Effect of Electroacupuncture on Natural-Killer Cells and Tumor Size in Patients with Cervical Squamous-Cell Carcinoma: A Randomized Controlled Trial

Wita Saraswati, MD, PhD,^{1,2} Erry Gumilar Dahlan, MD, PhD,^{1,2}
Koosnadi Saputra, MD, PhD,^{3,4} and Thomas Cahyono Sutrisno, MD⁴

ABSTRACT

Background: A natural-killer (NK) cell is a cytotoxic lymphocyte that responds to tumor formation. Electroacupuncture (EA) in patients with cancer, who had chemotherapy, enabled them to maintain their T-cell counts (CD3+, CD4+, and CD8+) and NK-cell activity.

Objective: To evaluate NK-cell levels and tumor sizes in patients with cervical squamous-cell carcinoma (SCC) after EA on ST 36 (*Zusanli*).

Design: This study was a randomized, experimental clinical study with a pre–post-test, control group design. Setting The study was performed in the obstetrics and gynecology department, Gynecology Oncology Division of the Rumah Sakit Dokter Soetomo General Hospital, in Surabaya, Jawa Timur, Indonesia, conducted from February 2016 until May 2016.

Subjects: Patients with cervical SCC, stages IIb–IIIb (locally advanced)—determined clinically and histopathologically—all of whom had 50 mg/m²/week of cisplatin chemotherapy. The patients were divided, consecutively, into 2 groups by simple random sampling.

Intervention: The controls received only received the chemotherapy, while treatment-group patients also had EA 1 day on bilateral ST 36 after each chemotherapy cycle.

Outcome Measures: Primary outcomes were percent of NK cells and tumor size. Before the first treatment and after the fourth one, all patients underwent peripheral blood examinations (complete blood counts, serum creatinine and blood–urea–nitrogen levels, and flow-cytometry) to determine percentages of NK cells, and pelvic magnetic resonance imaging to measure cervical tumor sizes. Secondary outcomes were pain, nausea, vomiting, and appetite.

Results: There was a significant increase in number of NK cells ($P=0.00$) and a significant decrease in tumor sizes ($P=0.03$) in the treatment group, compared to the control group. There was a significant increase in appetite ($P=0.00$) in the treatment group, compared to baseline, but there were no significant differences in pain ($P=0.061$), nausea ($P=0.399$), and vomiting ($P=0.854$).

Conclusions: Patients with cervical SCC at stages IIb–IIIb, who received 4 cycles of chemotherapy using 50 mg/m²/week of cisplatin and EA at ST 36, had increased NK-cell percentages in their peripheral blood and had reductions in their cervical tumors.

Keywords: electroacupuncture, natural-killer cells, cervical squamous-cell carcinoma

¹Obstetrics and Gynecologic Department, Rumah Sakit Dokter Soetomo General Hospital, Surabaya, Jawa Timur, Indonesia.

²Faculty of Medicine, Universitas Airlangga, Surabaya, Jawa Timur, Indonesia.

³Acupuncture Research Laboratory in Health Services, Ministry of Health of the Republic of Indonesia, Surabaya, Jawa Timur, Indonesia.

⁴Academy of Acupuncture Surabaya, Jawa Timur, Indonesia.

INTRODUCTION

CERVICAL CANCER IS A GYNECOLOGIC CANCER with the highest percentage (74.12%) of occurrences, compared to ovarian cancer (11%), gestational trophoblastic neoplasia (5.5%), and other cancers (vulva, vagina, tuba, and endometrial cancers). Data collected from 2007 to 2011 by the Surveillance, Epidemiology and End Result program, and released in April 2014, showed that new cervical-cancer cases were 7.8/100,000 females per year, with a mortality rate of 2.4/100,000 females per year. The same data also indicated that ~0.7% females will be diagnosed with cervical cancer during their lifetimes.¹

In 2009, the National Cervical Cancer Coalition reported that, in the United States, ~10,000 females are diagnosed with cervical cancer every year and ~3700 females die due to cervical cancer.² Females in developing countries comprise 85% of patients with cervical cancer worldwide (an estimated 473,000 cases), with an estimated mortality rate of 253,500 deaths per year. Cervical cancer is still the main cancer that causes death in most developing countries. The cervical cancer incidence in Indonesia in 2008 was 13,762 cases, with 7493 deaths. Most patients were seen when their cancers were at late stages (II–IVb); this comprised ~66.4% of these patients.³

