Assessment of chronic benign and cancer pain using blood plasma biomarkers

Richard Rokyta [1], Olga Haklova [2], Anna Yamamotova [1]

1 Charles University in Prague, Third Faculty of Medicine, Department of Normal, Pathological and Clinical Physiology, Prague, Czech Republic.
2 Faculty Hospital Brno, Pain Center, Brno, Czech Republic

Correspondence to: Prof. Richard Rokyta, MD., DSc.
Charles University in Prague, Third Faculty of Medicine
Department of Normal, Pathological and Clinical Physiology
Ke Karlovu 4, 120 00 Praha 2, Czech Republic.
TEL/FAX: +420 224923827; E-MAIL: richard.rokyta@lf3.cuni.cz

Submitted: 2009-09-15 Accepted: 2009-09-30 Published online: 2009-11-11

Key words: **chronic pain; cancer pain; biochemical markers of pain**

Neuroendocrinol Lett 2009; **30**(5):637–642 **PMID:** 20035266 NEL300509A08

Abstract

OBJECTIVE: The objective was a systematic study of the biochemical markers which are descriptive for the dynamics of pain processes.
MATERIALS AND METHODS: The patients who had not been systematically treated for pain prior to their participation in this study consisted of 20 non-oncological (mean age 56.5 years) and 20 oncological patients (mean age 64.8 years). Pain intensity, assessed using the visual analogue scale (VAS) on a scale from 0–10, and the following biochemical parameters were measured during the initial patient workups: blood serum total protein, glucose, total cholesterol, HDL cholesterol, LDL cholesterol, atherosclerotic indexes, triacylglyceroles, apolipoprotein A, apolipoprotein B, albumin, alpha1 globulin, alpha2 globulin, beta globulin and gamma globulin. Biochemical measurements were repeated as soon as VAS assessments fell below 5. Therapy in non-oncological patients involved administration of NSA and weak opioids; while oncological patients received NSA, medium strength and strong opioids, and antidepressants.
RESULTS: Prior to therapy, concentration of albumin in serum, HDL cholesterol, and apolipoprotein A were lower, whereas CRP and alpha1 globulin were higher in oncological patients compared to non-oncological patients. After therapy, levels of glucose and alpha1 globulin were significantly higher and levels of apolipoprotein A were lower in oncological patients compared to non-oncological patients. Irrespective of diagnosis, patients treated with antidepressants showed higher levels of gamma globulin compared to non treated patients.
CONCLUSIONS: We can conclude that observed biochemical markers in patients with malignancies are more similar to the values of patients with chronic benign pain than to the values of patients with acute pain.

ORIGINAL ARTICLE

INTRODUCTION

Chronic pain currently affects 20–30% of citizens in developed countries and represents one of the worst types of pain. Pain, especially associated with tumors, can lead to serious mental and physical stress in patients. Therefore early and accurate diagnosis of chronic pain is very important. It is widely accepted that 90% of chronic pain may be successfully treated with well managed therapy. The methods most commonly used are methods involving subjective evaluations that attempt to define pain intensity, its threshold, and its affective component, i.e. unpleasantness (Huskisson, 1974; Hicks *et al.* 2001). The simplest and most widely used method is the VAS; other methods involve different types of psychological questionnaires (e.g. McGill Questionnaire and others) (Melzack, 1987). These methods are affected by subjective error that can only be reduced through qualitative and quantitative investigations; which explains why there have always been attempts to develop effective and objective pain evaluation methods.

Electrophysiological methods are frequently used to achieve objectivity (Treede *et al.* 2003; Vaculín *et al.* 2004). However, these methods are too complicated and sometimes too invasive to be used in routine clinical practice. Imaging methods like CT, fMRI, and PET (Apkarian *et al.* 2001; Borsook *et al.* 2007*)* are very progressive, but they are expensive and too time consuming for routine clinical utilization. However, these methods have brought new insights into the central structures activated by pain. These insights have inspired us to search for peripheral pain markers. Today biochemical markers are routinely tested during basic hospital investigations (e.g. glycaemia, lipid and protein spectrum, cholesterol and its subtypes, indicators of inflammation, stress hormones, and levels of mediators or their metabolites). These substances are most often tested in urine, blood, and cerebrospinal fluid (CSF), but can also be tested in tissues and saliva (Krikava *et al.* 2004; Schell *et al.* 2008; Shirasaki *et al.* 2007; Mannes *et al.* 2003).

