. The improvement in clinical outcomes in our study for CPSP patients after treatment with a TNFα inhibitor further implicates the role of TNFα in this condition as well.

The underlying mechanism(s) at the molecular level responsible for the improvement in clinical outcomes in our study are challenging to define. TNFα has been recently shown to induce the increased expression of the TRP family of calcium-channel-related thermal nociceptors [66] and this action of TNFα would increase pain sensitivity via nociceptor sensitization [66,67]. TNFα receptor signaling is also required for development and function of primary nociceptors in sensory neurons [68]. The alleviation of CPSP in the present study

supports this mechanism as possibly underlying changes in the central nervous system in response to etanercept actions shown here and reported elsewhere [28,69]. However, the improvements in somatosensory perception in both arms demonstrated after treatment with etanercept are more difficult to explain. Despite the reduction in CPSP in the group administered perispinal etanercept, our study did not demonstrate a clear improvement in thermal sensory thresholds, although there was a trend toward normalization of cold pain thresholds in the etanercept group which correlated significantly with the decrease in CPSP within this group. Hence, the gain in responsiveness to both peripheral pressure and thermal stimuli suggest a normalization/restoration was also occurring in somatosensory perception by the central nervous system, along with the reduced CPSP. The small sample size likely precluded detecting more highly significant differences in thermal and pressure sensitivity. TNFα has a plethora of other roles in the central nervous system for regulating neuronal function, synaptic plasticity and neurotransmitter activity related to pain [70–73] which may also underlie mechanisms associated with the persistence of CPSP and the effects of anti-TNFα detected here.

6.2. TNFα and neuromuscular function

In view of the above neurosensory aspects of TNFα, understanding TNFα's role in control of neuromuscular function is limited. An unprecedented finding of this trial was that the majority (90%) of patients within the etanercept group showed significant rapid enhancements in both active and passive shoulder flexion ROM by their paretic arm. This change in movement was irrespective of effects on their pain levels, with three out of the 10 patients in the etanercept group regaining their full use and complete 180 degrees of active shoulder flexion. This result indicates that a relaxation in the extent of spasticity was likely occurring in the arm muscles of patients treated with perispinal etanercept, improving both their active and passive control of movement. TNFα has previously been demonstrated to be associated with muscle atrophy, particularly during cachexia in diseases such as cancer [74], and increased TNFα levels have been linked with skeletal muscle loss/atrophy as a common sequelae associated with individuals with chronic stroke symptoms [75], as well as sarcopenia [76]. The rapid improvement (after first treatment) in active shoulder flexion provides evidence that such sequelae may be reversible and points to TNFα also being involved in regulating neuromuscular function. Hence, when the results with loss of pain, changes in sensory (thermal and pressure) perception and improved mobility are considered together, occurring almost immediately following first treatment (with no significant changes in the control group), the data provide the first confirmatory supportive evidence from a randomized parallel double blinded clinical trial for rapid and wide-ranging benefits achievable by treating stroke patients with perispinal etanercept.

It would be remiss not to discuss the risks from etanercept for potential adverse effects. The risk profile for serious infections with etanercept is similar to that observed with the other TNFα blockers (reviewed in [38]) and in some cases, maybe lower [40]. Among patients with autoimmune diseases, compared to treatment with nonbiologic regimens, initiating anti-TNFα has not been associated with greater risk of serious adverse events (defined as requiring hospitalization for serious infections) [77]. Consistent data from observational studies also suggest that the rate of serious infections was mainly increased over the first 6–12 months of ongoing use (reviewed in [78]). Millions of doses, with many at 50 mg being twice the presently tested dose (25 mg), are commonly being applied in much higher dosage numbers (often up to 50–100 doses per year per patient) over many years of chronic use for the treatment of autoimmune diseases. Hence, the short-term use of two 25 mg doses presently administered over a 2-week period for perispinal etanercept treatment has a relatively low-risk side-effect profile and is well tolerated. It would also seem to offer advantages over the current use of sedative drugs such as opioids or gabapentanoids for treatment of pain in stroke patients [79,80].

