THE EFFECT OF LOW INTENSITY AND MEDIUM FREQUENCY ELECTRIC FIELDS ON THE NEUTROPHIL TO LYMPHOCYTE RATIO IN THE BLOOD OF RAT BRAIN TISSUE IN VIVO FROM A SAFETY ASPECT

Muhamad Husni Thamrin Hamdani[1], Joni Wahyuhadi[1], Irwan Barlian Immadoel Haq[1], Budi Utomo[2]

[1]Department of Neurosurgery, Faculty of Medicine, Universitas Airlangga/Dr. Soetomo General Academic Hospital
[2]Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga

Background: One of the most frequently found primary brain tumors in the adult population is glioblastoma. The Neutrophil-to-Lymphocyte Ratio (NLR) in brain tumors shows that higher NLR predicts poorer outcomes. The Electro Capacitive Cancer Treatment (ECCT) has demonstrated higher efficacy in vitro and in vivo clinically, while safety aspects have not been observed and require further research. This study aims to analyze the effect of ECCT using low intensity electric fields at medium frequency on NLR in the blood of rat brain tissue in vivo from a safety aspect.
Methods: This research used a true experimental design with a post test-only control group design. The electric field strengths applied were 30 Vpp and 50 Vpp, with durations of 24 hours, 48 hours, and 72 hours corresponding to the treatments used in ECCT studies. This study utilized female Rattus norvegicus rats, aged 4-6 weeks, weighing 120-150g.
Results: The results of the analysis showed that the group treated with low intensity and medium frequency electric fields using 30 Vpp with a frequency of 100 KHz for a duration of 72 hours produced a higher ratio of neutrophils to lymphocytes.
Conclusion: There is an effect of low intensity and medium frequency electric fields on the ratio of neutrophils to lymphocytes in the blood in rat brain tissue in vivo from a safety aspect.

Keywords: ECCT, Electric Field, Neutrophil, Lymphocyte, NLR

Background

Cancer is estimated to cause 1 in 6 deaths globally according to the WHO (World Health Organization, 2018). As many as 70% of deaths caused by cancer come from developing countries. Indonesia reported that cancer patients were estimated to be 1.8 per 1000 population in 2018, while in 2013 it was reported to be 1.3 per 1000 population (Riskesdas, 2018). The most common primary brain tumor in the adult population is glioblastoma, which accounts for 61.5% of all glioma cases in the United States. Glioblastoma is the brain tumor with the worst prognosis (Ostrom *et al.*, 2018).

Gliomas are tumors that originate from glia cells and their precursor lines. Based on its extensive growth pattern and infiltrative to the surrounding central nervous system parenchyma, it is called diffuse glioma. While non diffuse glioma with a regular growth pattern resulting in a more regular tumor shape. Diffuse gliomas are divided into astrocytic, oligodendroglial, or mixed. Gliomas with round nuclei are classified as oligodendrogliomas, while tumors with irregular or hyperchromatic nuclei are classified as astrocytomas. (D. N. Louis *et al.*, 2016).

The prognostic role of neutrophil-to-lymphocyte ratio (NLR) in brain tumor patients with glioma was found that increased NLR was associated with worse overall survival in glioma patients. The prognosis factor against brain tumors is proved that the presence of chronic inflammation or inflammatory status is closely related to brain tumor pathogenesis and tumor progression. Neutrophil-to-lymphocyte ratio (NLR) is a parameter calculating the ratio of peripheral blood neutrophils and lymphocytes. In recent years, several studies observing the prognostic role of NLR in brain tumors proved that higher NLR predicts worse outcomes in brain tumors (Zhang *et al.*, 2017).

Tumor Treating Fields (TTF) is another cancer therapy using an electric field device, this device has been used in the United States for brain tumor cases with glioblastoma. Treatment with TTF has been shown to provide significant clinical benefits for GBM patients. It uses an electric field with a voltage of 100 - 300 kHz and a frequency < 2 V/cm, which can penetrate rapidly dividing cancer cells and disrupt essential processes and cellular structures leading to apoptotic cell death (Davies, Weinberg and Palti, 2013). It is used by attaching electrodes to the scalp, whereas the ECCT product description does not mention that such a procedure needs to be done for this device (Branter, Basu and Palti, 2013; Branter, Basu and Smith, 2018).

This study aims to analyze the effect of ECCT administration in the form of low-intensity and intermediate-frequency electric fields on the ratio of neutrophil lymphocytes in the blood of normal rat brain tissue in *vivo* from a safety aspect so that it can provide benefits and new knowledge.

Methods

The type of research used in this study is true experimental with post-test only control group design. This study was conducted to determine the effect of low-intensity and intermediate-frequency electric fields on the ratio of neutrophil lymphocytes *in* the blood in normal rat brain tissue in *vivo* from a safety aspect. The strength of the electric field given was 30 Vpp and 50 Vpp with a duration of 24 hours, 48 hours, and 72 hours in accordance with the treatment in the study using ECCT. This study used female *Rattus norvegicus* rats, aged 4-6 weeks weighing 120-150g which were declared healthy by the consultant veterinarian.

Grouping of replicates into test groups was done by simple randomization using a lottery. Each group was color-coded and each sample received a numbering code.

The independent variable in this study is a low intensity electric field of 30 and 50 VPP with an intermediate frequency of 100 kHz. The dependent variable in this study is Inflammation in the brain tissue of *Rattus norvegicus* rats assessed based on the number of neutrophils in the blood after the influence of electric fields with low intensity and intermediate frequency. Inflammation in the brain tissue of *Rattus norvegicus* rats assessed based on the number of lymphocytes in the blood after the influence of electric fields with low intensity and intermediate frequency. Inflammation in the brain tissue of *Rattus norvegicus* rats as assessed by the ratio of neutrophil lymphocytes in the blood after the influence of electric fields with low intensity and intermediate frequency.