Previously we published research indicating that some markers showed changes during acute and chronic pain (Rokyta *et al.* 2008; Stancak *et al.* 2008). We were one of the first to measured levels of free radicals in pain conditions, both in experimental and clinical practice. Our findings revealed that the most important changes take place in hydroxyl and nitroxide radicals and in singlet oxygen (Pekarkova *et al.* 2001; Rokyta *et al.* 2003; Rokyta *et al.* 2004).

In this paper we have focused on describing simple biochemical blood markers that can be used in patients with tumor pain.

Tab. 1. Demographic characteristics and a brief overview of treatments in non-oncological and oncological patients.

	Chronic pain	Cancer pain
Number of patients	20	20
Sex: F/M at the beginning F/M at the end	20/0 20/0	11/9 6/4
Age (Mean ± SD) years	56.5 ± 11.4	64.8 ± 8.9
Therapy (number of patients)		
NSA	15	16
Morphine and its derivates	17	12
Fentanyl	0	10
Antidepressants	8	10

MATERIAL AND METHODS

The investigation started with 40 patients; 20 non-oncological patients and 20 oncological patients. The most frequent diagnosis in non-oncological group was low back pain (n=7) and failed back surgery syndrome (FBSS) (n=8). The other 5 patients in the group suffered from cervicobrachial syndrome, polyneuropathy, post-herpetic neuralgia, arthritis, and osteoporosis. Oncological patients were diagnosed as follows: breast carcinoma (n=4), carcinoma of GIT and pancreatic gland (n=4), carcinoma of uterus (n=2), lung cancer (n=2), carcinoma of prostate gland, urinary bladder, and kidneys (n=4), carcinoma of orofacial cavity and larynx (n=3) and leukemia (n=1).

The patients were examined in the outpatient unit of the Center for Pain of the Faculty Hospital in Brno. All patients signed an informed consent and the study was approved by the Ethical Committee of the Faculty Hospital in Brno. Treatment for pain did not begin until after the patient's initial examination.

During the initial investigation pain intensity was measured on the VAS scale (0–10) and blood samples were drawn for determination of the following biochemical blood serum values: total protein, albumin, glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triacylglyceroles, apolipoprotein A, apolipoprotein B, indexes of atherosclerosis. Apo A / Apo B, LDL/HDL, Klimov ((total cholesterol – HDL cholesterol) / HDL cholesterol) and CRP were also measured and electrophoresis of plasma proteins (albumin, alpha 1 globulin, alpha 2 globulin, beta globulin and gamma globulin) was performed.

The same VAS measurement was repeated 4 times during treatment (approximately every 3 weeks). Biochemical measurements were repeated when the pain intensity assessment fell below 5 on the VAS scale. The biochemical results were obtained from the biochemical laboratory at the Faculty Hospital in Brno.

Therapy for non-oncological patients consisted most often of administration of NSA (n=15) and tramadole (n=14). As necessary, the above mentioned drugs were used in combination with: opioids (oxycodon,

dihydrocodeine, morphine sulphate – MST), antiepileptics (clonazepam, gabapentin, carbamazepine), antidepressants (fluoxetine, tricyclic antidepressants), paracetamol, or coxibs.

For the oncological patients, NSAs were the most frequently administered drugs (n=16). Other medications were used as follows: indomethacine (n=5), opioids: phentanyl (n=10), morphine (n=6), morphine sulphate (n=1), dihydrocodeine (n=1); antidepressants: TCA, amitriptyline (n=5), fluoxetine (n=3), antiepileptics: clonazepam (n=1), gabapentin (n=1), hypnotics (n=1), and paracetamol.

A brief overview of treatments and further demographic characteristics are presented in Table 1.