Some discussion is warranted regarding the observed power of this study as a guide and reference in relation to future trials. *A posteriori* power analysis from this single study showed that the small sample size was sufficient, particularly based on the goniometry change in ROM by shoulder flexion at 76%, with a high level of significance. On the other hand, the primary outcome measure of pain analysis showed a 53–-56% power with a lower level of significance with this power estimate below the level predicted *a priori*. These findings suggest that the size of the cohorts used was close to the minimum sample size for statistical significance and would likely improve with increased numbers. A limitation of the present study was that it did not improve all the secondary outcomes of the trial, most likely because of the small sample size (total n = 22) which precluded sufficient power to detect statistically significant responses for some of these measures. In addition, these findings may prove to be of value as a guide for proceeding with caution when considering the design and planning for future-related trials in that the improvements in BDI, STS and FAS tests in both groups (Table 3) indicated that the control group may have experienced a placebo effect within these secondary parameters, possibly reflecting the control group participants' positive beliefs in obtaining some benefit on trial. Alternatively, this could also have been an effect from the saline control acting as a dilution factor after injection into the cerebrospinal venous system or may reflect the relatively short duration of the study with measures spanning over a four-week period. In this regard, placebo effects have been previously reported to occur in some pain trials [81,82]. A small effect was noted with a trend toward lower pain levels detected after first treatment within the control group, but this was not significant. A possible reason for this would be the self-realization of the control patients during the trial that they were not obtaining any obvious improvement.

Several questions arise from this study with the intent of optimizing patient outcomes in the longer term, particularly relating to the underlying causes for the variable response rates, with some but not all our stroke patients showing dramatic improvements after perispinal etanercept. With further

studies, it may be possible to expand on the application of perispinal etanercept therapy by identifying more precisely the determining factors for those patients who show significant responses, as well as the longevity of their effects. Our study's focus was on chronic stroke patients (average of 4 to 5 years since stroke) and the primary endpoint was 30 days after the first treatment. Thus, the role of anti-TNFα in earlier stages of stroke, or the longevity of treatment effects cannot be determined from our data, nor can we address the possibility of greater improvements that might be obtained with further perispinal etanercept treatments. Hence, dose optimization clearly needs to be established, including determining the best regimen, the dosing level to be delivered via the cerebrospinal venous system, the optimal timing of stage of stroke and the intervals between successive treatments. Larger trials would also allow the underlying mechanisms to be further evaluated, with our current trial being underpowered to detect changes in thermal or pressure sensitivity/pain, albeit with a trend toward normalization.

Despite the relatively small sample size, the effect size and power of this study was sufficiently adequate for the primary outcome measure of pain, as was predicted based on the previously reported open-label results in stroke using the vertical pain assessment score [32]. Hence, together with the presently documented outcomes from small total participant numbers, our results bode well for any follow-up trials. It is advisable that follow-up studies consider the possibility of the short-term placebo effects as seen with some of the secondary exploratory measures examined here. These effects would likely dissipate over long-term evaluation as patients in the control group undergo the self-realization that they are on the saline treatment, given a lack of significant effects on their pain levels, or on their functional mobility.

7. Conclusions

This randomized, double-blinded, controlled parallel trial design significantly improved health outcomes in CPSP, particularly for reducing average and worst daily pain levels after treatment with perispinal etanercept. Improvements were also obtained with secondary outcome measures of pain and functional mobility. The reduced pain severity has provided significant ongoing benefits for some patients, at least over the medium term offering several months of reprieve (noted from follow-up post-trial) and points to a key role-played by TNFα in the manifestation of CPSP.

Putting our findings into the wider evidential context, etanercept has been shown to improve neurological outcomes in six different experimental animal models of stroke (reviewed in [29]). The supportive evidence from animal models together with the findings from this randomized clinical trial and the favorable outcomes from open-label use in over a thousand stroke patients during the past 9 years [32,43]; reviewed in [29]) and the recent case report of immediate resolution of hemispatial neglect and CPSP after perispinal etanercept [31] should arguably justify the availability of perispinal etanercept therapy for chronic stroke being assigned a higher priority. Furthermore, encouragement should be offered promoting further studies to be undertaken so that this treatment gains wider recognition and the acceptance required by the regulatory authorities. The above results *in toto* provide solid evidential support for the efficacy of perispinal etanercept therapy in improving outcomes with chronic stroke. It also emphasizes the need for further studies to identify in detail how to better exploit this information for alleviating the suffering experienced by stroke victims and to avoid the presently used and often ineffective drugs currently in clinical practice with their higher associated health risks including noted features of sedation and dizziness [83, 84].

