Prognostic significance of CD4/CD8 ratio in patients with advanced cervical cancer

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Abstract. Cervical cancer is caused by HPV infection, which types mainly 16, 18, 31, and 45. CD4+ T cells are the most important cells in viral clearance. CD4+ and CD8+ are involved in the apoptotic process and the CD4/CD8 ratio was assessed in response to the treatment of NAC in advanced cervical cancer. We conducted a case-control study with 30 patients of advanced cervical cancer and 30 patients as the control. Samples were taken from the vein and processed using hemocytometry. Samples were checked three times for cases when the stage of cervical cancer was enforced, before patients underwent 2nd and 3rd series of NAC. The majority of controls were 39.73 years and for the cases were 48.3 years. Patients with CD4/CD8 ratio ≥ 1.2069 had 1.5 times higher risk to cervical cancer than patients with CD4/CD8 ratio < 1.2069, but it was insignificant (OR = 1.495, 95% CI = 0.540-4.136, p = 0.605). Before therapy, they had lower CD4 and CD8 value compared to control. The higher the CD4/CD8 ratio before the 3rd series of NAC was, the higher the risk of having no response towards NAC treatment in advanced cervical cancer patients. Moreover, most of our patients had either complete or partial response.

1. Introduction
HPV infection can cause cervical cancer. HPV 16 is the most common HPV type in cervical cancer, together with HPV 18, 31 and 45 which accounts for around 80% of all cervical cancer. Genital HPV infection is common for around 75% of all sexually active individuals infected at least once during their lifetime. However, only a minority of HPV-infected women developed cervical cancer. As a result of an adequate cellular immune response, most infections are cleared within 12 months. T cells, in particular, CD4+ T cells were the most important cells involved in viral clearance [1].

Inflammatory cells, particularly lymphocytes surrounded the tumor cells. The cells of the adaptive immune system perform surveillance and can eliminate the nascent tumor. T and B lymphocytes, as immune cells, play an important role in the immunological surveillance and can help in the elimination of tumor cells. The higher levels of particular lymphocyte subpopulations in the circulating blood were correlated to the tumor development and poor prognosis in breast cancer patients. However, the influence of the CD4+/CD8+ ratio on the outcome in patients with cancer was still unclear [2].

The treatment modality for cervical cancer was chosen based on disease status at the time of diagnosis. The treatment of primary concurrent chemoradiation is effective for patients with advanced stage (IIB to IVA) according to International Federation of Gynecology and Obstetrics (FIGO). In the developing country, like Indonesia where the modality of radiotherapy in cervical cancer is very limited with a fairly high case, therapy with neoadjuvant chemotherapy becomes an option in
advanced cases such as IIB-IIIB, prior to surgery. However, its efficacy has been investigated over the past two decades [3].

To assess the therapeutic response of cervical cancer towards NAC is by measuring cervical tumor size change by either clinical examination or measurement modalities such as ultrasound, CT scan or MRI. The change of cervical tumors size with NAC in accordance with the mechanism of action with chemotherapy drugs is the death of tumor cells (apoptosis). The process of apoptosis involves many immunological processes.

Several pieces of evidence indicated that impaired cell-mediated immune responses were important for both HPV infections and HPV associated neoplasm. Human Leukocyte Antigen (HLA) comprises a family of highly polymorphic genes encoding a set of transmembrane proteins that present peptide epitopes to specific antigen receptors on T cells. HLA’s expression may prevent tumor invasion and progression to the invasive stage and also participate in the local immune response. The down-regulation of HLA class I expression can be found in the development of several tumors, such as malignant melanoma, carcinoma of breast, cervix, and lungs, and Burkitt’s lymphoma. In addition, the upregulation of HLA class II expression can be found in certain cancer, such as breast and cervical cancer [4].

The CD4+ T cells may activate B cells to produce an antibody that could destruct a pathogen and assist in the maturation of CD8+ T cells. These polymorphisms are mainly clustered in the antigen-binding groove and thereby affect the peptide-binding specificity of HLA molecule. Therefore, polymorphisms in HLA complex partially account for interindividual variation in disease susceptibility or progression rate for long-term viral infections such as HIV, human T-cell lymphotropic virus type I, hepatitis B and C and also HPV. Many studies have indicated specific HLA class II alleles as candidates for involvement in the onset of CIN or invasive cervical cancer. However, some studies show no association [4].