Human papilloma virus (HPV) causes 99.7% cervical cancers. HPV has a small diameter, <55 nm. There are more than 100 types of HPV. HPV types 16, 18, 31, 33, 35, 45, 51, 52, 56, and 58 are often found in cervical cancers and in precancerous lesions. HPV types 16 and 18 cause 70% of cervical cancers.^{4,5}

In the early stage of cervical cancer (stages I–IIa), the treatment is surgery (conization, trachelectomy, or simple or radical hysterectomy). Treatment for advanced stages (IIb–IVb) are chemotherapy and radiation as a single treatment or a combination of both. These sophisticated and expensive treatments sometimes do not produce good results, especially in late-stage cancers. Moreover, there is a lack of information on cancer treatments to improve patients' quality of life (QoL). In Indonesia, cervical-cancer 5-year survival rates are 50% for stage I, 40% for stage II, 20% for stage III, and 0% for stage IV.⁶ One problem in a patient with cancer is an immune-system defect that was present before the malignancy occurred. This defect will increase during chemotherapy and radiation, lowering the patient's immunity (e.g., a leucocyte-count decrease after chemotherapy); furthermore, that patient's QoL will deteriorate.^{7–9}

Neoadjuvant chemotherapy is a cervical-cancer treatment strategy used before radical hysterectomy or radiotherapy (concurrent with chemotherapy or radiotherapy alone) are attempted. Cisplatin is the most-studied agent and has shown a consistent clinical response. Most active combinations in cervical cancer therapy include cisplatin. Cisplatin, like other chemotherapy agents, has severe side-effects that

include myelosuppression (a decrease of blood cells and platelet production in bone marrow).⁸

A natural-killer (NK) cell is a cytotoxic lymphocyte that is important in the innate immune system. The NK cell functions as an analogue to T-cytotoxic lymphocytes in the adaptive immune response in vertebrates. NK cells have an immediate response to cells that are infected by viruses and also respond to tumor formation. Generally, immune cells will detect the major histocompatibility complex (MHC) that presents on the surface of infected cells. This will trigger a cytokine release, which causes lysis or apoptosis. NK cells are unique because they have the ability to recognize stressed cells without antibodies and MHC. This ability enables a faster immune response.

NK cells are called *natural-killer cells* because they do not need to be activated to kill cells that have lost their "self" markers (MHCs).¹⁰ NK cells play a role in the innate immune response to viruses and intracellular microbes as well as to transformed cells (tumor cells). NK cells comprise 5%–15% of the mononuclear cells in the blood and spleen. NK cells do not have immunologic memory. NK cells have CD16 and CD56 surface markers to differentiate them from T-lymphocytes and B-lymphocytes. Simultaneous surface markers CD16 and CD56 without expression of CD3 (CD3-CD56+/CD16+) are currently used to identify NK cells in peripheral blood; these characterize >90% of the NK-cell populations in normal adults.^{11,12} NK cells lysis certain tumor targets spontaneously *in vivo* and *in vitro* without prior sensitization. NK cells are also able to eliminate metastatic cells in circulation.

Several surveys have indicated that acupuncture is the most-recommended complementary and alternative medicine by conventional biomedical practitioners.^{13,14} Electroacupuncture (EA) is a kind of acupuncture that has been used widely, as well as percutaneous electrical nerve stimulation, which is considered to be complementary to traditional acupuncture. For EA, a small electric current is used to stimulate nerves and muscles. Compared to traditional acupuncture and other forms of acupuncture, EA became popular quickly.^{14,15}

In one study, EA applied to 28 patients with cancer who had chemotherapy were able to maintain their T-cell counts (CD3+, CD4+, and CD8+) and NK-cells' activity, which are normally lowered in patients who are receiving chemotherapy.¹⁶ Another study showed that continuous stimulation on ST 36 (*Zusanli*), once per day for 3 days, increased NK-cell activity in normal rats but did not affect spleen NK-cell populations.¹⁷ The researchers suggested that the increase of NK-cell activity was mediated by increases of interferon- γ (IFN- γ) and β -endorphin that were caused by EA.¹⁷

The purpose of the current study was to evaluate NK-cell levels and tumor sizes in patients with cervical squamous-cell carcinoma (SCC) after EA on ST 36.

MATERIALS AND METHODS

Study Design

This was a randomized experimental clinical study with a pre–post-test, control-group design.

Location

This study was conducted in the obstetrics and gynecology department, Gynecology Oncology Division, of the Rumah Sakit Dokter Soetomo General Hospital, in Surabaya, Jawa Timur, Indonesia. The study was conducted from February 2016 until May 2016.