The electric field procedure was conducted using a cage designed by CTech Labs. This cage was electrified with voltages of 30 Vpp and 50 Vpp using an oscillator. The electric field was applied according to the treatment with a duration of 24 hours, 48 hours, and 72 hours.

Mice before dissection using intraperitoneal injection of ketamine (75 mg/kg) with xylazine (10 mg/kg) had their blood taken intracardiac using a 3 cc syringe by an expert veterinarian. Samples were stored in EDTA blood tubes for further analysis based on the number of neutrophils, lymphocytes and neutrophil-lymphocyte ratio for further analysis.

Data were collected using data collection sheets (DFS). Data were presented in the form of tabulations, graphs, diagrams, text, or writing. Data were processed using a computer with the statistical program SPSS 20 edition and analyzed statistically with one-way ANOVA to compare more than 2 groups.

The research was conducted at Stem Cell Laboratory of Universitas Airlangga and Clinical Pathology Laboratory of Faculty of Veterinary Medicine Universitas Airlangga. This research was conducted after obtaining ethical approval from the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga.

Results

Descriptive data on the mean lymphocyte levels in the control and ECCT treatment groups can be seen in table 1. The highest lymphocyte levels were in the control group (67.00 ± 3.571), and the lowest in the 50 Vpp treatment group (63.64 ± 3.219). Lymphocyte levels at 24 hours, 48 hours, and 72 hours were the lowest in the 50 Vpp, 50 Vpp, and 50 Vpp treatment groups, respectively. The lowest cumulative lymphocyte levels were in the 50 Vpp treatment group.

Table 1 Lymphocyte levels on control and ECCT exposure group

	Mean 24 hours	**Mean 48 Hours**	**Mean 72 hours**	**Total Mean**
Control	67,00 ± 3,571	67,00 ± 3,571	67,00 ± 3,571	67,00 ± 3,571
30 Vpp	64,22 ± 3,346	63,50 ± 2,777	64,29 ± 3,353	64,29 ± 3,353
50 Vpp	62,89 ± 1,167	62,50 ± 2,507	61,57 ± 2,149	63,64 ± 3,219

Descriptive data on the mean neutrophil levels in the control and ECCT treatment groups can be seen in Table 2. The highest neutrophil level was in the 50 Vpp treatment group (28.94 ± 3.181), and the lowest in the control group (25.22 ± 3.456). Neutrophil levels at 24 hours, 48 hours, and 72 hours were highest in the 50 Vpp, 50 Vpp, and 30 Vpp treatment groups, respectively. Cumulative neutrophil levels were highest in the 50 Vpp treatment group.

Table 2 Neutrophil levels on control and ECCT exposure group

	Mean 24 hours	**Mean 48 Hours**	**Mean 72 hours**	**Total Mean**
Control	25,22 ± 3,456	25,22 ± 3,456	25,22 ± 3,456	25,22 ± 3,456
30 Vpp	27,89 ± 3,371	29,13 ± 2,588	31,50 ± 1,604	28,32 ± 3,591

50 Vpp	30,89 ± 1,764	29,38 ± 1,188	30,71 ± 1,380	28,94 ± 3,181

Descriptive data on the mean NLR levels that resemble the trend of mean neutrophil levels can be seen in table 5.3. The highest NLR level was in the 50 Vpp treatment group (0.439 ± 0.0788), and the lowest in the control group (0.356 ± 0.0726). NLR levels at 24 hours, 48 hours, and 72 hours were highest in the 50 Vpp, 50 Vpp, and 30 Vpp treatment groups, respectively. The highest cumulative NLR level was in the 50 Vpp treatment group.

Table 3 NLR levels on control and ECCT exposure group

	Mean 24 hours	**Mean 48 Hours**	**Mean 72 hours**	**Total Mean**
Control	0,356 ± 0,0726	0,356 ± 0,0726	0,356 ± 0,0726	0,356 ± 0,0726
30 Vpp	0,422 ± 0,0667	0,425 ± 0,0707	0,500 ± 0,0535	0,424 ± 0,0819
50 Vpp	0,489 ± 0,0601	0,438 ± 0,0518	0,486 ± 0,0378	0,439 ± 0,0788

Lymphocyte levels at 24 hours of ECCT exposure

Normality test showed that the data distribution of lymphocyte levels on ECCT exposure for 24 hours was normal ($p>0.05$). Analysis with One-Way Anova showed a value of $p=0.019$ which means there is a significant difference between groups ($p<0.05$). *Post-hoc* analysis showed significant findings in the comparison between the control group and 50 Vpp with $p=0.016$.

Table 4 Comparison of lymphocyte levels in ECCT exposure for 24 hours

Group	**Comparison**	**p**
Control	30 Vpp	0,127
Control	50 Vpp	0,016*
30 Vpp	50 Vpp	0,600

*p<0,05

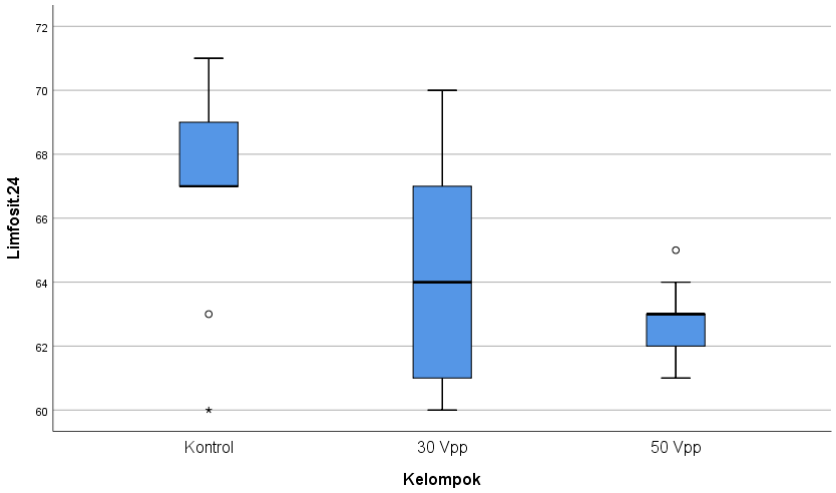

Figure 1 Comparison of lymphocytes with 24-hour exposure of control group with 30 Vpp and 50 Vpp

