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Do low levels of Beta-endorphin in the cerebrospinal fluid indicate defective top-down inhibition in patients with chronic neuropathic pain? A cross-sectional, comparative study

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ABSTRACT

Objective: Pain medicine still lacks mechanism-specific biomarkers to guide diagnosis and treatment, and defective top-down modulation is an important factor in the pathophysiology of chronic pain conditions. Using modern analytical tools and advanced multivariate statistical analysis, the aim of this study was to revisit two classical potential biomarkers of pro- and anti-nociception in humans (Substance P and Beta-endorphin), focusing particularly on the cerebrospinal fluid (CSF).

Design: Cross-sectional, comparative, observational study.

Subjects: Patients with chronic, post-traumatic and/or post-surgical, neuropathic pain refractory to conventional treatment (n=15) and healthy controls (n=19) were included.

Methods: Samples were taken from CSF and blood, and levels of Substance P and Beta-endorphin were investigated using a Luminex technology kit.

Results: We found low levels of Beta-endorphin in the CSF of neuropathic pain patients (66 ± 11 pg/ml) compared to healthy controls (115 ± 14 pg/ml) ($p=0.017$). Substance P levels in the CSF did not differ (20 ± 2 pg/ml, 26 ± 2 , $p=0.08$). However, our multivariate data analysis showed that belonging to the patient group was associated with low levels of both substances in the CSF. A higher correlation between the levels of Beta-endorphin and Substance P in CSF was found in healthy controls than in patients ($r_s=0.725$, $p<0.001$ vs $r_s=0.574$, $p=0.032$).

Conclusions: Patients with chronic neuropathic pain due to trauma or surgery had low levels of Beta-endorphin in the CSF. We speculate that this could indicate a defective top-down modulation of pain in chronic neuropathic pain. Our results also illustrate the importance of taking a system-wide, multivariate approach when searching for biomarkers.

Key-words: Beta-endorphin; biomarker; cerebrospinal fluid; neuropathic; pain; Substance P

1. Introduction

The nociceptive input to the brain is dependent on an intricate balance between, on the one hand, nociceptive signalling from the periphery and, on the other hand, modulation by descending neural pathways (1, 2). The best studied top-down descending pathway is the PAG-RVM system, which links the periaqueductal grey (PAG) to the spinal cord via the rostral ventromedial medulla (RVM) (3). The PAG and the RVM contain a high density of opioid receptors (4). Noradrenergic pathways originating in e.g., the locus coeruleus are also involved in downward pain modulation (5), and these circuits are partially integrated into the PAG-RVM system (1). Another important related concept is that of Diffuse Noxious Inhibitory Controls (DNIC), also known as *counter irritation*. The DNIC phenomenon means that nociceptive signalling from the periphery is inhibited by applying another noxious stimulus to a remote area of the body (6). The term “diffuse” relates to the fact that DNIC works non-somatotopically, i.e. its response is general regardless of where the noxious stimulus is applied (1). Hence, the concept of pain modulation at the spinal level has evolved considerably since Melzack & Wall first presented their groundbreaking work almost half a century ago (7).

The concept of “pain biomarkers” is sometimes used when discussing the future of pain medicine (8-11). As pain by definition is a subjective experience (9), the neologism “nocimarker” will be used instead in the present paper to denote attempts to find objective, measurable correlates to the neurobiological processes involved in different pain conditions (i.e., correlates to nociceptive pathophysiology, not pain) (12). A long-term vision for pain medicine could be the possibility of basing the prescription of analgesics on a mechanistic understanding of different pain types. Such a vision requires the discovery of mechanism-specific markers (13). Today, analgesics are often prescribed on a trial-and-error basis.

Given the above-mentioned balance between nociceptive input from the periphery and top-down modulating systems, noci-markers studies should focus on both pro- and anti-nociceptive proteins. One well-known anti-nociceptive neuropeptide is the endogenous opioid Beta-endorphin (BE) (14). Interestingly, the cerebrospinal fluid (CSF) can act as a transport medium for BE synthesised by hypothalamic neurons. Hence, BE may have effects on distant cerebral and spinal regions by means of so-called “long-distance volume transmission” in the CSF (15). The PAG, with its high density of opioid receptors and its anatomical proximity to the CSF, is potentially interesting in this respect.