Participants

The participants were patients in the hospital who had cervical SCC. Each patient received oral and written explanations about the study and signed informed consent to participate.

Inclusion criteria were patients who:

- (1) had cervical SCC in stages IIB–IIIB (locally advanced), which were determined clinically and histopathologically with performance states <3 (according to the World Health Organization¹⁸)
- (2) were scheduled to have chemotherapy with 50 mg/m²/week of cisplatin (patients with hemoglobin levels at 10–16 g/dL, leucocyte counts >3,000/mm³, and creatinine clearances >45 mL/minute)
- (3) were willing to have EA treatment
- (4) were willing and able to have blood samples collected through phlebotomy
- (5) had never had any chemotherapy before
- (6) did not have conditions that were contraindications for chemotherapy and/or EA
- (7) indicated agreement to join the study by signing informed consent forms.

Exclusion criteria were patients who: (1) had other severe comorbid conditions (such as uncontrolled diabetes mellitus or malignancies other than SCC); (2) underwent therapy for other chronic conditions; and/or (3) had skin problems on the ST 36 area.

Dropout criteria were patients who: (1) did not finish the treatment according to the procedure (4 cycles of chemotherapy and/or 4 cycles of EA); and (2) had severe side-effects or complications because of chemotherapy, EA, or intolerable subjective symptoms. Death of a patient was also considered a “dropout” in the study.

Sampling was conducted by consecutive sampling. Patients were divided into 2 groups by simple random sampling. The number of samples was calculated using the following formula:

$$n1 = n2 = \frac{(Z\alpha\sqrt{PQ} + Z\beta\sqrt{P_1Q_1} + P_2Q_2)^2}{(P_1 - P_2)^2}$$

$$(Z\alpha = 1.96; Z\beta = 0.842)$$

The number of samples required was 22 patients in each group.

Interventions

Patients in the control group received 4 cycles of chemotherapy with 50 mg/m²/week of cisplatin.

Patients in the acupuncture group also received the same 4 cycles of chemotherapy. In addition, these patients had EA 1 day after each chemotherapy cycle. One stainless-steel, 0.25 × 40 mm, acupuncture needle (Huanqiu) was used to make a puncture at each ST 36 point (3 finger *cun* below the patella and 1 finger-breadth lateral to the anterior crest of the tibia, on the tibialis anterior muscle). The points were punctured until the needles were at a depth of 10–15 mm, without further attempts to elicit a needling sensation. The needles were connected to an EA device (a KWD-808 I Multi-Purpose Health Device with 6 channels). The electrical stimulation given was a continuous wave, at 2 Hz, 0.2 mA, for 30 minutes. This EA was administered by a certified acupuncturist at the hospital.

Peripheral blood examinations and pelvic magnetic resonance imaging (MRI) were conducted in both groups before the first treatment and after the fourth treatment. Blood examinations included complete blood counts, serum creatinine levels, blood–urea–nitrogen (BUN) testing, and flow-cytometry to determine the patients’ percentage of NK cells. The pelvic MRIs were performed to measure the size of the cervical tumors. Pain was evaluated using a numeric pain scale, nausea and vomiting were evaluated with a modified Rhodes Index of Nausea and Vomiting,¹⁹ and appetite was evaluated using motivation-to-eat visual analogue scale questions.²⁰

Ethics

As noted before, all patients gave written consent to be in the study. It was approved by, and received ethical clearance from, the ethical committee of the hospital (*Ethical Clearance*: 123/Panke.KKE/II/2015).

RESULTS

Participants flow

Fifty three patients were recruited from the oncology and gynecology clinic and obstetrics and gynecology ward of the hospital. The patients were divided randomly, by using simple random sampling, into a control group and an acupuncture group. There were 25 patients in the control group and 28 patients in the acupuncture group (Fig. 1).

Seven patients (25%) in the acupuncture group and 9 patients (36%) in the control group did not complete the study. They were dropped out of the study for the following reasons: (1) deteriorated kidney function (marked by increases of BUN and serum creatinine) so the patients could

FIG. 1. Participants' flowchart. CBC, complete blood count; BUN, blood-urea-nitrogen; MRI, magnetic resonance imaging; EA, electroacupuncture.

no longer receive cisplatin; (2) repeated massive bleeding that required immediate radiation to stop the bleeding; or (3) they did not come to the hospital for treatment.

Background Characteristics

There were no differences in ages, stages of cancer, and pretreatment laboratory values between the 2 groups (Table 1).