Lymphocyte levels at 48 hours of ECCT exposure

Normality test showed that the data distribution of lymphocyte levels on ECCT exposure for 48 hours was normal (p>0.05). Analysis with One-Way Anova showed a value of p=0.013 which means there is a significant difference between groups (p<0.05). *Post hoc* analysis showed significant findings in the comparison between the control group and 50 Vpp with p=0.015.

Table 5 Comparison of lymphocyte levels at 48 hours of ECCT exposure

Group	Comparison	p
Control	30 Vpp	0,064
Control	50 Vpp	0,015*
30 Vpp	50 Vpp	0,787

*p<0,05

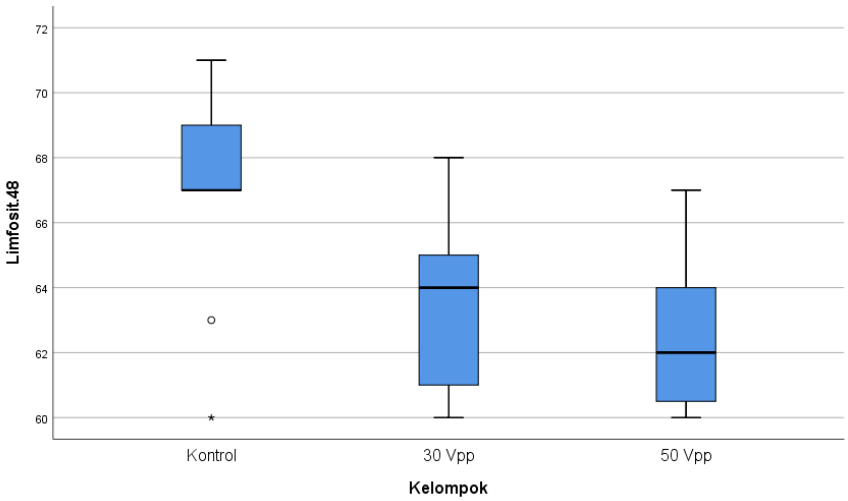

Figure 2 Comparison of lymphocytes with 48 hours exposure of control group with 30 Vpp and 50 Vpp

Lymphocyte levels at 72 hours of ECCT exposure

Normality test showed that the data distribution of lymphocyte levels in ECCT exposure for 72 hours was abnormal ($p<0.05$). *Kruskal-Wallis* analysis showed $p=0.006$, which means there is a significant difference between groups ($p<0.05$). *Post-hoc* analysis showed significant findings in the comparison between the control group and 30 Vpp and 50 Vpp with p values of 0.010 and 0.007 respectively.

Table 6. Comparison of lymphocyte levels in ECCT exposure for 72 hours.

Group	Comparison	p
Control	30 Vpp	0,010*
Control	50 Vpp	0,007*
30 Vpp	50 Vpp	0,314

*p<0,05

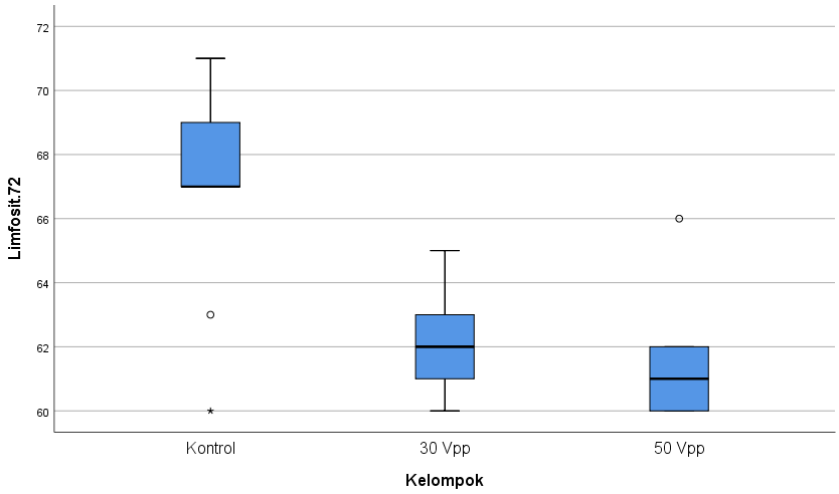

Figure 3 Comparison of lymphocytes with 72 hours exposure of control group with 30 Vpp and 50 Vpp

Neutrophil levels at 24 hours of ECCT exposure

Normality test showed that the data distribution of neutrophil levels on ECCT exposure for 24 hours was abnormal ($p<0.05$). *Kruskal-Wallis* analysis showed $p=0.006$ which means there is a significant difference between groups ($p<0.05$). *Post-hoc* analysis showed significant findings in the comparison between the control group and 50 Vpp with $p=0.002$.

Table 7 Comparison of neutrophil levels at 24 hours of ECCT exposure.