Substance P (SP) has long been considered to be a pro-nociceptive neuropeptide (16, 17). SP is released from primary afferents in the spinal dorsal horn and probably plays a role for central sensitization processes (18). Even though SP has never achieved the status of a noci-marker in clinical pain medicine, it has been convincingly shown that levels of SP are elevated in the CSF of fibromyalgia syndrome patients, compared with healthy controls (19, 20).

The primary objective of this study was to investigate the concentrations of both SP and BE in the CSF of patients with chronic neuropathic pain, compared with healthy controls. We also wanted to relate CSF levels of SP and BE to one another, as a possible indicator of the balance between pro- and anti-nociceptive factors. Secondary objectives were to analyse samples from a more easily available body fluid (plasma), and to investigate if intercorrelations existed between the two fluids.

2. Material and methods

2.1 Procedures

For every subject in this study, body fluid sampling was undertaken as follows: First, a 10 ml venous blood sample was drawn using an EDTA tube. Then, intrathecal access was obtained by lumbar puncture and a 10 ml sample of CSF was taken. In all cases and for the two body fluids, each sample was immediately cooled on ice and transported to the Painomics® laboratory, Linköping University Hospital. The blood samples were centrifuged for 10 minutes at 1000 x g within 30 minutes of blood collection, plasma was removed, aliquoted and stored at -70°C until analysis. The CSF samples were checked for blood contamination and centrifuged for 10 min at 1000 x g to discard any cellular debris. The supernatant was removed to a new tube and stored as 1 ml aliquots at -70°C.

2.2 Subjects

2.2.1 Patients

All 15 pain patients included in this study were participating in an on-going clinical trial of intrathecal bolus injections of the analgesic ziconotide (data not presented in the present paper). Inclusion criteria were: 1) patient, at least 18 years of age, suffering from chronic (≥ 6 months) neuropathic pain due to trauma or surgery, who had failed on conventional pharmacological treatment; 2) average Visual Analogue Scale Pain Intensity (VASPI) last week ≥ 40 mm; 3) patient capable of judgment, i.e. able to understand information regarding the drug, the mode of administration and evaluation of efficacy and side effects; 4) signed informed consent.

Exclusion criteria were: 1) limited life expectancy (investigator's judgement); 2) intrathecal

chemotherapy; 3) known or suspected intracranial hypertension; 4) known liver or kidney disease, defined as serum transaminases, total bilirubin, alkaline phosphatase or creatinine >1.2 x upper limit of normal; 5) advanced cardio-pulmonary disease (investigator's judgment); 6) ongoing infection, whether systemically or locally in the lumbar area; 7) coagulopathy (including medication with warfarin, clopidogrel and heparin); 8) allergy to ziconotide or any of the excipients in the ziconotide vial; 9) history of psychiatric disorders which in the investigator's opinion would put the patient at risk; 10) pregnant or lactating woman ; 11) participation in another clinical trial during the last 30 days.

After informed consent, the following data were registered: basic demographic data; pain diagnosis; pain duration; present and past medical history; concomitant medication. A physical examination was performed. Then, within a month, the patients came back for body fluid sampling as described above (section 2.1). After CSF sampling, the patient received an intrathecal bolus injection of ziconotide according to the protocol of the clinical trial. For patients characteristics and comparison with healthy controls, see **Table I** and **II**.

2.2.2 Healthy controls

Nineteen healthy controls were recruited by local advertisement at the Faculty of Health Sciences, Linköping University, Sweden, and by contacting healthy subjects from earlier studies. After informed consent, a structured interview was conducted to ensure the absence of any significant medical condition.

2.2.3 Ethics

The healthy controls protocol was approved by the Regional Ethics Committee in Linköping (RECL), Sweden (Dnr M136-06 and Dnr 2012/94-32). The clinical trial, from which patient

data were derived, was conjointly approved by the Swedish Medical Products Agency (EudraCT 2010-018920-21) and by the RECL (Dnr 2011/48-31). The clinical trial was monitored by the Linköping Academic Research Centre (LARC) and was conducted according to the standards of Good Clinical Practice (GCP). All lumbar punctures were performed by a specialist in Anaesthesiology (EB).

