ORIGINAL ARTICLES

C- REACTIVE PROTEIN IN RECENTLY OPERATED PERIPHERAL ARTERIAL DISEASE PATIENTS AND TREATED WITH ELECTROACUPUNCTURE POSTOPERATIVELY

Marius E. Sinteân¹, Petru M. Deutsch¹, Alina Lupu¹, Mircea Focșa², Mihai Ionac²

REZUMAT

Obiectiv: Proteina C reactivă (CRP), reactant de fază acută și marker independent al inflamației în ateroscleroză, a fost studiată pre și post chirurgical la pacienții cu boală arterială periferică cronică obstructivă (BAPCO). Noi am investigat posibilitatea ca electroacupunctura (EA) să influențeze evoluția valorilor plasmatici post operatorii ale CRP. Material și metode: În acest studiu au fost inclusi 50 de pacienți cu boală vasculară periferică, operați consecutiv cu by-pass femuro-popliteal la Institutul de Boli Cardiovasculare Timișoara și care au acceptat introducerea în studiu. EA a constat în electrostimularea următoarelor puncte de acupunctură: Neiguan (Pc 6), Zusanli (St 36), Sanyinjiao (Sp 6), Taiyaun (P 9), acestea fiind considerate a fi active în reglarea fluxului sanguin de către medicina tradițională chineză. Lotul de pacienți tratat cu EA include 30 de pacienți iar lotul de control include 20 de pacienți. Rezultate: Ambele loturi au prezentat o creștere a valorilor CRP în ser. Acești pacienți cu BAPCO se prezintă deja în preoperator cu valorile medii crescute ale CRP (lot acupunctură =10,79 mg/L și lot control =11,03 mg/L), din care 80% sunt peste 3 mg/L. Valorile medii ale CRP post operatorii sunt mai scăzute în lotul tratat cu acupunctură comparativ cu lotul de control dar diferențele nu sunt semnificative statistic. Concluzii: Valorile plasmatici crescute ale CRP, mai mari de 3 mg/L pentru 80% din pacienții vasculari, sugerează că aceștia au o formă mai severă de ateroscleroză cu un risc crescut de evenimente cardiovasculare. Tratamentul cu EA nu influențează semnificativ statistic valorile post operatorii ale PCR. Cuvinte cheie: proteina C reactivă, ateroscleroză, boală arterială periferică, electroacupunctură, by-pass femuro-popliteal

ABSTRACT

Objective: C- reactive protein (CRP), acute phase reactant and independent marker of inflammation in atherosclerosis, was observed pre and post-vascular surgery at patients with peripheral arterial disease (PAD). We searched for the possible influence of electroacupuncture (EA) therapy on postoperative CRP levels. Material and methods: Fifty consecutive patients with vascular by-pass surgery performed at Timisoara Institute of Cardiovascular Medicine, during the period of July 2007 - July 2008, who gave their informed consent were enrolled in the study. EA treatment consisted in electro-stimulation for 30 minutes of the following acupuncture points: Neiguan (Pc 6), Zusanli (St 36), Sanyinjiao (Sp 6), Taiyaun (P 9). These are acupoints are considered active in blood flow regulation by traditional Chinese medicine (TCM). The acupuncture group consisted of 30 patients and the remainder 20 patients formed the control group. Results: Both study groups showed that surgery increase CRP; in the serum of the operated patients. These patients with PAD have their CRP preoperative levels already increased (means of 10.79 mg/L and lot control =11.03 mg/L), of which 80% are above 3 mg/L. The baseline CRP values are decreased in the EA group compared with control group, but there is no statistical significance. Conclusion: Elevated preoperative CRP > 3.0 mg/L for 80% of patients suggests that the PAD patients have a more severe atherosclerosis with a relative high risk for recurrent cardiovascular events. EA treatment does not influence significantly the postoperative CRP values. Key Words: C-reactive protein, atherosclerosis, peripheral artery disease, electroacupuncture, femoro-popliteal by-pass

INTRODUCTION

Recently, methods of complementary medicine are of growing interests. Acupuncture is an important area of research. There are four areas of cardiovascular disease (CVD) for which acupuncture eventually may be indicated. These include ischemic heart disease, hypertension, heart failure, and arrhythmias.¹

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Our current knowledge on the effect of acupuncture in peripheral artery disease (PAD) is limited, at best. We chose the perioperative period of the management of the disease because there are already well established some beneficial facts of reducing pain and postoperative side effects. Our study looked for a biological parameter - the high sensitive C-reactive protein (CRP), as an endpoint parameter of the electroacupuncture (EA) therapy.