Antidepressants were indicated in both groups of patients either for psychiatric comorbidity (depression) and/or neuropathic pain. None of the patients were treated with antidepressants before entering the study. Prior pain management had been sporadic and non-systematic, making it impossible to establish precise pain treatment histories.

The group of non-oncological patients consisted of women only (average age = 56.5 years), while the group of oncological patients consisted of 11 women and 9 men (average age = 64.8 years). Ten oncological patients died during the 8 month study (4 men and 6 women).

Statistical analysis

Differences in the values of laboratory clinical findings in non-oncological and oncological patients were analyzed using the Student's t-test for two independent samples. Changes within diagnostic groups throughout the therapy were evaluated using the Student's paired t-test or the Kruskal-Wallis nonparametric test. Changes in pain intensity throughout treatment were evaluated by analysis of variance for repeated measures.

RESULTS

Regardless of the differential diagnosis, no differences were found in the non oncological patients in either biochemical parameters or pain intensity (data not presented because of the small number of subjects in each group). With the exception of lower levels of total protein, oncological patients who died during the observation period did not differ at the beginning of the study from those who finished the therapy.

The two groups did not differ in pain intensity assessments prior to treatment. The results of admission biochemical data and data obtained after pain assessments below 5 on the VAS are presented in Table 2. Pain intensity, as assessed using the VAS method, decreased significantly in both groups; in non-oncological patients the average VAS decreased from 8.5 to 4.2, in oncological patients the average VAS decreased from 9.0 to 4.1 (ANOVA $F_{(4,116)}$=2.5, p=0.048) (Figure 1).

The only biochemical parameter to change after therapy was albumin levels in non-oncological patients which decreased after therapy (t=3.4, p=0.003). No changes were found in the oncological patients who completed the study.

Comparison of biochemical values in non-oncological and oncological patients before and after therapy

Before starting analgesic treatment, the following values were lower in the oncological patients compared to the non-oncological patients: albumin (p=0.015), HDL cholesterol (p=0.003) and apolipoprotein A (p=0.01). The opposite was true for CRP (p=0.029) and alpha 1 globulin (p=0.008), which were higher in the oncological patients.

The oncological group showed significantly higher levels of glucose (p=0.034) and alpha 1 globulin after therapy than the non-oncological group. Levels of HDL cholesterol (p=0.005) and apolipoprotein A (p=0.0003) were lower in the oncological group compared to non-oncological group.

After therapy all indexes of atherosclerosis in the oncological patients were higher than in the non-oncological patients (Klimov p=0.023), LDL/HDL (p=0.004) and ApoA/ApoB (p=0.043). It is necessary to mention that values for the oncological patients after therapy were obtained from a reduced number of patients due to the death of ten members of this group.

The effect of antidepressant therapy on VAS and biochemical parameters

There was no difference in the intensity of pain in the non-oncological patients at the end of the therapy with respect to adjuvant therapy with antidepressants. The surviving oncological patients that used antidepressants (n=7) reported lower pain intensity than the oncological patients not taking antidepressants (n=3) (Figure2). Despite the small number of patients, it is interesting that out of 10 patients using antidepressants, survived 7, while out of 10 patients not using antidepressants, only three patients survived (tested by chi square χ^2=1.09, p=0.29).

Patients taking antidepressants had, regardless of diagnosis, higher levels of gamma globulin compared to patients not treated with antidepressants. In the oncological group the difference was statistically significant (KW=7.02; p=0.008) (Figure 3).

DISCUSSION

At the beginning of the study the oncological patients showed: lower levels of serum albumin, HDL cholesterol, apolipoprotein A and higher levels of CRP and alpha 1 globulin than the non-oncological patients.

After therapy the oncological patients showed higher levels of glucose and alpha 1 globulin and lower levels of apolipoprotein A than the non-oncological patients. After therapy the groups also differed in the level of HDL cholesterol which was lower in patients with

Tab. 2. Changes in blood plasma biochemical markers in non-oncological and oncological patients before and after analgesic treatment.