Author contributions

All authors agree to be accountable for all aspects of the work and were involved in the conception, design and implementation of the trial. The SJ Ralph and AD Smith analyzed and interpreted the data and prepared the manuscript. All authors were involved in revising the content and final approval of the version to be published.

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Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Data sharing statement

The data collected for the study, including participant data and a data dictionary can be made available to other investigator researchers who provide a methodologically sound and approved proposal. Individual participant data that underlie the results reported in this article, after de-identification (text, tables, figures, and appendices) can be made available beginning 3 months and ending 36 months following article publication. Proposals should be directed to the corresponding author to gain access and requests must meet with investigator approval and a signed data access agreement must be in place.

Reviewer Disclosures

Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Treister AK, Hatch MN, Cramer SC, et al. Demystifying poststroke pain: from etiology to treatment. Pm R. 2017 Jan;9(1):63–75. PubMed PMID: 27317916; PubMed Central PMCID: PMCPMC5161714.
2. Oh H, Seo W. A comprehensive review of central post-stroke pain. Pain Manag Nurs. 2015 Oct;16(5):804–818. PubMed PMID: 25962545.
3. Akyuz G, Kuru P. Systematic review of central post stroke pain: what is happening in the central nervous system? Am J Phys Med Rehabil. 2016 Aug;95(8):618–627. PubMed PMID: 27175563.
4. Haroutounian S, Ford AL, Frey K, et al. How central is central poststroke pain? The role of afferent input in poststroke neuropathic pain: a prospective, open-label pilot study. Pain. 2018 Jul;159(7):1317–1324. PubMed PMID: 29570507.
5. Mulla SM, Wang L, Khokhar R, et al. Management of central post-stroke pain: systematic review of randomized controlled trials. Stroke. 2015 Oct;46(10):2853–2860. PubMed PMID: 26359361.
6. Fang M, Zhong L, Jin X, et al. Effect of inflammation on the process of stroke rehabilitation and poststroke depression. Front Psychiatry. 2019;10:184. PubMed PMID: 31031649; PubMed Central PMCID: PMCPMC6470379.
7. Tuttolomondo A, Pecoraro R, Pinto A. Studies of selective TNF inhibitors in the treatment of brain injury from stroke and trauma: a review of the evidence to date. Drug Des Devel Ther. 2014;8:2221–2238. PubMed PMID: 25422582; PubMed Central PMCID: PMCPMC4232043.
8. Cevik O, Adiguzel Z, Baykal AT, et al. The apoptotic actions of platelets in acute ischemic stroke. Mol Biol Rep. 2013 Dec;40 (12):6721–6727. PubMed PMID: 24057255.
9. Zaremba J, Losy J. Early TNF-alpha levels correlate with ischaemic stroke severity. Acta Neurol Scand. 2001 Nov;104(5):288–295. PubMed PMID: 11696023.
10. Zaremba J, Skrobanski P, Losy J. Tumour necrosis factor-alpha is increased in the cerebrospinal fluid and serum of ischaemic stroke patients and correlates with the volume of evolving brain infarct. Biomed Pharmacother. 2001 Jun;55(5):258–263. PubMed PMID: 11428551.
11. Barone FC, Arvin B, White RF, et al. Tumor necrosis factor-alpha. A mediator of focal ischemic brain injury. Stroke. 1997 Jun;28 (6):1233–1244. PubMed PMID: 9183357.
12. Tarkowski E, Rosengren L, Blomstrand C, et al. Intrathecal release of pro- and anti-inflammatory cytokines during stroke. Clin Exp Immunol. 1997 Dec;110(3):492–499. PubMed PMID: 9409656; PubMed Central PMCID: PMCPMC1904815.
13. Zaremba J, Losy J. The levels of TNF-alpha in cerebrospinal fluid and serum do not correlate with the counts of the white blood cells in acute phase of ischaemic stroke. Folia Morphol (Warsz). 2001;60(2):91–97. PubMed PMID: 11407149.
14. Zaremba J. Contribution of tumor necrosis factor alpha to the pathogenesis of stroke. Folia Morphol (Warsz). 2000;59 (3):137–143. PubMed PMID: 10974781.
15. Lambertsen KL, Finsen B, Clausen BH. Post-stroke inflammation-target or tool for therapy? Acta Neuropathol. 2019 May;137(5):693–714. PubMed PMID: 30483945; PubMed Central PMCID: PMCPMC6482288.