2.3 Analytical methods

SP and BE were quantified by using the MILLIPLEX® MAP Human Neuropeptide Magnetic Panel Kit, HNPMAG-35K (EMD Millipore Corporation, Billerica, MA, USA). This is a Luminex technology kit, which enables simultaneous quantification of SP and BE in the same assay and comprises all components necessary (buffers, standards and microplate) for the whole assay procedure. SP and BE from plasma samples were extracted by acetonitrile precipitation method according to the manufacturer's recommendations. 50 µl of the extracted plasma samples and 50 µl of CSF were analysed in the Luminex 200 instrument (Life Technologies, Invitrogen Stockholm, Sweden). The concentrations were calculated by reference to a seven-point five-parameter logistic standard curve for each substance using MasterPlex QT 2010 (MiraiBio Inc., San Diego, CA, USA).

2.4 Statistics

$P \leq 0.05$ was considered significant in all statistical tests.

2.4.1 Traditional statistics

For traditional statistics, the IBM Statistical Package for the Social Sciences (SPSS, IBM Corporation, Somers, NY, USA) version 20.0 was used. Unless stated otherwise, data are reported as mean \pm SEM in the text and in **Table I**. However, to give an appropriate picture of the spread of the data, boxplots are used in **Figure 1**. For comparisons between groups, we

performed the Mann Whitney U test or, for categorical data, the Chi-square test or Fisher's exact test. Spearman's non-parametric rank correlation coefficient (r_s) was used for correlation analysis.

2.4.2 Multivariate Data Analysis

Traditional statistical methods can quantify level changes of individual substances but disregard interrelationships between them and thereby ignore system-wide aspects (21). Classical methods assume variable independence when interpreting the results (22). To handle these drawbacks, a multi/megavariate regression method was used.

For multivariate analyses, SIMCA-P+ version 13.0 (Umetrics AB, Umeå, Sweden) was used. *Principal Component Analysis* (PCA) was used to extract and display systematic variation in the data matrix. All variables were log transformed prior to statistical analysis if necessary. A cross validation technique was used to identify nontrivial components. Variables loading upon the same component are correlated and variables with high loadings but with different signs are negatively correlated. Variables with high loadings which had 95% jack-knife uncertainty confidence interval non equal to zero were considered as significant. Hence, the most important of these were those with high absolute loadings. Significant variables with high loadings (positive or negative) are more important for the component under consideration than variables with lower absolute loadings. In the present study, PCA was used for checking for multivariate outliers. Outliers were identified using the two powerful methods available in SIMCA-P+: 1) score plots in combination with Hotelling's T^2 (identifies strong outliers); 2) distance to model in X-space (identifies moderate outliers). No multivariate outliers were found.

Partial Least Square Regression (PLS) (i.e., PLS-OPLS/O2PLS) was used for multivariate regression analysis. The importance of the variables is measured as a Variable Influence on Projection (VIP) value. This indicates the relevance of each X-variable pooled over all dimensions and Y-variables – the group of variables that best explain Y. $VIP \geq 1.0$ was considered significant. Coefficients (PLS scaled and centred regression coefficients; coeffs) were used to note the direction of the relationship (positive or negative). Multiple linear regression (MLR) or logistic regression (LR) could possibly have been alternatives in the regressions, but these methods assume that the regressor (X) variables are fairly independent. If multi-collinearity (i.e., high correlations) occurs among the X-variables, the regression coefficients become unstable and their interpretability breaks down. MLR and LR also assume that a high subject-to-variables ratio is present (e.g., >5) and such requirements are not required for PLS. In fact, PLS regression can handle subject-to-variables ratios < 1 (23).

3. Results

BE and SP could be detected in CSF and plasma in all healthy controls and in 14 out of 15 patients (analytic technical error for the samples of one patient). Hence, the results are based on 14 patients and 19 controls. Patients were significantly older than healthy controls (59 ± 3 years vs. 32 ± 3 years, $p<0.001$) (**Table I**).