	Chronic pain		Cancer pain		Significance of T-test for independent samples (*p*-values)		Number of patients at the beginning/end (N)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	chronic	cancer
Pain intensity (VAS)	8.50±1.00	4.25±1.94	9.05±0.83	4.10±1.96	0.065	0.844	20/20	20/10
Total protein (g/l) (64–82)	72.20±4.31	69.85±4.69	69.32±4.68	70.77±4.57	0.052	0.623	20/20	19/9
Albumin (g/l) (36–54)	41.70±3.01	37.95±3.20	39.10±3.22	37.90±4.27	0.015	0.973	20/20	17/8
Glucose (mmol/l) (3.3–6.1)	6.07±2.51	5.23±1.61	5.86±1.79	6.62±1.63	0.756	0.034	20/20	20/10
Total cholesterol (mmol/l) (3.8–5.7)	5.66±1.23	5.04±1.06	5.03±1.19	5.08±1.02	0.110	0.912	20/20	20/10
HDL-cholesterol (mmol/l) (1.1–1.6)	1.62±0.29	1.46±0.28	1.30±0.36	1.15±0.22	0.003	0.005	20/20	20/10
LDL-cholesterol (mmol/l) (2.0–4.0)	3.36±1.19	2.76±0.88	2.99±0.96	3.16±0.78	0.299	0.234	19/19	20/10
TRG (mmol/l) (0.6–2.0)	1.60±0.76	1.90±1.26	1.65±1.10	1.69±0.69	0.884	0.633	20/20	20/10
Apo A-I (g/l) (1.19–2.12)	1.33±0.21	1.32±0.16	1.15±0.19	1.09±0.08	0.010	0.0003	17/20	20/10
Apo B (g/l) (0.72–1.45)	1.03±0.27	0.91±0.26	0.89±0.22	0.99±0.19	0.103	0.377	17/20	20/10
Klimov (1.0–4.5)	2.67±1.17	2.57±1.11	3.20±1.57	3.58±1.02	0.244	0.023	19/20	20/10
LDL/HDL (1.0–3.0)	2.19±1.04	1.93±0.77	2.52±1.07	2.87±0.78	0.335	0.004	19/19	20/10
ApoA/ApoB (1.4 – 1.6)	1.42±0.70	1.59±0.65	1.37±0.46	1.14±0.21	0.800	0.043	17/20	20/10
CRP (mg/l) (0–10.0)	1.27±2.23	3.92±8.17	15.02±27.00	18.10±30.22	0.029	0.057	20/20	18/9
A/G	1.33±0.18	1.39±0.20	1.26±0.36	1.26±0.32	0.503	0.209	20/20	18/10
Albumin (%) (52–65)	56.76±3.23	58.35±4.25	56.26±4.65	55.03±6.67	0.703	0.107	20/20	18/10
Alpha 1 globulin (%) (2.0–4.0)	3.00±0.44	3.02V0.37	3.72v1.06	3.73v0.83	0.008	0.002	20/20	18/10
Alpha-2 globulin (%) (9.0–14.0)	12.72±1.60	12.18±1.79	12.74±2.36	11.85±2.61	0.970	0.686	20/20	18/10
Beta globulin (%) (9.0–15.0)	11.65±1.56	11.790±1.67	12.23±1.44	12.03±1.44	0.241	0.695	20/20	18/10
Gamma globulin (%) (10.0–19.0)	15.83±3.22	15.18±3.05	15.03±3.23	17.36±6.56	0.458	0.220	20/20	18/10

Normal physiological ranges (in parenthesis) were provided by the Department of Clinical Biochemistry, Faculty Hospital Brno.

tumor pain and the ratio of atherosclerotic indexes, which were higher in patients with tumor pain.

Schell *et al.* (2008) reported a decrease of HDL cholesterol levels with increasing pain in people with benign pain (e.g. neck, shoulder, upper and lower back pain). They also found decreasing levels of albumin, growth hormone, and neuropeptide Y in blood plasma. These same values were seen to increase as pain levels decreased.