16. Lambertsen KL, Biber K, Finsen B. Inflammatory cytokines in experimental and human stroke. J Cereb Blood Flow Metab. 2012 Sep;32 (9):1677–1698. PubMed PMID: 22739623; PubMed Central PMCID: PMCPMC3434626.
17. Nguyen TV, Frye JB, Zbesko JC, et al. Multiplex immunoassay characterization and species comparison of inflammation in acute and non-acute ischemic infarcts in human and mouse brain tissue. Acta Neuropathol Commun. 2016 Sep 6;4(1):100. PubMed PMID: 27600707; PubMed Central PMCID: PMCPMC5011964.
18. Beridze M, Sanikidze T, Shakarishvili R, et al. Selected acute phase CSF factors in ischemic stroke: findings and prognostic value. BMC Neurol. 2011 Mar 30;11:41. PubMed PMID: 21450100; PubMed Central PMCID: PMCPMC3078848.
19. Kim JW, Park MS, Kim JT, et al. The impact of tumor necrosis factor-alpha and Interleukin-1beta levels and polymorphisms on long-term stroke outcomes. Eur Neurol. 2018;79(1–2):38–44. PubMed PMID: 29161722.
20. Dziewulska D, Mossakowski MJ. Cellular expression of tumor necrosis factor a and its receptors in human ischemic stroke. Clin Neuropathol. 2003 Jan–Feb;22(1):35–40. PubMed PMID: 12617192.
21. Ogawa N, Kawai H, Terashima T, et al. Gene therapy for neuropathic pain by silencing of TNF-alpha expression with lentiviral vectors targeting the dorsal root ganglion in mice. PLoS One. 2014;9(3):e92073. PubMed PMID: 24642694; PubMed Central PMCID: PMCPMC3958473.
22. Gerard E, Spengler RN, Bonoiu AC, et al. Chronic constriction injury-induced nociception is relieved by nanomedicine-mediated decrease of rat hippocampal tumor necrosis factor. Pain. 2015 Jul;156(7):1320–1333. PubMed PMID: 25851457; PubMed Central PMCID: PMCPMC4474806.
23. Ignatowski TA, Spengler RN. Targeting tumor necrosis factor in the brain relieves neuropathic pain. World J Anesthesiology. 2018;7 (2):10–19.
24. Sommer C, Schafers M, Marziniak M, et al. Etanercept reduces hyperalgesia in experimental painful neuropathy. J Peripher Nerv Syst. 2001 6;Jun(2):67–72. PubMed PMID: 11446385.
25. Sommer C, Schäfers M. Mechanisms of neuropathic pain: the role of cytokines. Drug Discov Today. 2004 Dec 01;1(4):441–448.
26. Uceyler N, Sommer C. Cytokine regulation in animal models of neuropathic pain and in human diseases. Neurosci Lett. 2008 Jun 6;437(3):194–198. PubMed PMID: 18403115.
27. Griessenauer CJ, Raborn J, Foreman P, et al. Venous drainage of the spine and spinal cord: a comprehensive review of its history, embryology, anatomy, physiology, and pathology. Clin Anat. 2015 Jan;28(1):75–87. PubMed PMID: 24677178.
28. LaMacchia ZM, Spengler RN, Jaffari M, et al. Perispinal injection of a TNF blocker directed to the brain of rats alleviates the sensory and affective components of chronic constriction injury-induced neuropathic pain. Brain Behav Immun. 2019 Jul 31;82:93–105. PubMed PMID: 31376497.
    - • **Key study identifying perispinal etanercept entry into the choroid plexus and treatment reduced neuropathic pain in a rat model.**
29. Tobinick EL. Perispinal delivery of CNS drugs. CNS Drugs. 2016 Jun;30(6):469–480. PubMed PMID: 27120182; PubMed Central PMCID: PMCPMC4920856.
    - •• **Comprehensive review of the evidence for the mechanism whereby perispinal delivery of large drug molecules can enter into the brain.**
30. Zanella JM, Burright EN, Hildebrand K, et al. Effect of etanercept, a tumor necrosis factor-alpha inhibitor, on neuropathic pain in the rat chronic constriction injury model. Spine (Phila Pa 1976). 2008 Feb 1;33(3):227–234. PubMed PMID: 18303453.
31. Tobinick E. Immediate resolution of hemispatial neglect and central post-stroke pain after perispinal etanercept: case report. Clin Drug Investig. 2019 Oct 22. PubMed PMID: 31642048. doi:10.1007/s40261-019-00864-8
    - • **Adding to the supportive evidence for rapid improvement in human stroke patients and associated pain with perispinal etanercept.**
32. Tobinick E, Kim NM, Reyzin G, et al. Selective TNF inhibition for chronic stroke and traumatic brain injury: an observational study