Primary Outcomes

There was a significant decline in the percentage of NK cells in the control group ($P < 0.05$). The acupuncture

group had a slight increase in percentage of NK cells, although this increase was not significant ($P > 0.05$). There were no statistically significant differences in tumor sizes ($P > 0.05$) before and after treatment in both groups (Table 2).

Secondary Outcomes

There were no significant pain reductions or nausea and vomiting score changes in both groups. The control group had reductions in appetite, while the acupuncture group had an increase in appetite (Table 3).

TABLE 1. CHARACTERISTICS OF WOMEN AT TRIAL ENTRY BY ALLOCATED TREATMENT GROUP

Characteristics	Control group (n = 16)	Acupuncture group (n = 21)	P-value
Age in years	48.75 (6.58)	48.09 (8.97)	>0.05
Stage of cancer			
IIb	10 (62.5)	16 (76.2)	>0.05
IIIb	6 (37.5)	5 (23.8)	>0.05
Body weight (kg)	54.53 (10.99)	60.57 (11.30)	>0.05
Hemoglobin (g/dL)	12.29 (1.15)	11.95 (1.15)	>0.05
Leucocyte (cells/mm ³)	8721 (23)	9354 (33.0)	>0.05
BUN (mg/dL)	8.06 (2.86)	9.52 (2.27)	>0.05
Serum creatinine (mg/dL)	0.95 (0.17)	0.96 (0.30)	>0.05
Number of NK cells (%)	11.11 (6.28)	7.74 (3.32)	>0.05
Cervix tumor volume (cm ³)	26.11 (12.31)	59.38 (49.73)	>0.05
Pain scores	1.75 (1.39)	1.67 (1.42)	>0.05
Appetite scores	3.63 (2.55)	5.05 (2.01)	>0.05
Nausea scores	3.31 (0.70)	3.67 (1.71)	>0.05
Vomiting scores	15.00 (0.00)	14.71 (0.95)	>0.05

BUN, blood-urea-nitrogen; NK, natural killer.

Summary of Changes after Treatment

The acupuncture group had a higher increase in percentage of NK cells, compared to the control group. The acupuncture group also had decreases in tumor size, while the control group had increases in tumor size. There were no statistically significant differences between both groups after treatment in pain scores or nausea and vomiting scores. The acupuncture group had an increase in appetite, while the control group had a decrease in appetite (Table 4).

DISCUSSION

Number of NK Cells

The prevalence of myelosuppression caused by cisplatin ranges from 25% to 30%. Myelosuppression is decreased in bone-marrow activity that causes a decrease in thrombocyte, erythrocyte, and leucocyte counts.²¹ In the current study,

TABLE 2. PRIMARY STUDY OUTCOMES BY TREATMENT GROUP

Outcomes	Before	After	P-value
Number of NK cells (%)			
Control group, n = 16	11.11 (6.28)	6.04 (3.72)	0.00*
Acupuncture group, n = 21	7.74 (3.32)	8.81 (4.39)	0.144
Cervix tumor volume (cm ³)			
Control group, n = 16	26.11 (12.31)	27.44 (18.30)	0.764
Acupuncture group, n = 21	59.38 (49.73)	52.80 (53.89)	0.106

*Significant result.

NK, natural killer.

TABLE 3. SECONDARY STUDY OUTCOMES BY TREATMENT GROUP

Outcomes	Before	After	P-value
Pain scores			
Control group, n = 16	1.75 (1.39)	1.31 (1.01)	0.068
Acupuncture group, n = 21	1.67 (1.42)	1.38 (0.80)	0.061
Appetite scores			
Control group, n = 16	3.63 (2.55)	1.69 (2.60)	0.01*
Acupuncture group, n = 21	5.05 (2.01)	6.47 (1.93)	0.00*
Nausea scores			
Control group, n = 16	3.31 (0.70)	3.19 (0.40)	0.480
Acupuncture group, n = 21	3.67 (1.71)	3.33 (1.11)	0.399
Vomiting scores			
Control group, n = 16	15.00 (0.00)	14.88 (1.01)	0.180
Acupuncture group, n = 21	14.71 (0.95)	14.67 (1.06)	0.854

*Significant result.

leucocyte counts and hemoglobin levels were used to evaluate the myelosuppressive effect of cisplatin. Leucocytes consist of granulocyte cells (neutrophils, eosinophils, and basophils) and agranulocyte cells (lymphocytes and monocytes). Leucocytes comprise the main component of the immune system.