There was no difference in the intensity of pain evaluated on the VAS scale among patients with chronic non-tumor pain and tumor pain. We expected patients with

tumor pain to experience greater pain intensity before the onset of treatment than non-oncological patients. While this expectation is quite common, we were not able to confirm it in our sample of patients. This widely held expectation was seen in the work of Cohen *et al.* (1986), however, only for the evaluation of subjective pain discomfort and for pain intensity; although, emotional distress was significantly higher in patients with chronic benign pain. We did not follow the affective aspects of pain in our work. However it is well established that pain suffering is closely related to psycho-neuro-immunological changes (Rittner *et al.* 2008).

Perhaps because the oncological group of patients was small and half of the patients died prior to the end of the study, but some of our results seem create more questions than answers. One such question concerns whether the higher mortality seen in the tumor pain patients not treated with antidepressants is just a coincidence (although statistically non significant) or whether this may suggest some protective function associated with antidepressants. Meta-analyses from human and animal studies have concluded that several antidepressants can have a significant positive association with cancer protection, while others have shown a negative association (Steingart and Cotterchio, 1995); the effect seems to depend on the type of cancer and the type of antidepressant used.

Some observations in the research reports indicate that in depressed patients treated with antidepressants a normalization of immune parameters occurred; increased parameters were lowered whereas depressed immune parameters were restored (Neveu and Castanon, 1999). Similar normalization of serum cortisol was shown in patients with sever chronic pain treated with opioids (Tenant and Hermann, 2002). From these clinical studies, it is not possible to conclude whether antidepressants and/or opioids have a direct effect on the immune and endocrine system or whether their putative effects result from improved mood.

Lieb (2008) has described the multifaceted value of antidepressants in cancer therapeutics. Among the mechanisms of carcinogenesis he focused mainly on up regulation of cyclooxygenase and changes in prostaglandins, oncogene synthesis and expression, viral activation, signal disruption, accelerated cell replication, failed apoptosis, tumor initiation and promotion, angiogenesis, metastasis, immunosuppression, and

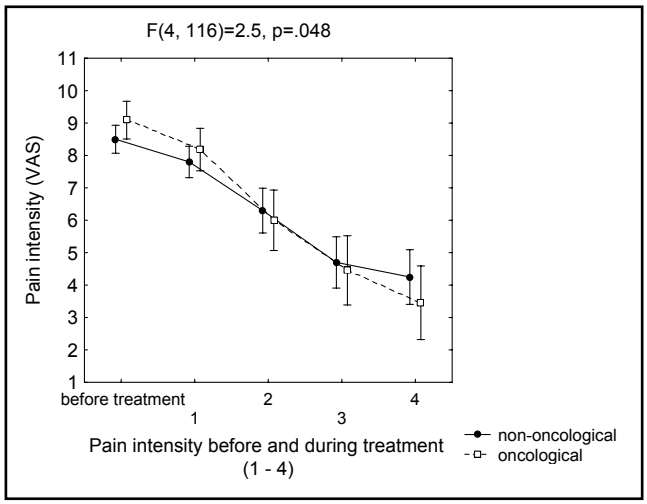

Fig. 1. Changes in pain intensity before and during treatment. Patients with chronic nonmalignant pain – solid line; patients with malignant pain – dashed line. Vertical bars denote 0.95 confidence intervals.