Group	Comparison	p
Control	30 Vpp	0,110
Control	50 Vpp	0,002*
30 Vpp	50 Vpp	0,054

*p<0,05

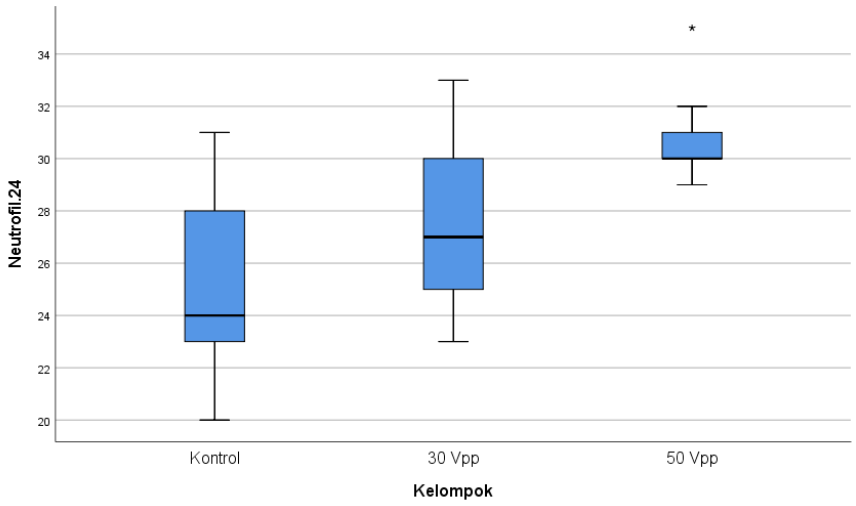

Figure 4 Comparison of neutrophils with 24-hour exposure of control group with 30 Vpp and 50 Vpp

Neutrophil levels at 48 hours of ECCT exposure

Normality test showed that the data distribution of neutrophil levels on ECCT exposure for 48 hours was normal (p>0.05). Analysis with One-Way Anova showed a value of p=0.005 which means there is a significant difference between groups (p<0.05). *Post-hoc* analysis showed significant findings in the comparison between the control group and 30 Vpp and 50 Vpp with p values of 0.015 and 0.010 respectively.

Table 8 Comparison of neutrophil levels at 48 hours of ECCT exposure.

Group	Comparison	p
Control	30 Vpp	0,015*
Control	50 Vpp	0,010*
30 Vpp	50 Vpp	0,980

*p<0,05

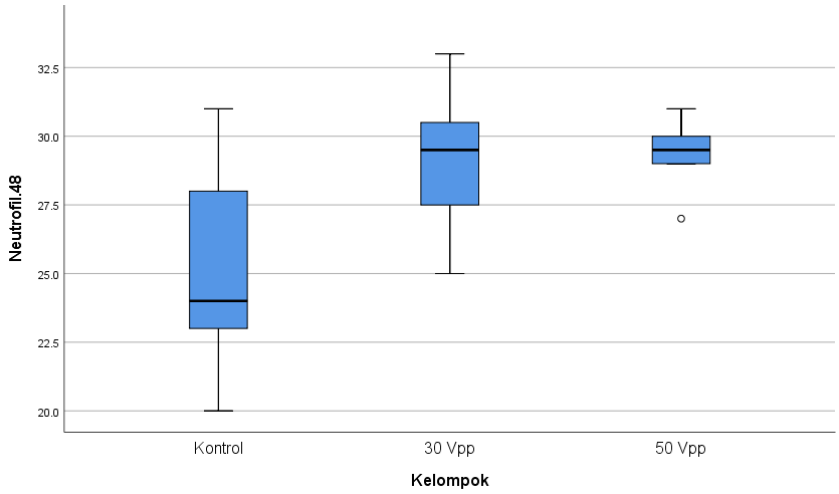

Figure 5 Comparison of neutrophils with 48 hours exposure of control group with 30 Vpp and 50 Vpp

Neutrophil levels at 72 hours of ECCT exposure

Normality test showed that the data distribution of neutrophil levels on ECCT exposure for 72 hours was abnormal ($p<0.05$). *Kruskal-Wallis* analysis showed $p=0.001$, which means there was a significant difference between groups ($p<0.05$). *Post-hoc* analysis showed significant findings in the comparison between the control group and 30 Vpp and 50 Vpp with p values of 0.002 and 0.005 respectively.

Table 9 Comparison of neutrophil levels at 72 hours of ECCT exposure.

Group	Comparison	p
Control	30 Vpp	0,002*
Control	50 Vpp	0,005*
30 Vpp	50 Vpp	0,310

*p<0,05

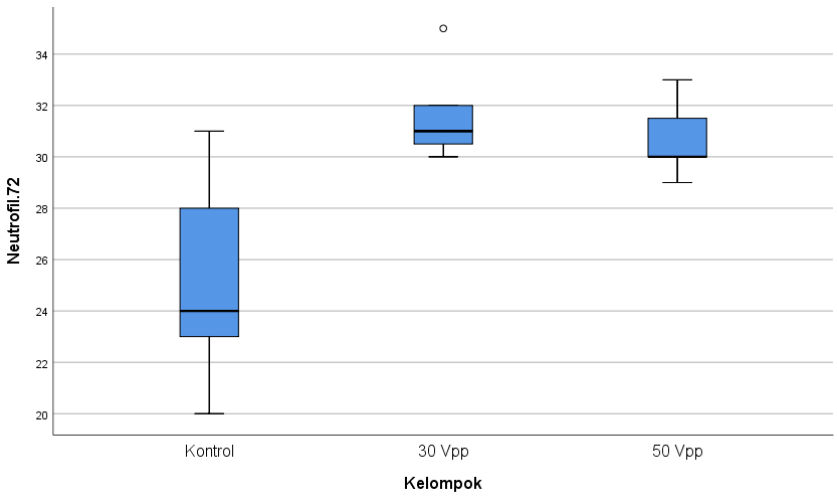

Figure 6 Comparison of neutrophils with 72 hours exposure of control group with 30 Vpp and 50 Vpp

NLR levels at 24 hours of ECCT exposure

Normality test showed that the data distribution of NLR levels in ECCT exposure for 24 hours was abnormal (p<0.05). *Kruskal-Wallis* analysis showed p=0.004, which means there is a significant difference between groups (p<0.05). *Post-hoc* analysis showed significant findings in the comparison between the control group and 50 Vpp with a p value of 0.002 and the 30 Vpp group and 50 Vpp with a p value of 0.045.