The nature, function, and specificity of the effector cells that drive the antitumoral immune response have been widely studied. Dendritic cells (DCs), macrophages, natural killer (NK), NK/T cells, neutrophils, cytokines, and complement proteins represent the innate immunity. Moreover, B lymphocytes, CD4+ T-helper lymphocytes and CD8+ cytotoxic lymphocytes (CTL) represent the adaptive immune cells. The general mechanism for tumor suppression has been principally attributed to CD4 T-helper lymphocytes. Cytokines and lymphokines from CD4+ T-helper can also activate the CD8+ CTL, NK killer, and macrophages [5].

The tumor cells have been the escape mechanism from the host defense because it can create a “local immunosuppressed environment” by producing various substances. The factors secreted by tumor cells include the Vascular Endothelial Growth Factor (VEGF), which inhibits the ability of the dendritic cells to activate T cells and promote angiogenesis; the transforming growth factor β (TGF-β), which suppresses the CD8T cells activation and promotes their differentiation in regulatory CD4T cells; the indoleamine 2, 3-dioxygenase (IDO), which modulates tryptophan level, when upregulated will lead to NK inactivation and T reg differentiation. Alternatively, tumor cells may lose the expression of the tumor antigen and/or the MHC-I or develop defects in the intracellular antigen presentation pathway. Finally, the upregulation of immune checkpoints, an inhibitory pathway which blocks the activated T cells, is believed to represent a crucial mechanism of immune-surveillance escape; the programmed death-1 (PD-1) signaling, consisting of the PD-1 receptor, expressed on the CD4+ and CD8+ cells, and its ligand. By the release of PD-1, the malignant cells are able to inhibit T cells proliferation and function [6].

It appears that the participating role in the apoptotic process is the role of CD4+ and CD8+, therefore in this assessment CD4/CD8 ratios were assessed in response to the treatment of NAC in advanced cervical cancer.

2. Methods
This descriptive research was done using a case-control study. This research was conducted from January to September 2017 at dr. Mohammad Hoesin Hospital Palembang. For the samples, there were 30 patients with advanced cervical cancer and 30 patients as control who met the inclusion
criteria. The protocol has been approved by the Ethical Committee, Faculty of Medicine Sriwijaya University.

The gynecology oncologists diagnosed advanced cervical cancer by clinical staging. To know the value of T-CD4+ and CD8+ lymphocytes, the serum was sampled from the vein and processed using hemocytometry. For cases, samples were checked at three times when the stage of cervical cancer was enforced, before patients underwent 2nd and 3rd series of NAC by Prodia laboratory. Pathologists analyzed and obtained conclusions of patients’ histology from the biopsy.

The frequency and distribution of data are described in tabular form. CD4+/CD8+ ratio before therapy was calculated and crossed between case and control and also therapeutic response to find the cut-off point. Besides that, CD4+/CD8+ ratio before therapy, before 2nd and 3rd series of NAC were calculated and crossed with therapeutic response. The data were analyzed using SPSS version 22.

3. Results

Table 1. Clinicopathologic characteristics.

| Characteristics                        | Control                | Group       |
|----------------------------------------|------------------------|-------------|
| Age (years)                            | 39.73 ± 8.84 (28-56)   | 48.30 ± 9.67 (32-72) |
| Mean ± SD (min-max) CD4 (cell/mm³)     | 747.17 ± 212.09 (305-1349) | 744.84 ± 401.72 (151-1598.2) |
| Mean ± SD (min-max) CD8 (cell/mm³)     | 608.53 ± 204.86 (298-1059) | 550.02 ± 329.74 (160.2-1519) |
| Mean ± SD (min-max) Ratio CD4/CD8 before therapy (cell/mm³) | 1.34 ± 0.53 (0.65-2.72) | 1.51 ± 0.71 (0.59-3.51) |
| Mean ± SD (min-max)                    |                        |             |

From the Mann-Whitney test, we got that there was a meaningful age difference between cervical cancer patients and control (p<0.05). The youngest patient in the control group was 28 years and the oldest one was 56 years. Besides that, for cases, the youngest one was 32 years and the oldest one was 72 years.