The concept of the involvement of inflammation in atherosclerosis has spurred the discovery and adoption of inflammatory biomarkers for cardiovascular risk prediction. CRP is currently the best-validated inflammatory biomarker. In the context of postoperative EA, the CRP, an acute phase reactant, can give a perspective of the effects of EA through neurovegetative modulation on the immune balancing role of some acupoints, as claimed by traditional Chinese medicine (TCM).

According to TCM, the acupuncture proposes itself to reset the harmony in the balance Yin/Yang, and into energy (“Qi”) and blood flow through the body by stimulating so called “acupuncture points” (there are 365 points organized in 14 canals or meridians of acupuncture). It is known that acupuncture is used successfully in the treatment of some chronic and acute pain conditions, including postoperative pain. EA uses electrical energy to stimulate the acupuncture needles inserted into skin.

In this study we observed the CRP values perioperatively and the effect of EA on the inflammatory reaction induced by surgery. The TCM claims that by acupuncture it can cool the “heat” in the body and reduce the inflammatory reaction and also that it can modulate at certain acupoints the immune response of the human body.

**MATERIALS AND METHODS**

This study enrolled 50 consecutive patients with PAD, Fontaine class IIb and III, who underwent surgery for peripheral revascularization (femuro-popliteal bypass) at Timisoara Institute of Cardiovascular Medicine, between July 2007 and July 2008. (Table 1) The study was approved by the institution’s ethical committee. The selection criterion was patients’ acceptance to participate in the study.

The exclusion criteria were: diabetes mellitus (DM) with glycemia > 200 mg/dL during hospital stay, type 1 DM or DM requiring insulin for control; chronic renal insufficiency with creatinine > 2 mg/dL; severe chronic obstructive pulmonary disease (COPD) requiring steroid treatment and hospital admittance in the past; hepatic disease: elevated liver function tests > 2 x upper normal limit; coagulopathies; vasculites and autoimmune diseases; acute arterial occlusions (traumatic), and arterio-venous malformations and signs of skin infection – ulcers of the inferior limbs. Patients with signs and/or symptoms of infection, on antibiotic treatment have been excluded from this study.

Overall, 51 patients were enrolled but one was excluded because his CRP base line levels were twenty times greater (128.6 mg/L) than normal CRP, the only clinical finding was an erythema of the feet but no ulcer on the legs or signs of infection.

In our clinic, the vascular surgery for PAD patients is done routinely by using regional anesthesia, mostly epidural catheters. The operated patients, in the second or the third day post-operatively, at minimum 12 hours after removal of epidural catheter, received an EA treatment. EA consisted in electrostimulation of the following acupuncture points: Neiguan (Pc 6), Zusanli (St 36), Sanyinquiao (Sp 6), Taiyaun (P 9), these

<table>
<thead>
<tr>
<th>DATA</th>
<th>Age (mean) (SD)</th>
<th>Sex</th>
<th>PAD Fontaine</th>
<th>Other medical conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>CRP-ctr (No: 20)</td>
<td>61.29 (7.06)</td>
<td>66.66 (1.52)</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>CRP-Acp (No: 30)</td>
<td>59.88 (8.48)</td>
<td>71.33 (10.78)</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td>Total No: (50)</td>
<td>60.43 (7.91)</td>
<td>69.0 (7.34)</td>
<td>44</td>
<td>6</td>
</tr>
</tbody>
</table>