Table 10 Comparison of NLR levels at 24 hours of ECCT exposure.

Group	Comparison	p
Control	30 Vpp	0,058
Control	50 Vpp	0,002*
30 Vpp	50 Vpp	0,045*

*p<0,05

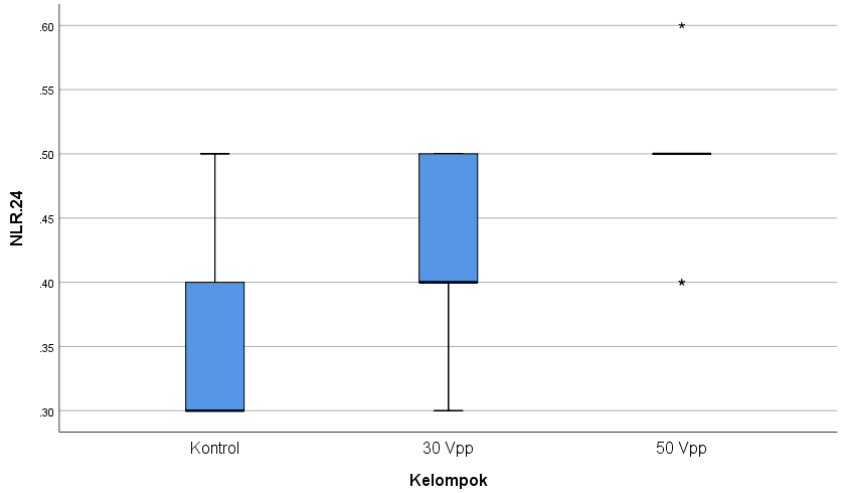

Figure 7 Comparison of NLR with 24-hour exposure of control group with 30 Vpp and 50 Vpp

NLR levels at 48 hours of ECCT exposure

Normality test showed that the data distribution of NLR levels in ECCT exposure for 48 hours was abnormal (p<0.05). *Kruskal-Wallis* analysis showed p=0.047 which means there is a significant difference between groups (p<0.05). *Post hoc* analysis showed significant findings in the comparison between the control group and 50 Vpp with p=0.022.

Table 11 Comparison of NLR levels in ECCT exposure for 48 hours.

Group	Comparison	p
Control	30 Vpp	0,064
Control	50 Vpp	0,022*
30 Vpp	50 Vpp	0,765

*p<0,05

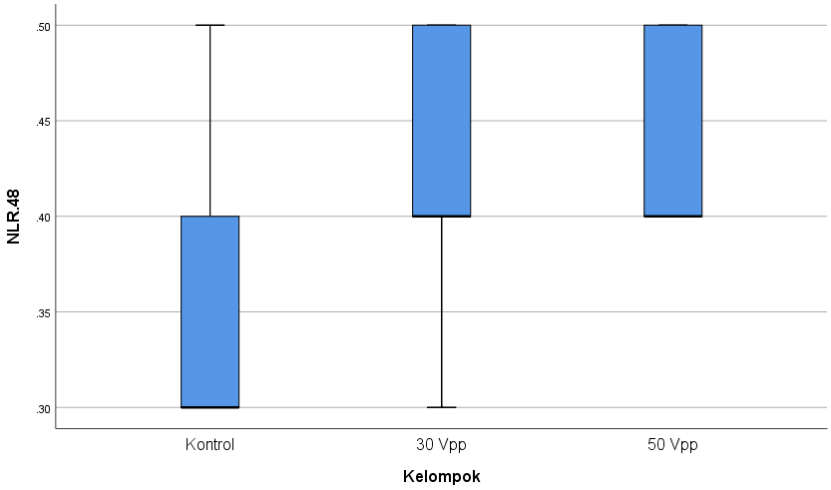

Figure 8 Comparison of NLR with 48 hours exposure of control group with 30 Vpp and 50 Vpp

NLR levels at 72 hours of ECCT exposure

Normality test showed that the data distribution of NLR levels in ECCT exposure for 72 hours was abnormal ($p<0.05$). *Kruskal-Wallis* analysis showed $p=0.001$, which means there is a significant difference between groups ($p<0.05$). *Post hoc* analysis showed significant findings in the comparison between the control group and 30 Vpp and 50 Vpp with p values of 0.002 and 0.003 respectively.

Table 12 Comparison of NLR levels in ECCT exposure for 72 hours.

Group	Comparison	p
Control	30 Vpp	0,002*
Control	50 Vpp	0,003*
30 Vpp	50 Vpp	0,562

*p<0,05

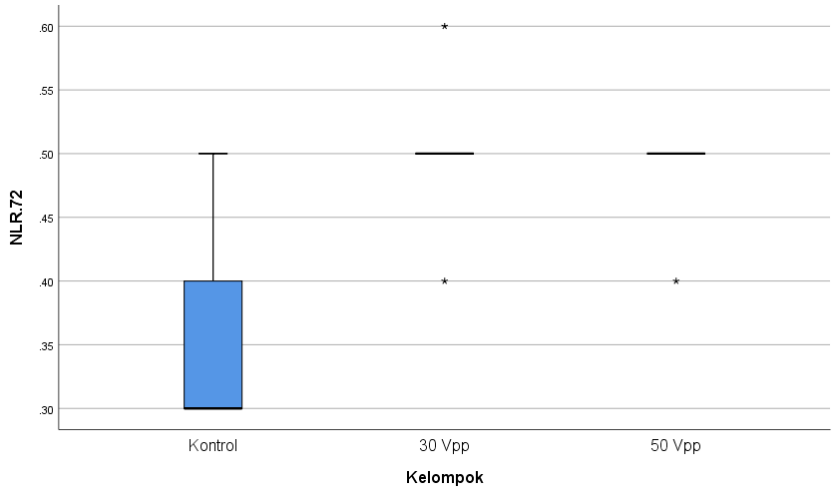

Figure 9 Comparison of NLR with 72-hour exposure of control group with 30 Vpp and 50 Vpp