involving 629 consecutive patients treated with perispinal etanercept. CNS Drugs. 2012 Dec;26(12):1051–1070. PubMed PMID: 23100196.

•• **Review of extensive open-label stroke patient treatment with perispinal etanercept to improve outcomes.**

33. Ignatowski TA, Spengler RN, Dhandapani KM, et al. Perispinal etanercept for post-stroke neurological and cognitive dysfunction: scientific rationale and current evidence. CNS Drugs. 2014 Aug;28 (8):679–697. PubMed PMID: 24861337; PubMed Central PMCID: PMCPMC4110406.
34. Gronseth GS, Messe SR. Practice advisory: etanercept for poststroke disability: report of the guideline development, dissemination, and implementation subcommittee of the American Academy of Neurology. Neurology. 2016 Jun 7;86(23):2208–2211. PubMed PMID: 27272034; PubMed Central PMCID: PMCPMC4898316.
35. Paller AS, Siegfried EC, Pariser DM, et al. Long-term safety and efficacy of etanercept in children and adolescents with plaque psoriasis. J Am Acad Dermatol. 2016 Feb;74(2):280–7 e1–3. PubMed PMID: 26775775.

• **Establishing that etanercept is generally well tolerated and safe over long-term use in children.**

36. Esposito M, Giunta A, Mazzotta A, et al. Efficacy and safety of subcutaneous anti-tumor necrosis factor-alpha agents, etanercept and adalimumab, in elderly patients affected by psoriasis and psoriatic arthritis: an observational long-term study. Dermatology. 2012;225(4):312–319. PubMed PMID: 23295383.

• **Establishing safety and tolerability of etanercept in the elderly.**

37. Mease PJ, Kivitz AJ, Burch FX, et al. Etanercept treatment of psoriatic arthritis: safety, efficacy, and effect on disease progression. Arthritis Rheum. 2004 Jul;50(7):2264–2272. PubMed PMID: 15248226.
38. Azevedo VF, Galli N, Kleinfelder A, et al. Etanercept biosimilars. Rheumatol Int. 2015 Feb;35(2):197–209. PubMed PMID: 24980068; PubMed Central PMCID: PMCPMC4308636.
39. Chadwick L, Zhao S, Mysler E, Moots RJ. Review of Biosimilar Trials and Data on Etanercept in Rheumatoid Arthritis. Curr Rheumatol Rep. 2018 Nov 9;20(12):84. PMID:30411183.
40. Senabre-Gallego JM, Santos-Ramirez C, Santos-Soler G, et al. Long-term safety and efficacy of etanercept in the treatment of ankylosing spondylitis. Patient Prefer Adherence. 2013 Sep 23;7:961–972. PubMed PMID: 24101863; PubMed Central PMCID: PMCPMC3790868.
41. Strangfeld A, Listing J, Herzer P, et al. Risk of herpes zoster in patients with rheumatoid arthritis treated with anti-TNF-alpha agents. JAMA. 2009 Feb 18;301(7):737–744. PubMed PMID: 19224750.
42. Tobinick E. Perispinal etanercept advances as a neurotherapeutic. Expert Rev Neurother. 2018 Jun;18(6):453–455. PubMed PMID: 29695205.
43. Tobinick E. Rapid improvement of chronic stroke deficits after perispinal etanercept: three consecutive cases. CNS Drugs. 2011 Feb;25(2):145–155. PubMed PMID: 21254790.