No = Number of patients, CRP = C-reactive protein, CRP-ctr = CRP control group, CRP-Acp = CRP acupuncture group, M = male, F = female; PAD = peripheral artery disease, HBP = high blood pressure, CHD = coronary artery disease, CVA = cerebral vascular accidents, COPD = chronic obstructive pulmonary disease.
are acupoints considered active in blood flow and vessel regulation by TCM. The needle used was “Tewa J, 0.25 x 40 mm”. The needles were inserted through the skin between 5 and 10 mm deep looking to obtain “De Qi” sensation at the moment of insertion. After the needles were inserted in the acupoints as mentioned above, each needle was stimulated with the neurostimulating device for a period of 30 minutes.

EA was performed with the Cefar Acus 4 set as: frequency 2 Hz, time 180 ms, with variable amplitude between 0 and 5 mA determined by each patient by his level of comfort at each point of acupuncture or visible muscle twitch. CRP was measured with Cobas Intergra 400 Plus. The normal values are between 0-6.0 mg/L. The statistical data was processed with the statistical software package SPSS. Fisher’s Exact Test, paired T test and ANOVA were used to assess the statistical significance.

In this study we looked if EA modified the inflammatory reaction induced by surgery through the means of PCR. The CRP was measured before surgery (baseline) and twice after surgery, in the second or third day and second time in the fourth to fifth day post operation, respectively.

RESULTS

CRP was measured in the two groups of patients: one labeled as CRP –control (CRP-ctr) and the other as CRP acupuncture (CRP-acp). The CRP – control group consisted of the 20 patients who underwent vascular by-pass surgery and had their CRP serum levels checked at base line – before surgery and after surgery in the second or third day for the first sample and the second sample at the 4-5th day post surgery. The second group of 30 vascular surgical patients CRP - acupuncture (CRP-acp) received an acupuncture treatment and the serum levels of CRP were determined in the same way as in the control group. Both groups were similar regarding baseline characteristics. Regarding the age of the patients, no significant difference was found between the two groups (Fisher test, p=0.672) but when we compared males and females, we found that the female patients were older (ANOVA, p < 0.01). (Fig. 1) The descriptive statistics of the study groups are shown in Table 2.

Our baseline CRP values in 80% of cases are above 3 mg/L, considered to be high cardiovascular risk prediction as recommended by AHA, and this is a sign of the severity of the PAD in our study population. Less than 50% of the patients had CRP in the normal range of the assay (0-6.00 mg/L). The expected behavior for an acute phase reactant (CRP) is shown in Table 2. Immediately after surgery the CRP levels rise (means increased more than 14 folds) and then by the 5th postoperative day they are slowly decreasing but maintaining elevated levels, 7-9 times higher than the baseline values (p < 0.001).

In Figure 2 we can observe that, both in the controls and in the acupuncture treated patients, the CRP blood level follows the same pattern: from base line rise to a peak level at 48-72 hours and then decrease to the levels shown in the second sample in the 4-5th post operative day.

Table 2. Statistics: C-reactive protein, control and acupuncture groups

<table>
<thead>
<tr>
<th>DATA</th>
<th>CRP ctr base</th>
<th>CRP ctr peak</th>
<th>CRP ctr late</th>
<th>CRP acp base</th>
<th>CRP acp peak</th>
<th>CRP acp late</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>30</td>
<td>30</td>
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</tr>
<tr>
<td>Mean</td>
<td>10.79</td>
<td>157.11</td>
<td>91.19</td>
<td>11.03</td>
<td>152.20</td>
<td>71.41</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>9.84</td>
<td>75.67</td>
<td>58.49</td>
<td>8.78</td>
<td>41.37</td>
<td>43.99</td>
</tr>
<tr>
<td>Percentiles</td>
<td>25</td>
<td>4.16</td>
<td>102.50</td>
<td>45.86</td>
<td>4.64</td>
<td>125.58</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.65</td>
<td>157.10</td>
<td>77.30</td>
<td>9.12</td>
<td>156.67</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>13.90</td>
<td>196.52</td>
<td>143.57</td>
<td>13.92</td>
<td>182.75</td>
</tr>
</tbody>
</table>

CRP = C-reactive protein, CRP ctr = CRP control lot, CRP acp = CRP acupuncture lot.