Lymphocyte levels at 30 Vpp exposure

Normality test showed that the data distribution of lymphocyte levels at 30 Vpp ECCT exposure was normal (p>0.05). Analysis with ANOVA showed a value of p=0.014 which means there is a significant difference between groups (p<0.05). *Post-hoc* analysis showed significant findings in the comparison between the control group and the 72-hour exposure with a p value of 0.010.

Table 13 Comparison of lymphocyte levels at 30 Vpp exposure

Group	Comparison	p
Control	24 Hours	0,213
Control	48 Hours	0,092
Control	72 Hours	0,010*

*p<0,05

Lymphocyte levels at 50 Vpp exposure

Normality test showed that the data distribution of lymphocyte levels in 50 Vpp ECCT exposure was normal (p>0.05). Analysis with *Kruskal-Wallis* test showed a value of p=0.008 which means there is a significant difference between groups (p<0.05). *Post-hoc* analysis showed significant findings in the comparison between the control group and 24-hour, 48-hour, and 72-hour exposure with p values of 0.014, 0.017 and 0.007, respectively.

Table 14 Comparison of lymphocyte levels at 50 Vpp exposure

Group	Comparison	p
Control	24 Hours	0,014*
Control	48 Hours	0,017*
Control	72 Hours	0,007*

*p<0,05

Neutrophil levels at 30 Vpp exposure

Normality test showed that the data distribution of neutrophil levels at 30 Vpp ECCT exposure was normal (p>0.05). Analysis with *Kruskal-Wallis* test showed a value of p=0.003 which means there is a significant difference between groups (p<0.05). *Post-hoc* analysis showed significant findings in the comparison between the control group and 48 and 72 hours exposure with p values of 0.020 and 0.002, respectively.

Table 15 Comparison of neutrophil levels at 30 Vpp exposure

Group	Comparison	p
Control	24 Hours	0,110
Control	48 Hours	0,020*
Control	72 Hours	0,002*

*p<0,05

Neutrophil levels at 50 Vpp exposure

Normality test showed that the data distribution of neutrophil levels in 50 Vpp ECCT exposure was normal (p>0.05). Analysis with *Kruskal-Wallis* test showed a value of p=0.002 which means there is a significant difference between groups (p<0.05). *Post-hoc* analysis showed significant findings in the comparison between the control group and 24-hour, 48-hour, and 72-hour exposure with p values of 0.002, 0.015 and 0.005, respectively.

Table 16 Comparison of neutrophil levels at 50 Vpp exposure

Group	Comparison	p
Control	24 Hours	0,002*
Control	48 Hours	0,015*
Control	72 Hours	0,005*

*p<0,05

NLR levels at 30 Vpp exposure

Normality test showed that the data distribution of NLR levels at 30 Vpp ECCT exposure was normal (p>0.05). Analysis with *Kruskal-Wallis* test showed a value of p=0.005 which means there is a significant difference between groups (p<0.05). *Post hoc* analysis showed significant findings in the comparison between the control group and the 72-hour exposure with a p value of 0.002.

Table 17 Comparison of NLR levels at 30 Vpp exposure

Group	Comparison	p
Control	24 Hours	0,058
Control	48 Hours	0,064
Control	72 Hours	0,002*

*p<0,05

NLR levels at 50 Vpp exposure

Normality test showed that the data distribution of NLR levels at 50 Vpp ECCT exposure was abnormal (p<0.05). Analysis with *Kruskal-Wallis* test showed a value of p=0.002 which means there is a significant difference between groups (p<0.05). *Post-hoc* analysis showed significant findings in the comparison between the control group and 24-hour, 48-hour, and 72-hour exposure with p values of 0.002, 0.022 and 0.003, respectively.

Table 18 Comparison of NLR levels at 50 Vpp exposure.

Group	Comparison	p
Control	24 Hours	0,002*
Control	48 Hours	0,022*

Control	72 Hours	0,003*

*p<0,05

Discussion

Eletro Capacitive Cancer Treatment (ECCT) is a cancer treatment method using a source of low-intensity and low-frequency (frequency <100 KHz and intensity <30 Vpp) electrostatic waves that produce electrical polarization in a near-field region bounded by a number of capacitive electrodes. This technology was developed by Warsito and the team at CTech Labs Edwar Technology Company to cure cancer and is claimed to kill brain cancer cells. (C-Tech Labs, 2015). The modality of this ECCT therapy tool for brain cancer therapy has an optimal configuration for therapy, capable of generating an electric field with a wider area used for cancers positioned in most brain tissues (Andiani *et al.*, 2017). Electric field therapy exerts various effects on cell processes and disrupts mitosis, the carefully regulated process of cell division, which is more common in cancerous than healthy tissue. This does not affect normal cells and only affects cells that divide more frequently than normal cells, i.e. cancer cells (Carlson *et al.*, 2021).

Exposure to low-intensity and intermediate-frequency electric fields in normal tissues disrupted microtubule function can reorganize so as not to cause cells to be in a state of mitotic arrest and does not affect normal cells, this does not cause apoptosis. *Endoplasmic reticulum* stress (*ER* Stress) can reorganize so as not to activate the unfolded protein response (UPR) complex and immunogenic cell death, this does not cause apoptosis and necrosis. This process therefore does not cause inflammation.