• **A pioneering study outlining the potential for rapid improvement of chronic stroke deficits by perispinal etanercept treatment.**

44. Tobinick E. Perispinal etanercept: a new therapeutic paradigm in neurology. Expert Rev Neurother. 2010 Jun;10(6):985–1002. PubMed PMID: 20518613.
45. Tobinick E. Perispinal etanercept for neuroinflammatory disorders. Drug Discov Today. 2009 Feb;14(3–4):168–177. PubMed PMID: 19027875.
46. Tobinick E. Perispinal etanercept produces rapid improvement in primary progressive aphasia: identification of a novel, rapidly reversible TNF-mediated pathophysiologic mechanism. Medscape J Med. 2008 Jun 10;10(6):135. PubMed PMID: 18679537; PubMed Central PMCID: PMCPMC2491668.
47. Tobinick E. Perispinal etanercept for treatment of Alzheimer's disease. Curr Alzheimer Res. 2007 Dec;4(5):550–552. PubMed PMID: 18220520.
48. Tobinick EL, Gross H. Rapid cognitive improvement in Alzheimer's disease following perispinal etanercept administration. J Neuroinflammation. 2008 Jan 9;5:2. PubMed PMID: 18184433; PubMed Central PMCID: PMCPMC2211476.
49. Tobinick E, Davoodifar S. Efficacy of etanercept delivered by perispinal administration for chronic back and/or neck disc-related pain: a study of clinical observations in 143 patients. Curr Med Res Opin. 2004 Jul;20(7):1075–1085. PubMed PMID: 15265252.
50. Smajlovic D. Strokes in young adults: epidemiology and prevention. Vasc Health Risk Manag. 2015;11:157–164. PubMed PMID: 25750539; PubMed Central PMCID: PMCPMC4348138.
51. Chuang LL, Wu CY, Lin KC, et al. Relative and absolute reliability of a vertical numerical pain rating scale supplemented with a faces pain scale after stroke. Phys Ther. 2014 Jan;94(1):129–138. PubMed PMID: 24029301.
52. Chiti G, Pantoni L. Use of montreal cognitive assessment in patients with stroke. Stroke. 2014 Oct;45(10):3135–3140. PubMed PMID: 25116881.
53. Fullerton KJ, McSherry D, Stout RW. Albert's test: a neglected test of perceptual neglect. Lancet. 1986 Feb 22;1(8478):430–432. PubMed PMID: 2868349.
54. Mead G, Lynch J, Greig C, et al. Evaluation of fatigue scales in stroke patients. Stroke. 2007 Jul;38(7):2090–2095. PubMed PMID: 17525397.
55. Champod AS, Gubitz GJ, Phillips SJ, et al. Clock drawing test in acute stroke and its relationship with long-term functional and cognitive outcomes. Clin Neuropsychol. 2019 Jul;33(5):817–830. PubMed PMID: 29985104.
56. Niesters M, Aarts L, Sarton E, et al. Influence of ketamine and morphine on descending pain modulation in chronic pain patients: a randomized placebo-controlled cross-over proof-of-concept study. Br J Anaesth. 2013 Jun;110(6):1010–1016. PubMed PMID: 23384733.
57. Nothnagel H, Puta C, Lehmann T, et al. How stable are quantitative sensory testing measurements over time? Report on 10-week reliability and agreement of results in healthy volunteers. J Pain Res. 2017;10:2067–2078. PubMed PMID: 28919806; PubMed Central PMCID: PMCPMC5587201.
58. Di Costanzo L, Ayala F, Megna M, et al. The risk of herpes zoster in the anti-TNF-alpha era: a case report and review of the literature. J Dermatol Case Rep. 2013 Mar 30;7(1):1–4. PubMed PMID: 23580906; PubMed Central PMCID: PMCPMC3622506.
59. Keszler M, Gude T, Heckert K, et al. Pain syndromes associated with cerebrovascular accidents. In: Freedman MK, Gehret JA, Young GW, editors. Challenging neuropathic pain syndromes Chapter 19. Elsevier; 2018. p. 155–165.
60. Bushnell C, Bettger JP, Cockroft KM, et al. Chronic stroke outcome measures for motor function intervention trials: expert panel recommendations. Circ Cardiovasc Qual Outcomes. 2015 Oct;8(6 Suppl 3):S163–9. PubMed PMID: 26515205; PubMed Central PMCID: PMCPMC5289112.
61. Pickering AE, Thornton SR, Love-Jones SJ, et al. Analgesia in conjunction with normalisation of thermal sensation following deep brain stimulation for central post-stroke pain. Pain. 2009 Dec 15;147(1–3):299–304. PubMed PMID: 19833434; PubMed Central PMCID: PMCPMC2789248.
62. Hess A, Axmann R, Rech J, et al. Blockade of TNF-alpha rapidly inhibits pain responses in the central nervous system. Proc Natl Acad Sci U S A. 2011 Mar 1;108(9):3731–3736. PubMed PMID: 21245297; PubMed Central PMCID: PMCPMC3048151.