3.1 Cerebrospinal fluid

BE levels in the CSF of patients were lower than in healthy controls (66 ± 11 pcg/ml vs. 115 ± 14 pcg/ml, $p=0.017$), but SP levels did not differ (20 ± 2 pcg/ml vs. 26 ± 2 pcg/ml, $p=0.08$) (**Figure 1**).

For all subjects taken together, we found a significant correlation between the levels of BE and SP in CSF ($r_s=0.686$, $p<0.001$). A higher correlation ($r_s=0.725$, $p<0.001$) was found between SP and BE in healthy controls than in patients ($r_s=0.574$, $p=0.032$) (**Figure 2**); both correlations were significant. For five patients and five controls, the total CSF protein concentration was determined, and was found to be 533 ± 93 $\mu\text{g/ml}$ and 451 ± 93 $\mu\text{g/ml}$, respectively (mean \pm SD, $p=0.251$).

3.2 Plasma

Plasma levels of BE did not differ between patients and healthy controls (897 ± 66 pcg/ml vs. 1029 ± 143 pcg/ml, $p=0.659$). Plasma levels of SP did not differ between patients and healthy controls (15.1 ± 2.2 pcg/ml, vs. 15.0 ± 1.4 pcg/ml, $p=0.985$).

For all subjects taken together, we found a significant correlation between the levels of BE and SP in plasma ($r_s=0.564$, $p=0.001$). A significant and high correlation ($r_s=0.680$, $p=0.001$) was found between SP and BE in healthy controls while no significant correlation existed in patients ($r_s=0.302$, $p=0.316$).

3.3 Correlations between plasma and CSF levels of the two substances

No correlation was found between CSF and plasma levels, neither for BE ($r_s=0.307$, $p=0.088$) nor for SP ($r_s=0.315$, $p=0.079$).

3.4 Multivariate data analysis

3.4.1 Regression of group membership using BE and SP in the two body fluids

First, we regressed group membership using the concentrations of the two substances in the two body fluids as regressors (X-variables). The significant regression ($R^2=0.15$, $Q^2=0.09$) revealed that the two substances in CSF but not in plasma were significant (in descending order, the sign indicating the direction of the correlation): CSF-BE (VIP=1.52 (-)), CSF-SP and (VIP=1.18(-)). Hence, belonging to the patient group was associated with low concentrations of CSF-BE and CSF-SP.

3.4.2 Possible influences of age upon the concentrations of BE and SP

As reported above, there was a significant difference in age between the two groups of subjects. In order to scrutinize this, we regressed the concentrations of the two substances in the two body fluids (i.e. four separate regression analyses) using age, group membership, BMI and gender as regressors (X-variables).

For CSF-BE, we found that both age (VIP=1.31(-)) and group (VIP=1.17(-)) were significant

regressors ($R^2= 0.44$, $Q^2=0.35$). For plasma-BE, we found that neither age nor group were significant regressors ($R^2= 0.10$, $Q^2=-0.05$).

In regression of CSF-SP, group (VIP= 1.40(-)) and age (VIP=1.25 (-)) were significant regressors ($R^2= 0.14$, $Q^2=-0.04$). For plasma-SP, we found that neither age nor group were significant regressors ($R^2= 0.07$, $Q^2=-0.05$).

3.4.3 Regression of pain intensity and pain duration

It was not possible to significantly regress pain intensity or pain duration in the group of patients using the concentrations of BE and SP in the two body fluids, age and BMI as regressors.

3.4.4 Do the pharmacological treatments influence the concentrations of BE and SP?

Based on the pharmacological variables presented in **Table I**, it was investigated if significant correlations existed in the patient group between these variables and the concentrations of the two substances in the two body fluids. However, no significant correlations were found.

4. Discussion

Using traditional statistics, the major finding of this study was that patients with chronic neuropathic pain had low CSF-BE levels, whereas CSF-SP did not differ compared to healthy controls. However, our multivariate data analysis showed that belonging to the patient group was associated with lower levels of both CSF-BE and CSF-SP. Hence, our results illustrate the importance of taking a system-wide, multivariate approach when searching for noci-markers.