Figure 1. The age and the sex of PAD patients. (F+M = age mean values of the combined female and male patient groups).
The mean CRP values of the acupuncture treated patients are decreased, compared with the control group, but not statistically significant. There were no significant differences between the groups when we looked at other variables: the age and sex of the patients, the severity of the PAD or the associated diseases.

**DISCUSSION**

The current phase of interest in CRP and cardiovascular disease started in the 1990s with observations of increased CRP concentrations in some patients with “active coronary syndromes” and some individuals with acute myocardial infarction tested very soon after onset of pain, before the acute phase response to infarction could have started. Patients with established PAD have a cardiovascular event rate similar to patients with established coronary heart disease (CHD). The management of PAD is complex and facilitates the penetration of other treatment methods like palliative and alternative medicine, which are increasing in popularity and usage. The World Health Organization (WHO) has noted that acute infection and inflammation, dysfunction of autonomic nervous system, pain, each represent conditions for which acupuncture may be indicated.

Postoperative pain management remains a significant challenge for all healthcare providers. Well-designed clinical studies are required to investigate the role of non-pharmacological techniques in relieving postsurgical pain. The acupuncture treatment reduces the pain with a lower incidence of opioid-related side effects, improve movement and increase patient satisfaction. Perioperative acupuncture may be a useful adjunct for acute postoperative pain management. Specific acupuncture points, such as the Neiguan or Zusanli acupoints, overlying the median and deep peroneal nerves, respectively, have been used extensively for treatment of cardiovascular abnormalities. Neiguan (Pc 6), Zusanli (St 36), Sanyinjiao (Sp 6), Taiyaun (P 9) were the acupoints used in our study. These points are influential points for vessels (P 9) and regulate the flow of the heart Qi and blood, tonify the blood/ Qi the others, from TCM point of view.

The mechanism by which acupuncture is believed to benefit the subject is through its ability to modulate neural activity in several regions of the brain and thus reduce sympathetic outflow to the heart and vascular system. Regarding the pain perception, in one study acupuncture has been shown to reduce somatosensory evoked potentials to noxious stimuli in anaesthetized volunteers. In reviewing the peer-reviewed literature, it is apparent that there is growing body of scientific evidence supporting the efficacy of acupuncture and its many variants (e.g., percutaneous neuromodulation therapy, transcutaneous electrical nerve stimulation, percutaneous electrical nerve stimulation) in providing pain relief in patients with acute and chronic pain syndromes. The electrostimulation has been used at different levels of the nervous system, mainly spinal, peripheral nerves, and points of TCM acupuncture. The most favorable results have been observed with spinal cord stimulators in patients with peripheral vascular disease, complex regional pain syndrome, and peripheral neuropathy (e.g., diabetic or causalgic origin). The electrostimulation is combined with acupuncture in an effort to obtain a more powerful and sustained effect of the neuromodulation upon the peripheral nerves, and it is easier to quantify the therapy.

The biological effects of acupuncture include the regulation of a variety of neurohumoral factors and growth control factors. The neurophysiology model on the long-term effects of acupuncture emphasizes the trophic and anti-inflammatory effects of acupuncture. Its prediction on the peripheral effect of endorphin in acupuncture has been confirmed. The study by Tsuchiya et al. provides evidence that acupuncture enhances the generation of nitric oxide (NO), which serves an important regulatory function in controlling local blood flow. Stimulating acupuncture points can not only cause transient modulation of neurotransmission, but also alter the growth control signal transduction in various systems-leading to long-term effects.