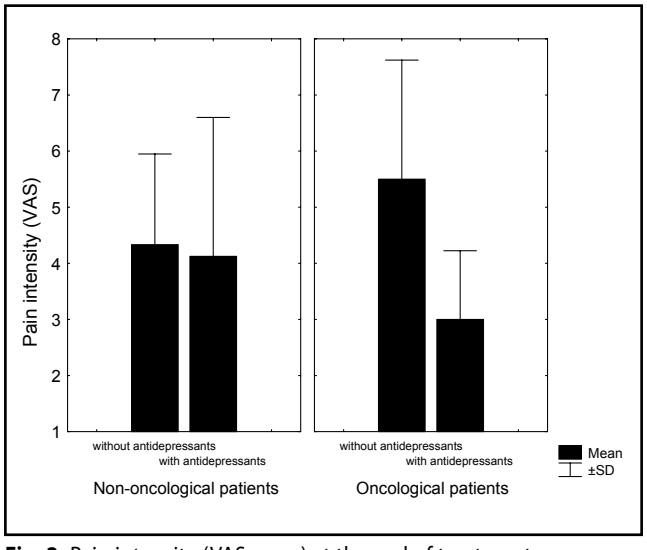

Fig. 2. Pain intensity (VAS score) at the end of treatment. Patients with chronic nonmalignant and malignant pain treated with antidepressants (right columns) patients treated without antidepressants (left columns). Antidepressants marginally reduced pain in cancer patients (KW-$H_{(1,11)}$ = 2.9, p=0.08).

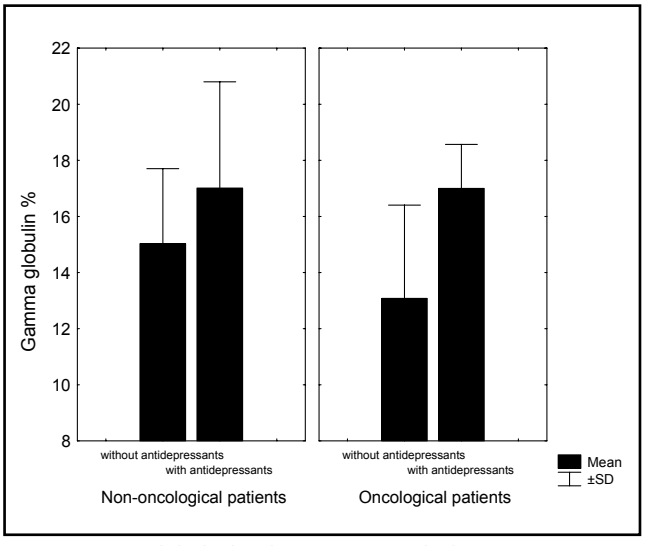

Fig. 3. Gamma globulin levels in patients with chronic nonmalignant and malignant pain associated with antidepressant treatment.
In both groups, patients treated with antidepressants had higher levels of gamma globulin: in non-oncological patients only marginally (KW-$H_{(1,20)}$ = 2.7, p=0.09), in oncological patients significantly higher (KW-$H_{(1,18)}$ = 7.0, p=0.008).

autoimmunity. In our patients treated with antidepressants, the level of gamma globulin was higher when compared to non treated patients. Van Hunsel *et al.* (1996) followed patients with depression and found, as we did, that depressive patients have low levels of gamma globulin, which rose significantly, after antidepressant treatment.

CONCLUSIONS

We observed that biochemical values in patients with tumor pain are more similar to those of chronic non-tumor pain patients than to patients with acute pain. These results show that routine biochemical markers differ between patients with acute pain and patients with chronic pain. We also found that underlying disease, causing the pain, has a significant influence; this is especially true in patients with chronic pain. We expected differences between tumor and non-tumor pain, but this was not fully confirmed by our results. It appears that the chronic nature and duration of pain is more important than whether the pain is tumor pain or non-tumor pain.

ACKNOWLEDGEMENTS

Supported by the Research Goal of the Ministry of Education of the Czech Republic 0021620816.