4.1 Beta-endorphin

The physiology of BE has recently been reviewed (15), and when discussing the results of the present study, the following points are helpful to keep in mind:

- BE is a 31 amino acids polypeptide with a molecular weight of 3465 Da, meaning that it is about 10 times “bigger” than the alkaloid morphine.
- There are probably two functionally different BE systems: one peripheral (release of BE by the pituitary into the systemic circulation) and one central (synthesis in hypothalamic pro-opio-melanocortin (POMC) neurons).
- An intact blood-brain barrier (BBB) hinders free exchange of BE between plasma and CSF.
- Even though these two systems are functionally different, there still may be some bi-directional exchange of BE: from plasma to brain in small areas lacking a BBB; from brain to plasma by means of transport mechanisms across capillaries.
- Hypothalamic POMC neurons can release BE directly into the CSF of the third ventricle.

- The CSF can serve as a transport medium for BE to distant brain or spinal sites. This is called “long-distance volume transmission” and is by no means unique for BE.
- For obvious anatomical reasons, the PAG is one of the first areas exposed to BE released into the third ventricle.

Hence, it makes physiological sense to speculate that low levels of CSF-BE could mirror a defective endogenous pain control system in our patients. Already in 1978, Almay et al reported that levels of unspecific “endorphins” was low in the CSF of patients with predominantly “neuralgic” pain, compared both to patients with what was labelled “psychogenic” pain and to healthy controls (24). Also, in 1988, Tonelli et al found low CSF-BE in patients scheduled for Spinal Cord Stimulation, compared to historic controls (25). Our findings can be said to partly replicate these old data, using today’s more specific and more reliable analytical tools. As a contrast, in 1988, Vaeroy et al found normal levels of CSF-BE in fibromyalgia patients (14).

For obvious reasons, it is difficult to study the natural temporal dynamics of putative noci-markers during the development of chronic pain in humans. Concerning CSF-BE, longitudinal studies before and after an invasive intervention have yielded inconclusive results, and/or results that are difficult to interpret due to the nature of the intervention (25, 26). In one of these studies (26), mean CSF-BE decreased by 56% in 9 patients 12-17 days after successful treatment with Dorsal Root Entry Zone Lesions. However, there was no control group at baseline, and the neuro-destructive nature of the intervention makes interpretation difficult.

An important interpretive issue in this study is the possible presence of any confounding factor, e.g. age or opioid medication. Notably, our healthy controls were markedly younger, and one study has described that CSF-BE decreases with age (27). However, other studies have failed to confirm this age-effect on CSF-BE (24, 28-30). At any rate, our multivariate data analysis showed that even when taking age into account, group membership was still a significant predictor for the levels of CSF-BE. We also found that pharmacological treatment did not significantly affect the concentrations of CSF-BE. All in all, the balance of evidence does not favour a simple confounding effect of age on CSF-BE. However, this has to be confirmed in an aged-matched study.

We found no correlation between plasma-BE and CSF-BE, and plasma-BE did not differ between groups. These results are in line with the above-mentioned view that there are two functionally different BE-systems (15). The analgesic actions of plasma BE are unclear (31).

4.2 Substance P

Our study is in line with two previous studies failing to show that CSF-SP is elevated in human chronic neuropathic pain, compared to healthy controls (32, 33). Given the fact that neuropathic pain processes probably primarily affect a limited portion of the spinal cord, one could argue that a putative localized excess of SP over-spilling into the CSF might be too small to be detectable, and hence not as a single substance sufficient to differ between groups. However, Strittmatter and co-workers found that, compared to patients with non-painful neurological (mostly neuromuscular) disease, mean CSF-SP was elevated by 33% ($p < 0.05$) in patients with trigeminal neuralgia (34), showing that high CSF-SP can be demonstrated by lumbar puncture far from the locus of putative overproduction of SP (provided this is not a Type I error). When trying to assess different studies of CSF-SP in human neuropathic pain, it

is important to remember that neuropathic pain is not a single entity. For instance, the neurobiological processes involved in trigeminal neuralgia are arguably different from the ones in the present study. Interestingly, when Almay et al subgrouped patients with neuropathic pain (who had low CSF-SP compared to healthy controls) according to localization of the lesion, they found that CSF-SP was lower in patients with painful polyneuropathy or neuropathy/radiculopathy of the extremities than in patients with central pain or neuropathy of the face and head (32).