•• **An early study in animal models showing that blocking TNF rapidly inhibited pain and nociceptive brain activity long before effects on the immune response.**

63. Fasick V, Spengler RN, Samankan S, et al. The hippocampus and TNF: common links between chronic pain and depression. Neurosci Biobehav Rev. 2015 Jun;53:139–159. PubMed PMID: 25857253.
64. Hasturk AE, Gokce EC, Yilmaz ER, et al. Therapeutic evaluation of tumor necrosis factor-alpha antagonist etanercept against traumatic brain injury in rats: ultrastructural, pathological, and biochemical analyses. Asian J Neurosurg. 2018 Oct–Dec;13 (4):1018–1025. PubMed PMID: 30459860; PubMed Central PMCID: PMCPMC6208262.

65. Shi K, Tian DC, Li ZG, et al. Global brain inflammation in stroke. Lancet Neurol. 2019 Jul 8;18:1058–1066. PubMed PMID: 31296369.
66. Meng J, Wang J, Steinhoff M, et al. TNFalpha induces co-trafficking of TRPV1/TRPA1 in VAMP1-containing vesicles to the plasmalemma via Munc18-1/syntaxin1/SNAP-25 mediated fusion. Sci Rep. 2016 Feb 18;6:21226. PubMed PMID: 26888187; PubMed Central PMCID: PMCPMC4758037.
67. Marrone MC, Morabito A, Giustizieri M, et al. TRPV1 channels are critical brain inflammation detectors and neuropathic pain biomarkers in mice. Nat Commun. 2017 May 10;8:15292. PubMed PMID: 28489079; PubMed Central PMCID: PMCPMC5436240.
68. Wheeler MA, Heffner DL, Kim S, et al. TNF-alpha/TNFR1 signaling is required for the development and function of primary nociceptors. Neuron. 2014 May 7;82(3):587–602. PubMed PMID: 24811380; PubMed Central PMCID: PMCPMC4046273.
69. Lu SC, Chang YS, Kan HW, et al. Tumor necrosis factor-alpha mediated pain hypersensitivity through Ret receptor in resiniferatoxin neuropathy. Kaohsiung J Med Sci. 2018 Sep;34(9):494–502. PubMed PMID: 30173779.
70. Liu Y, Zhou LJ, Wang J, et al. TNF-alpha differentially regulates synaptic plasticity in the hippocampus and spinal cord by microglia-dependent mechanisms after peripheral nerve injury. J Neurosci. 2017 Jan 25;37(4):871–881. PubMed PMID: 28123022; PubMed Central PMCID: PMCPMC5296781.
71. Chen SX, Liao GJ, Yao PW, et al. Calpain-2 regulates TNF-alpha expression associated with neuropathic pain following motor nerve injury. Neuroscience. 2018 Apr 15;376:142–151. PubMed PMID: 29477696.
72. Maggio N, Vlachos A. Tumor necrosis factor (TNF) modulates synaptic plasticity in a concentration-dependent manner through intracellular calcium stores. J Mol Med (Berl). 2018 Oct;96 (10):1039–1047. PubMed PMID: 30073573.