REFERENCES

1 Apkarian AV, Krauss BR, Fredrickson BE, Szeverenyi NM (2001). Imaging the pain of low back pain: functional magnetic resonance imaging in combination with monitoring subjective pain perception allows the study of clinical pain states. Neurosci Lett; **299**: 57–60.
2 Borsook D, Moulton EA, Schmidt KF, Becerra LR (2007). Neuroimaging revolutionizes therapeutic approaches to chronic pain. Mol Pain; **3**: 25.
3 Cohen RS, Brechner TF, Pavlos A, Reading AE (1986). Comparison of cancer pain and chronic benign pain patients on dimensions of pain intensity, affect, and approach to treatment. Clin J Pain; **1**: 205–209.
4 Hicks CL, von Baeyer CL, Spafford PA, van Korlaar I, Goodenough B (2001). The faces pain scale-revised: toward a common metric in pediatric pain measurement. Pain; **93**: 173–83.
5 Huskisson EC (1974) Measurement of pain. Lancet; **2**: 1127–1131.
6 Krikava K, Kalla K, Yamamotova A, Rokyta R (2004). Blood serum changes in patients with pain during bone fractures and acute pancreatitis. Neuro Endocrinol Lett; **25**: 62–69.
7 Lieb J (2008). The multifaceted value of antidepressants in cancer therapeutics. Eur J Cancer; **44**: 172–174.
8 Mannes AJ, Martin BM, Yang HY, Keller JM, Lewin S, Gaiser RR, Iadarola MJ (2003). Cystatin C as a cerebrospinal fluid biomarker for pain in humans. Pain; **102**: 251–256.
9 Melzack R (1987). The short-form McGill Pain Questionnaire. Pain; **30**: 191–197.
10 Neveu PJ, Castanon N. Is there evidence for an effect of antidepressant drugs on immune function? In: Dantzer R. et. al, eds. Cytokines, Stress, and Depression. New York: Plenum Publishers/ Kluwer Academic, 1999: 267–81.
11 Pekarkova I, Parara S, Holecek V, Stopka P, Trefil L, Racek J, Rokyta R (2001). Does exogenous melatonin influence the free radicals metabolism and pain sensation in rat? Physiol Res; **50**: 595–602.
12 Rittner HL, Brack A, Stein C (2008). Pain and the immune system. Br J Anaesth; **101**: 40–44.
13 Rokyta R, Holecek V, Pekarkova I, Krejcova J, Racek J, Trefil L, Yamamotova A (2003). Free radicals after painful stimulation are influenced by antioxidants and analgesics. Neuro Endocrinol Lett; **24**: 304–309.
14 Rokyta R, Stopka P, Holecek V, Krikava K, Pekarkova I (2004). Direct measurement of free radicals in the brain cortex and the blood serum after nociceptive stimulation in rats. Neuro Endocrinol Lett; **25**: 252–256.
15 Rokyta R, Yamamotová A, Šulc R, Trefil L, Racek J, Třeška V (2008). Assessment of biochemical parameters in pain manifestation of vascular origin. Clin Exp Med; **8**: 199–206.
16 Schell E, Theorell T, Hasson D, Arnetz B, Saraste H (2008). Stress biomarkers' associations to pain in the neck, shoulder and back in healthy media workers: 12-month prospective follow-up. Eur Spine J; **17**: 393–405.
17 Shirasaki S, Fujii H, Takahashi M, Sato T, Ebina M, Noto Y, Hirota K (2007).Correlation between salivary alpha-amylase activity and pain scale in patients with chronic pain. Reg Anesth Pain Med; **32**: 120–123.
18 Stancak A, Kozak J, Vrba I, Tintera J, Vrana J, Polacek H, Stancak M (2008). Functional magnetic resonance imaging of cerebral activation during spinal cord stimulation in failed back surgery syndrome patients. Eur J Pain; **12**: 137–148.
19 Steingart AB, Cotterchio M (1995). Do antidepressants cause, promote, or inhibit cancers? J Clin Epidemiol; 48: 1407–1412.
20 Tenant F, Hermann L (2002). Normalization of serum cortisol concentration with opioid treatment of severe chronic pain. Pain Medicine; **3**: 132–134.
21 Treede RD, Lorenz J, Baumgartner U (2003). Clinical usefulness of laser-evoked potentials. Neurophysiol Clin; **33**: 303–314.
22 Vaculin S, Franek M, Andrey L, Rokyta R (2004). Paradoxical firing of thalamic neurons under neuropathic pain state in rats. Neuro Endocrinol Lett; **25**: 407–410.
23 Van Hunsel F, Wauters A, Vandoolaeghe E, Neels H, Demedts P, Maes M (1996). Lower total serum protein, albumin, and beta- and gamma-globulin in major and treatment-resistant depression: effects of antidepressant treatments. Psychiatry Res; **65**: 159–169.