Patients with fibromyalgia syndrome have been shown to have high levels of CSF-SP (19, 20). Hence, one could speculate that SP might be considered as a noci-marker for *widespread* pain (the above mentioned findings of Strittmatter et al notwithstanding). Interestingly, CSF-SP levels are not elevated in patients with non-painful chronic fatigue syndrome, even though they share many of the other symptoms commonly described by fibromyalgia syndrome patients (35).

Recent veterinary work by Schmidt et al illustrates the interplay between SP and neuroinflammation (36). In dogs with painful syringomyelia (i.e. central neuropathic pain from the spinal cord), CSF-SP levels and IL-6 levels were higher than in dogs with non-painful syringomyelia, and the levels of the two substances intercorrelated. The authors suggest that release of these two potentially mutually interacting substances is a factor in the development of this type of pain. Indeed, SP is considered to be an activator of spinal glial cell activity, and glial cells play an important role in the pathophysiology of neuropathic pain (37, 38). Glial cells release multifunctional cytokines (TNF- α , IL-1 β , IL-6) that orchestrate the subsequent production of downstream cytokines and other proalgesic mediators (37, 39,

40). Hence, SP seems to be a mediator in a chronic central neuropathic pain condition like syringomyelia, at least in dogs.

4.3 Relationship between CSF-BE and CSF-SP

As argued in the introduction, the balance of pro- and anti-nociceptive factors is an important physiological question. Although of course statistical correlation does not imply biological causation, the finding of a positive correlation between CSF-BE and CSF-SP is still notable. This correlation was stronger in healthy controls than in patients (**Figure 2**). In patients with fibromyalgia, CSF-BE and CSF-SP do not correlate (14), whereas a positive correlation has been described in healthy children (29). Can the lower degree of correlation in our patients compared to controls indicate a dysregulated balance between pro- and antinociceptive functions? This is admittedly a speculation, albeit an interesting one.

On the basis of this present paper and others (14, 19, 20, 24, 25, 32, 33), one might perhaps hypothesize that peripheral neuropathic pain of the extremities and fibromyalgia are characterized by different combined patterns of CSF-BE and CSF-SP: the former would be associated with a tendency to low CSF-BE and low CSF-SP, whereas the latter would be associated with a tendency to normal CSF-BE and high CSF-SP. In both cases, normal nociceptive homeostasis would be disrupted, as indicated by less-than-normal correlations between CSF-SP and CSF-BE at group level, compared to healthy controls.

Finally, an important overall interpretative question has to do with the total protein concentration in the CSF: can our findings be explained by differences in total protein content between patients and controls? However, in five patients and five controls, we did not find

that patients had significantly lower CSF total protein concentrations. On the contrary, there was a tendency (albeit not significant) of patients having higher protein content than controls. Hence, we do not think that a confounding effect of total CSF protein on our results is likely.

4.4 Conclusion

In this study, patients with chronic peripheral neuropathic pain due to trauma or surgery had low CSF-BE, even when taking age and pharmacological treatment into account. We speculate that this could indicate an insufficient production of CSF-BE by hypothalamic neurons, resulting in defective top-down modulation (e.g. via the PAG-RVM system) of inputs from the periphery. Our results also illustrate the importance of taking a system-wide, multivariate approach when searching for noci-markers.

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Table I. Patients and healthy controls characteristics

Unless stated otherwise, data are presented as mean \pm SEM. Furthest to the right is the result of the statistical comparisons between patients and healthy controls. * denotes significant group difference.