EA stimulation at ST 36 regulates leucocytes and its subpopulations quantitatively and qualitatively. A study published in 2007 by Yamaguchi et al. showed that acupuncture can regulate the immune system and stimulate humoral and cellular immune activities, including NK-cell activity.²² The current study produced the same result. There was a significant increase in the average percentage of NK cells in the acupuncture group, compared to the control group ($P = 0.000$).

ST 36 is one of the specific points used to modulate immune-system activity. This modulation is transmitted by the same nerve pathway that causes an analgesic effect.²³ The theory is that acupuncture will increase NK-cell activity. The current study produced this result, supporting the theory. The average percentage of NK cells increased after acupuncture. The increase was not significant, compared to before acupuncture, but was significant, compared to the

TABLE 4. CHANGES AFTER TREATMENT

Outcomes	Control group (n = 16)	Acupuncture group (n = 21)	P-value
Number of NK cells (%)	-5.0731 (4.96)	0.8219 (3.30)	0.000*
Cervix tumor volume (cm ³)	3.5331 (17.04)	-8.2510 (45.21)	0.032*
Pain scores	-0.4375 (0.89)	-0.2857 (0.72)	0.582
Appetite scores	-4.9500 (15.19)	1.4190 (1.49)	0.000*
Nausea scores	-0.1250 (0.72)	-0.3333 (1.98)	0.820
Vomiting scores	-0.3125 (1.01)	-0.0476 (1.49)	0.575

*Significant result.

NK, natural killer.

control group. There was a decrease of average percentage of NK cells in the control group.

Interleukin (IL)-12 is a cytokine produced by macrophages and dendritic cells. IL-12 stimulates T-lymphocytes to differentiate into Th1. IL-12 also stimulates NK cells and T-lymphocytes to synthesize IFN- γ and therefore increase its cytotoxicity.¹¹

In a study, published in 2005, Kim et al. performed a microarray analysis to evaluate the effect of EA on ST 36 to gene-expression changes in mice splenocytes.²⁴ Four genes correlated with NK-cell activity. Four genes correlated with NK-cell activity. They were *PTK2*, *VCAM-1*, *PTP*, and *SHP-1*. That study showed that tyrosine kinase protein and *VCAM-1* had positive correlations with NK-cell activity, and that tyrosine phosphatase protein, and *SHP-1* had negative correlations with this activity. EA increased mRNA *CD94* twofold. *CD94* can activate or inactivate NK-cells, depending on which family of *NKG2* is bound. If *CD94* binds with *NKG2A*, the complex will recruit *SHP* tyrosine phosphatase and act as an inhibitory receptor through an *NKG2A* subunit, which has a immunoreceptor tyrosine-based inhibitory motif (ITIM). If *CD94* binds with *NKG2C*, which does not have an ITIM, the complex will trigger a receptor to bind *DAP-12*. The study showed significant increases of mRNA *CD94* expression and NK-cell activity and showed that EA induced *CD94* to bind with *NKG2C* and, therefore, increased NK-cell activity.²⁴

EA also increased VCAM-1 expression. VCAM-1 is a protein (encoded by the *V-CAM-1* gene) that is expressed by endothelial cells that are induced by various cytokines and that have an important role in anchoring lymphocytes. An increase of VCAM-1 expression was caused by IFN- γ , which is produced by activated NK cells. This increase of VCAM-1 production has an important role in NK-cells binding to target cells.²⁴

Therapeutic Response

There are some methods that can be used to evaluate therapeutic response in cervical cancer. The hospital in the current study used pelvic examinations to measure tumor masses and parametrium involvements. This method is highly subjective because it depends on the experience and competence of a gynecologic oncologist. In the last few years, pelvic MRIs have been used to evaluate therapeutic responses. Cervical tumor volume is determined by measuring the transverse antero-posterior and cranio-caudal diameters of the tumor mass, using T2-weighted MRI images and then calculating the size of the tumor according to an ellipse formula.²⁵ In this study, determinations were not made regarding complete response, partial response, stable disease, or progressive disease. Only cervical tumor volume was determined before and after treatment in both groups.

Both groups did not have significant changes in average cervical tumor volume. However, there was a significant

difference between the acupuncture and control groups ($P=0.032$).