CRP increased levels suggest that low-grade inflammation is involved in pathogenesis of atherosclerosis, especially in its thrombo-occlusive complications (MONICA Study). Since then, many prospective studies have shown that the inflammatory biomarker CRP is an independent predictor of future cardiovascular events that additionally predicts risk of incident hypertension and diabetes. Clear policy
statements endorsing selective CRP use among these intermediate-risk and high-risk groups would contribute to consensus.\textsuperscript{4,10,20} CRP may be a causal factor as well as a marker for inflammation, depending on the concentration. This concentration of CRP depends on the rates of production and clearance.\textsuperscript{21} The major part of the CRP present in the plasma comes from the liver, where the synthesis of CRP is mainly regulated by interleukin-6; small amounts of CRP can also be produced locally.\textsuperscript{21} Thus, CRP has numerous effects on endothelial cells that could support a pro-inflammatory, pro-thrombotic role.\textsuperscript{4,22,23}

In most populations, over 95\% of subjects have CRP values below 10 mg/L. The cut points of low CVD predictive risk (<1.0 mg/L), average risk (1.0 to 3.0 mg/L), and high risk (>3.0 mg/L) correspond to approximate tertiles of CRP in the adult population. In general, the high-risk category includes the right skewed tail of the distribution.\textsuperscript{24} Our findings reflect this situation, we have higher values of our base line CRP's data, with 80\% of CRP samples over 3.0 mg/L, despite the fact that 64\% of our patients have a less severe form of PAD - Fontaine IIB category. (Fig. 3) Elevated preoperative CRP above 3.0 mg/L suggests that our PAD patient study population is in high-risk prediction category for the severity of atherosclerosis. It is in conformity with the well-known fact, for example, that for vascular surgery, the prevalence of perioperative myocardial infarction and death is 5\% and 1.6\%, respectively. When the outcomes are assessed over a longer postoperative period (3-4 years) prevalence of myocardial infarction and death is even higher: 8.9\% and 9.1\%, respectively.\textsuperscript{25}

\textbf{Figure 3.} The pre-operative CRP mean values of the combined lots (50 patients) - control and EA treated patients (pre op CRP = pre-operative CRP)

Numerous reports are investigating the correlation of elevated CRP and coronary ischemic disease. Data suggest that increased levels of CRP before angioplasty or coronary artery bypass surgery have a higher risk of in-hospital mortality. Preoperative CRP value is an important (independent) risk factor for postoperative outcome, hospitalization and midterm survival for cardiac causes.\textsuperscript{26-28} Another study claim that a preoperative level of CRP > 5 mg/L did not predict in-hospital postoperative complications.\textsuperscript{29} In one paper, the multivariate analysis shows that age >75 years, peripheral vascular disease and C-reactive protein quartiles were the only independent predictors of mortality.\textsuperscript{27} There are less CRP reports involving peripheral vascular diseases. In such a study, Eldrup N. et all demonstrated that in PAD patients, CRP and ankle-brachial index predicted severe atherosclerosis.\textsuperscript{30}

There are studies that suggest CRP is not merely a marker of inflammation but, also, a modulator that drives direct biological effects on vascular cells. These in vivo observations support the hypothesis that CRP modulates NO metabolism and may have implications regarding the mechanisms by which CRP modulates vascular disease.\textsuperscript{22,21} CRP has been described to decrease the expression and bioactivity of endothelial nitric oxide synthase, which results in reduced bioavailability of nitric monoxide (NO) and a subsequent effect of vasodilatation.\textsuperscript{21} Our study considered the possibility that electroacupuncture, by diminishing the postoperative pain (secondary decrease acute phase reactants) and increasing the levels of NO,\textsuperscript{15,17} can modulate the expression of the CRP in the perioperative period. Figure 2 shows that, although the postoperative mean CRP levels are decreased in the EA group compared with control group, there is no clinical significance. In both study groups the expected pattern of an acute phase reactant, with peaking at 48-72 H and gradually declining, was found.

**CONCLUSION**

Elevated preoperative CRP > 3.0 mg/L for 80\% of patients suggests that the PAD patients have a more severe atherosclerosis with a relative high risk for recurrent cardiovascular events. EA treatment does not influence significantly the postoperative CRP values.

**REFERENCES**