Variables	Group	Patients (n=14)	Healthy controls (n=19)	Statistics p-value
Age (years)		59 \pm 3	32 \pm 3	<0.001*
Sex (% female)		43%	37%	0.727
Body Mass Index (kg/m ²)		25 \pm 0.86	24 \pm 0.55	0.117
Pain duration (months)		93 \pm 20	0	<0.001*
Pain intensity (0-100 mm) ¹		69 \pm 3	0	<0.001*
Opioid dose ² (mg/day) (median, range)		0 (0-480)	0	0.002*
On opioids (%)		43%	0%	0.003*
On tricyclics or duloxetine (%)		29%	0%	0.024*
On gabapentinoids (%)		29%	0%	0.024*
On paracetamol ³ (%)		50%	0%	0.001*
On NSAID ³ (%)		7%	0%	0.424

1: At inclusion, patients were asked to grade their average pain intensity for last week on a Visual Analogue Scale 0-100 mm, whereas the pain status of healthy controls was investigated by an extensive structured interview. All controls were free of pain.

2: In oral morphine equivalents.

3: Excluding treatment “as needed”. NSAID: Non-Steroidal Anti-Inflammatory Drug.

Table II. Detailed neuropathic pain patients characteristics

International Classification of Diseases (ICD-10) key: S14.2 – injury of nerve root of cervical spine; S34.2 – injury of nerve root of lumbar and sacral spine (i.e. failed back surgery syndrome with radiculopathy); S34.3 – injury of cauda equina; S54.9 – injury of unspecified nerve at forearm level; S74.0 – injury of sciatic nerve at hip and thigh level; S74.1 – injury of femoral nerve at hip and thigh level; S94.9 – injury of unspecified nerve at ankle and foot level; G62.9 – polyneuropathy.

Main cause of pain (ICD-10 diagnosis)	Concomitant diseases
S14.2	fibromyalgia syndrome
S34.2 and G62.9	history of alcohol dependence; psoriasis; tension headache
S34.2	hypertension; polymyalgia rheumatica
S34.2	hypertension; psoriasis
S34.2	mild angina; mild obstructive lung disease
S34.2	anemia; dyspepsia; hypertension
S34.2	none
S34.2	autonomic neuropathy; diabetes; dyspepsia; mild angina; panic anxiety disorder
S34.3	none
S54.9	none
S74.0	previous, no longer painful vertebral compressions; posterior vitreous detachment; postural hypotension
S74.1	none
S94.9	none
G62.9	none

Legends to the Figures

Figure 1: Boxplots of Beta-endorphin and Substance P in the cerebrospinal fluid (CSF), in pcg/ml. Median values are represented by horizontal lines and the interquartile ranges by boxes. The ends of the whiskers represent minimum and maximum values. Beta-endorphin levels differed significantly between groups ($p=0.017$).

Figure 2: Scatter plot of the relationship between Substance P and Beta-endorphin in the cerebrospinal fluid (CSF) of neuropathic pain patients (a) and healthy controls (b). All values are in pcg/ml.