The number of circulating white blood cells increases in the inflammatory process, the physiological response in the immune system to inflammation in the form of an increase in the number of neutrophils and a decrease in the number of lymphocytes causes an increase in the neutrophil *lymphocyte* ratio. In normal tissues, the *neutrophil* to *lymphocyte* ratio (NLR) is a simple parameter to easily assess the inflammatory status of the subject. NLR is an inflammatory biomarker that can be used as an indicator of systemic inflammation (Forget *et al.*, 2017). NLR plays a role in handling inflammation and immunity. NLR is superior to other leukocyte parameters. The prognostic value of NLR in peripheral blood, a marker of systemic inflammatory response, identified higher neutrophil markers and lower T-cell infiltration. Several studies analyzing the influence of NLR showed a significant correlation in univariate analysis between NLR >4 and worse median overall survival and in multivariate analysis NLR >4 remained an independent prognostic indicator for poor outcome (Lopes *et al.*, 2018).

This study analyzes the effect of the safety aspects of the effect of electric fields with low intensity and intermediate frequency using alternating current (*AC*) with voltages of 30 and 50 Vpp with a frequency of 100 KHz given with a cage-shaped device designed by CTech Labs with a duration of 24 hours, 48 hours, and 72 hours on female Wistar rats (*Rattus norvegicus*) assessed based on the number of neutrophils, lymphocytes, and NLR.

This study obtained the highest mean lymphocyte levels in the control group (67.00 ± 3.571) and the lowest in the 50 Vpp treatment group (63.64 ± 3.219). Mean lymphocyte levels at 24 hours, 48 hours, and 72 hours were lowest in the 50 Vpp treatment group (62.89 ± 1.167), 50 Vpp (62.50 ± 2.507), and 50 Vpp (61.57 ± 2.149), respectively. Analysis with *Kruskal-Wallis Test* showed a value of p=0.008 which means there is a significant difference between groups (p<0.05). *Post-hoc* analysis showed significant findings in the comparison between the control group and exposure to 50 Vpp for 24 hours, 48 hours, and 72 hours with p values of 0.014, 0.017 and 0.007, respectively.

The low lymphocytes in the group that received higher intensity exposure showed inflammatory activity caused by the lymphocyte complex. The study by Alamsyah *et al*. where

the group that received ECCT with low intensity had minimal tumor growth due to the inflammatory response caused by lymphocytes (Alamsyah *et al*., 2019). The presence of an electric exposure field can reduce the level of lymphocytes. The findings in this study are in line with a study conducted by Leal *et al*. where in patients with non-small cell lung cancer, there was a leukocyte reduction effect in 14%, which is categorized as a common therapeutic effect found in ECCT (Leal *et al*., 2019).

Alamsyah *et al.* found that the growth rate of breast tumors in experimental mice was significantly lower in the therapy group compared to the non-therapy group. The therapy group had a negative mean value of the specific growth rate, indicating that the non-contact electric field inhibited the growth of breast tumor cells that were actively dividing in the animals (Alamsyah *et al*., 2019). The decrease in lymphocytes which is inversely proportional to the intensity of ECCT illustrates the inflammatory process surrounding tumor cells (Alamsyah *et al*., 2021).

The study conducted by Setyaji *et al*. found that the group treated with ECCT saw a decrease in the CD4+/CD8+ ratio, which is a significant measure of immunological response. The decrease in CD4+/CD8+ ratio implies that ECCT can increase the cytotoxicity of CD8+ T lymphocytes, which play an important role in inducing tumor cell apoptosis. ECCT electric field therapy triggers anti-toxic mechanisms played by T *helper* and cytotoxic cells from lymphocytes, as well as macrophages (Gonzalez *et al*., 2018).

Non-Contact Electric Field has been found to prevent breast tumor growth in mice. This exposure also stimulates the production of CD8+ T lymphocytes, which can lead to tumor cell death and potentially aid in wound healing. Alamsyah *et al.* used an alternative intermediate frequency of 100 kHz to investigate the impact of non-contact electric field exposure on breast cancer formation in mice and noticed the stimulation of immune cells in fighting tumor cells. Non-contact electric field induced lymphocyte infiltration in the intervention group resulting in phagocytosis of dead cells and debris (Alamsyah et al., 2021).

This study found that the highest mean neutrophil levels were in the 50 Vpp treatment group (28.94 ± 3.181) and the lowest in the control group (25.22 ± 3.456). The mean neutrophil levels at 24 hours, 48 hours, and 72 hours were highest in the 50 Vpp treatment group (30.89 ± 1.764), 50 Vpp (29.38 ± 1.188), and 30 Vpp (31.50 ± 1.604), respectively. Analysis with *Kruskal-Wallis Test* showed a value of p=0.002 which means there is a significant difference between groups ($p<0.05$). *Post-hoc* analysis showed significant findings in the comparison between the control group and the exposure of 50 Vpp for a duration of 24 hours with a p value of 0.002. *Post-hoc* analysis showed significant findings in the comparison between the control group and 30 Vpp with a time duration of 72 hours with a p value of 0.002.

A general increase in neutrophil levels after being treated with ECCT indicates the potential to enhance the body's immune response and strengthen the body's ability to fight cancer (Setyaji *et al*., 2020, Pratiwi *et al*., 2021). Research by Setyaji *et al.* has shown that exposure to ECCT can significantly increase the concentration of neutrophils in the bloodstream. The study conducted on healthy individuals found that ECCT therapy led to an increase in the number of neutrophils compared to the control group (Setyaji *et al*., 2020). The increase in neutrophils may be attributed to the ability of ECCT to enhance immunological responses through stimulation of cytokines and chemokines, which attract neutrophils to sites of inflammation and in other findings may decrease the concentration of pro-inflammatory cytokines such as IL-18 that can inhibit neutrophil function (Pratiwi *et al*., 2021).