NK cells comprise a small part of tumor-infiltrating lymphocytes that produce spontaneous cytotoxic activity against tumor cells. NK cells also comprise the first line in tumor surveillance. It has been reported that NK cells have an important role in regulating tumor-cell proliferation and can be used as predictors to determine survival of patients with early stage cervical cancer.²⁶ In cervical cancer, lymphocyte subpopulation—especially CD8—distribution to the tumor site has implications regarding cancer progression and patient prognosis. However, for clinical relevance, intratumor NK-cell infiltration has not been established. Most NK cells' surveillance function involves their ability to extravasate and localize tumors (in tissues).

NK cells comprise 5%–15% of the mononuclear cells in the blood and spleen. NK cells are rarely found in other lymphoid tissues; rather, these cells are concentrated in certain organs such as the liver and pregnant uterus.^{11,12} A previous study showed that the normal range of NK cells in peripheral blood circulation is quite wide. The average number of these cells in normal adults is 250 cells/mL (range: 90–590 cells/mL). The average percentage of activated NK cells in healthy adults is 3.51% (range: 0.95%–7.67%) (Johanis, Department of Internal Medicine, Airlangga University, 2011 [unpublished material]).

The current study showed that the average percentage of NK cells in the peripheral blood circulation of patients with early stage SCC was higher, compared to normal women. The average percentage of NK cells before treatment in the control and acupuncture groups were $11.11 \pm 6.28\%$ and $7.73 \pm 3.32\%$, respectively. These high percentages of NK cells can be caused by infiltration of tumor cells in the blood circulation, micrometastases in lymphoid nodes, or secondary infections (viral) that can be occur in patients with late-stage cancer.

A previous study by the first author showed that, although there were increases in the percentages of NK cells in the peripheral blood circulation of patients with cervical cancer, NK cells' infiltration of tumor specimens was very low.²⁷ Another study also showed that only small numbers of NK cells infiltrated tumor tissue.²⁸ Therefore, the role of NKs cells in eliminating tumor cells was small. NK cells seemed unable to function and infiltrate tumor tissue in patients with cancer.²⁸

Development of precervical cancer lesions and cervical cancers are strongly related to the change of Th1 cytokine to Th2, a response that is marked by increases of IL-4 and IL-10 and decreases of IL-12 and IFN- γ . IL-10 decreases specific tumor immune responses by downregulating IFN- γ and IL-12 production. These will inhibit migration and activation of NK cells and cytotoxic T-lymphocytes (CTLs).²⁵

Loss of expression of MHC class I in cervical cancer makes tumor cells unable to convert tumor antigens to CTLs. However, this loss of expression of MHC class I allows tumor cells to be lysed by NK cells. Although expression of MHC class I is low, NK-cell infiltration is also low. Therefore, it can be concluded that low migration and infiltration of NK cells

into tumor tissue is related to insufficient chemokine chemotactic concentrations (IL-8, MIP-1 α , MIP-1 β , RANTES, IP-10, MCP-2, MCP3, IFN- γ , IL-12, and TNF- α) and low expression of chemokine receptors such as CCR1, CCR2, CCR4, CCR5, CCR6, CCR7, and CXCR3 to the cells. Inefficient NK-cell migration to tumor tissue could be related to immunosuppressive cytokine expression, especially that of IL-10 (Johanis, Department of Internal Medicine, Airlangga University, 2011 [unpublished material]).

Lymphocyte-cell distribution is affected by the parasympathetic nervous system. EA stimulation can stimulate parasympathetic nerves and, therefore, increase lymphocyte components in leucocyte subpopulations.²¹ Although the percentage of NK cells that infiltrated the tumor tissues was very low in the current study, it showed that there was a significant difference in cervical tumor volume reduction in the acupuncture group, compared to the control group ($P=0.032$).

Like other randomized clinical trials, this study had limitations. The dropout rate was quite high. Patients' compliance was limited due to various side-effects of chemotherapy and deterioration of the patients' conditions.

CONCLUSIONS

These study results suggest that patients with cervical SCC at stages IIb–IIIb, who received 4 series of chemotherapy using cisplatin 50 mg/m²/week and EA at ST 36, had increased percentages of NK cells in peripheral blood and had reductions of cervical tumor sizes.

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This study was approved, and received ethical clearance, by the ethical committee of the Rumah Sakit Dokter Soetomo General Hospital. (*Ethical Clearance*: 123/Panke.KKE/II/2015).

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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Address correspondence to:
 Thomas Cahyono Sutrisno, MD
 Academy of Acupuncture of Surabaya
 Indrapura 17
 Surabaya, Jawa Timur 60176
 Indonesia
 E-mail: dr_thomasc@yahoo.com