Figure 1

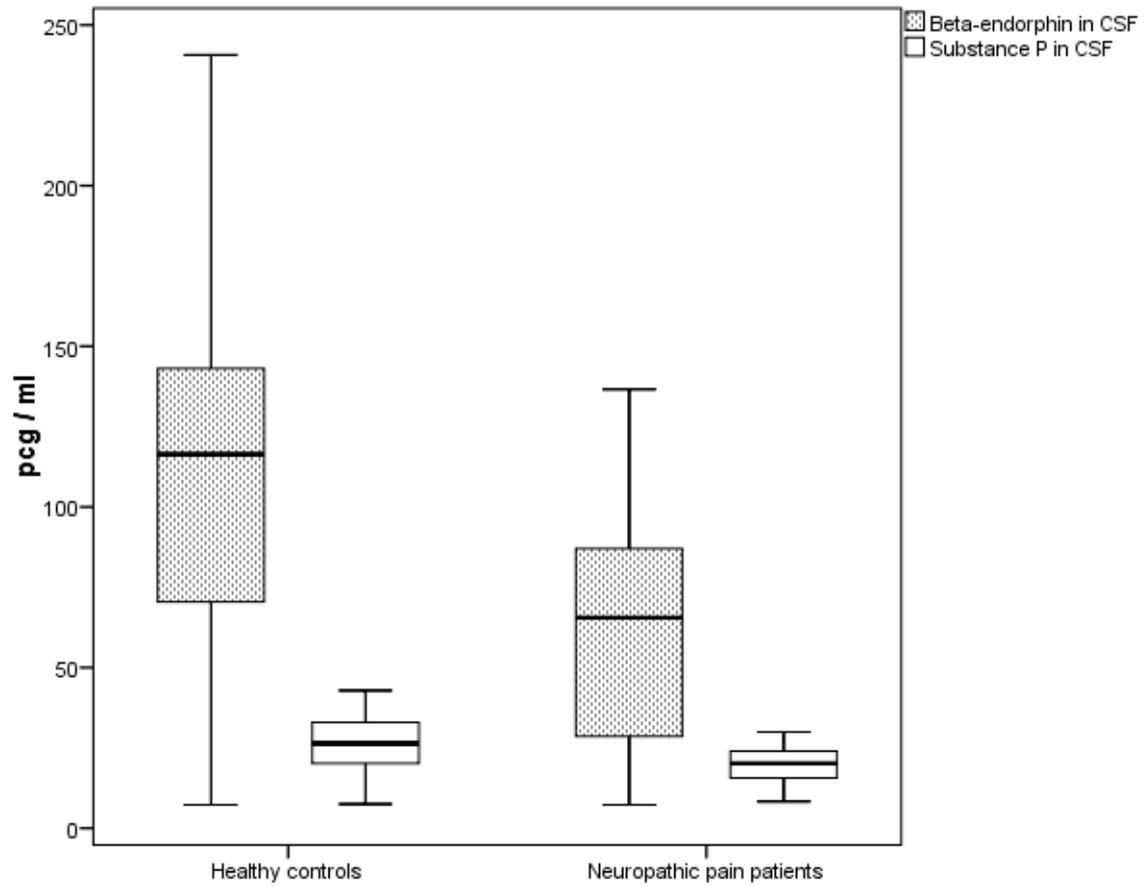
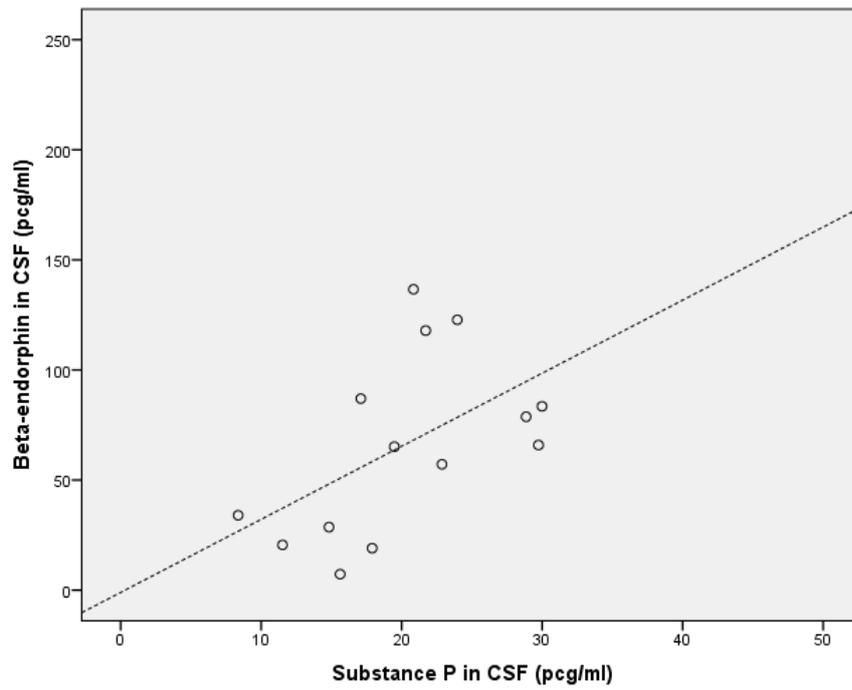


Figure 2

a)



b)