Studies suggest that ECCT has the ability to regulate inflammatory responses by specifically targeting cellular processes that control neutrophil production and activation. A research article published in the International Journal of Molecular Sciences emphasized that ECCT has the potential to reduce the production of pro-inflammatory cytokines may reduce neutrophil migration to the tumor side (Buonacera *et al*., 2019).

This study found that the highest mean NLR levels were in the 50 Vpp treatment group (0.439 ± 0.0788), and the lowest in the control group (0.356 ± 0.0726). Mean NLR levels at 24 hours, 48 hours, and 72 hours were cumulatively highest in the 50 Vpp (0.489 ± 0.0601), 50 Vpp (0.438 ± 0.0518), and 30 Vpp (0.500 ± 0.0535) treatment groups, respectively. *Post-hoc* analysis showed significant findings in the comparison between the control group and the exposure to 50 Vpp for a duration of 24 hours with a p value of 0.002. *Post-hoc* analysis showed significant findings in the comparison between the control group and the exposure to 30 Vpp with a time duration of 72 hours with a p value of 0.002.

The NLR of 3.0 is the most widely used cut-off value. The NLR in both the control and intervention groups was still below the cut-off value, indicating that ECCT administration at 30 Vpp and 50 Vpp was still not toxic when compared to the control group. An increase in NLR above the cut-off was found to be a separate predictive factor for worse overall survival (Taguchi et al., 2015). Zheng et al. demonstrated a statistically significant decrease in neutrophil count among patients receiving neoadjuvant treatment. Increased NLR was an independent prognostic factor for reduced overall survival according to multivariate analysis (Pirozzolo *et al*., 2013; Zheng *et al*., 2024).

Electro Capacitive Cancer Treatment (ECCT) has a major impact on NLR, which is a measure of the body's inflammatory response and immunological function in individuals with cancer. Increased NLR is often associated with poorer prognosis and decreased overall survival in many types of cancer. According to a study conducted by Diakos *et al*. better survival rate after whole-brain radiation therapy for brain metastases from non-small cell lung cancer was substantially predicted by an NLR of less than 5.0 (Diakos i, 2014).

Research conducted by Corbeau *et al*. showed that ECCT can reduce the number of neutrophils, increase the number of lymphocytes, and result in a decrease in NLR. Systematic studies have shown that NLR can be used as a predictive indicator in breast cancer having the potential to improve treatment effectiveness (Corbeau *et al*., 2019).

Conclusion

There is an effect of low intensity and medium frequency electric fields on the ratio of neutrophils to lymphocytes in the blood of rat brain tissue in vivo from a safety aspect.

Reference

1. Andiani, L., Endarko, Al Huda, M., & Taruno, W. P. (2017). A novel method for analyzing electric field distribution of electro capacitive cancer treatment (ECCT) using wire mesh electrodes: A case study of brain cancer therapy. *EuroMediterranean Biomedical Journal*, *12*(38), 178–183. https://doi.org/10.3269/1970-5492.2017.12.38
2. Branter, J., Basu, S., & Smith, S. (2018). Tumour treating fields in a combinational therapeutic approach. In *Oncotarget* (Vol. 9, Issue 93, pp. 36631–36644). https://doi.org/10.18632/oncotarget.26344
3. Carlson, K. W., Tuszynski, J. A., Dokos, S., Paudel, N., Dreeben, T., & Bomzon, Z. (2021). How Do Tumor-Treating Fields Work? *Brain and Human Body Modeling 2020*, 19–35. https://doi.org/10.1007/978-3-030-45623-8_2
4. C-Tech Labs. (2015). Eletro Capacitive Cancer Treatment (ECCT). *C-Tech Labs Edwar Technology*.
5. Davies, A. M., Weinberg, U., & Palti, Y. (2013). Tumor treating fields: A new frontier in cancer therapy. *Annals of the New York Academy of Sciences*, *1291*(1), 86–95. https://doi.org/10.1111/nyas.12112
6. Forget, P., Khalifa, C., Defour, J. P., Latinne, D., Van Pel, M. C., & De Kock, M. (2017). What is the normal value of the neutrophil-to-lymphocyte ratio? *BMC Research Notes*, *10*(1), 1–4. https://doi.org/10.1186/s13104-016-2335-5

7. Lopes, M., Carvalho, B., Vaz, R., & Linhares, P. (2018). Influence of neutrophil–lymphocyte ratio in prognosis of glioblastoma multiforme. *Journal of Neuro-Oncology*, *136*(1), 173–180. https://doi.org/10.1007/s11060-017-2641-3
8. Louis, D. N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., Ohgaki, H., Wiestler, O. D., Kleihues, P., & Ellison, D. W. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: A summary. *Acta Neuropathologica*, *131*(6), 803–820. https://doi.org/10.1007/s00401-016-1545-1
9. Ostrom, Q. T., Cote, D. J., Ascha, M., Kruchko, C., & Barnholtz-Sloan, J. S. (2018). Adult glioma incidence and survival by race or ethnicity in the United States from 2000 to 2014. *JAMA Oncology*, *4*(9), 1254–1262.
10. Riskesdas. (2018). *Hasil Utama Riskesdas 2018*.
11. World Health Organisation. (2018). Latest global cancer data. *International Agency for Research on Cancer*, *September*, 13–15.
12. Zhang, J., Zhang, S., Song, Y., He, M., Ren, Q., Chen, C., Liu, Z., Zeng, Y., & Xu, J. (2017). Prognostic role of neutrophil lymphocyte ratio in patients with glioma. *Oncotarget*, *8*(35), 59217.