73. Yao P, Wang S, Xin W, et al. Upregulation of tumor necrosis factor-alpha in the anterior cingulate cortex contributes to neuropathic pain and pain-associated aversion. Neurobiol Dis. 2019:104456:1–13.
74. Patel HJ, Patel BM. TNF-alpha and cancer cachexia: molecular insights and clinical implications. Life Sci. 2017 Feb 1;170:56–63. PubMed PMID: 27919820.
75. Hafer-Macko CE, Yu S, Ryan AS, et al. Elevated tumor necrosis factor-alpha in skeletal muscle after stroke. Stroke. 2005 Sep;36 (9):2021–2023. PubMed PMID: 16109906.
76. Thoma A, Lightfoot AP. NF-kB and inflammatory cytokine signalling: role in skeletal muscle atrophy. Adv Exp Med Biol. 2018;1088:267–279. PubMed PMID: 30390256.
77. Grijalva CG, Chen L, Delzell E, et al. Initiation of tumor necrosis factor-alpha antagonists and the risk of hospitalization for infection in patients with autoimmune diseases. JAMA. 2011 Dec 7;306 (21):2331–2339. PubMed PMID: 22056398; PubMed Central PMCID: PMCPMC3428224.
78. Jani M, Barton A, Hyrich K. Prediction of infection risk in rheumatoid arthritis patients treated with biologics: are we any closer to risk stratification? Curr Opin Rheumatol. 2019 May;31(3):285–292. PubMed PMID: 30789850; PubMed Central PMCID: PMCPMC6443047.
79. Fleet JL, Dixon SN, Kuwornu PJ, et al. Gabapentin dose and the 30-day risk of altered mental status in older adults: a retrospective population-based study. PLoS One. 2018;13(3):e0193134. PubMed PMID: 29538407; PubMed Central PMCID: PMCPMC5851574.
80. Khodneva Y, Muntner P, Kertesz S, et al. Prescription opioid use and risk of coronary heart disease, stroke, and cardiovascular death among adults from a prospective cohort (REGARDS study). Pain Med. 2016 Mar;17(3):444–455. PubMed PMID: 26361245; PubMed Central PMCID: PMCPMC6281131.
81. Carvalho C, Caetano JM, Cunha L, et al. Open-label placebo treatment in chronic low back pain: a randomized controlled trial. Pain. 2016 Dec;157(12):2766–2772. PubMed PMID: 27755279; PubMed Central PMCID: PMCPMC5113234.
82. Vachon-Presseau E, Berger SE, Abdullah TB, et al. Brain and psychological determinants of placebo pill response in chronic pain patients. Nat Commun. 2018 Sep 12;9(1):3397. PubMed PMID: 30209286; PubMed Central PMCID: PMCPMC6135815.
83. Toth C. Pregabalin: latest safety evidence and clinical implications for the management of neuropathic pain. Ther Adv Drug Saf. 2014 Feb;5(1):38–56. PubMed PMID: 25083261; PubMed Central PMCID: PMCPMC4110876.
84. Chau DL, Walker V, Pai L, et al. Opiates and elderly: use and side effects. Clin Interv Aging. 2008;3(2):273–278. PubMed PMID: 18686750; PubMed Central PMCID: PMCPMC2546472.