From Mann-Whitney test, we got that there was a meaningless CD4/CD8 ratio difference before therapy between Ca cervix patients and control (p>0.05). The mean CD4/CD8 ratio before therapy in control and case group were 1.34 and 1.51 respectively.

By using the ROC curve test, we found that the cut-off point of CD4/CD8 ratio before therapy was 1.2069 which has unfavorable discrimination (AUC = 0.554, 95% CI = 0.407-0.702, p=0.469). From 30 cervical cancer patients, there were 17 patients (56.7%) with CD4/CD8 ≥ 1.2069. Patients with CD4/CD8 ≥ 1.2069 had 1.5 times higher risk to cervical cancer than patients with CD4/CD8 < 1.2069, but it was insignificant (OR = 1.495, 95% CI = 0.540-4.136, p=0.605).

By using the ROC curve test, we found that the cut-off point of CD4/CD8 before therapy and the therapeutic response was 1.325 which had unfavorable discrimination (AUC = 0.528, 95% CI = 0.310-0.747, p=0.815).
Figure 2. (a) ROC curve, (b) Cut-off point of CD4/CD8 ratio before therapy and cervical cancer.

Table 2. CD4/CD8 ratio before therapy and cervical cancer.

| Characteristics (cells/mm³) | Group      | Total | OR (95% CI) | p value |
|-----------------------------|------------|-------|-------------|---------|
| CD4/CD8 ≥ 1.2069            | 17         | 14    | 31          | 1.495   | 0.605  |
| CD4/CD8 < 1.2069            | 13         | 16    | 29          |         | 1      |
| Total                       | 30         | 30    | 60          |         | 1      |

Figure 3. (a) ROC curve, (b) Cut-off point of CD4/CD8 ratio before therapy and therapeutic response.

From 30 cervical cancer patients, there were 8 patients (26.7%) who had no response. Four (50.0%) of them, had CD4/CD8 ratio ≥ 1.325. Patients with CD4/CD8 ratio ≥ 1.325 and < 1.325 had similar risk of having no response, but it was insignificant (OR = 1.000, 95% CI = 0.198-5.045, p=1.000).

Table 3. CD4/CD8 ratio before therapy and therapeutic response.

| Characteristics (cells/mm³) | Therapeutic Response | Total | OR (95% CI) | p value |
|-----------------------------|----------------------|-------|-------------|---------|
| CD4/CD8 ≥ 1.325             | Negative             | 4     | 15          | 1.000   | 1.000  |
| CD4/CD8 < 1.325             | Positive             | 11    | 15          |         | 1      |
| Total                       |                      | 8     | 30          |         | 1      |

By using the ROC curve test, we found that the cut-off point of CD4/CD8 ratio before 2nd series of NAC and the therapeutic response was 1.505 which had unfavorable discrimination (AUC = 0.580, 95% CI = 0.331-0.828, p=0.511).

From 30 cervical cancer patients, there were 8 patients (26.7%) who had no response. Four (50.0%) of them, had CD4/CD8 ratio before 2nd series of NAC ≥ 1.505. Patients with CD4/CD8 ratio before 2nd series of NAC ≥ 1.505 and < 1.505 had similar risk of having no response, but it was insignificant (OR = 1.000, 95% CI = 0.198-5.045, p=1.000).
Figure 4. (a) ROC curve, (b) Cut-off point of CD4/CD8 ratio before 2nd series of NAC and therapeutic response.

Table 4. CD4/CD8 ratio before 2nd series of NAC and therapeutic response.

| Characteristics (cells/mm$^3$) | Therapeutic Response | Total | OR (95% CI) | p value |
|-------------------------------|----------------------|-------|-------------|---------|
| CD4/CD8 ≥ 1.505               | Negative             | 4     | 11          | 15      | 1.000   |
| CD4/CD8 < 1.505               | Positive             | 11    |             |         | 1.000   |
| Total                         |                      | 15    |             |         | 1.000   |

By using the ROC curve test, we found that the cut-off point of CD4/CD8 ratio before 3rd series of NAC and the therapeutic response was 1.485 which had fair discrimination (AUC = 0.747, 95% CI = 0.565-0.930, p=0.041).

From 30 cervical cancer patients, there were 8 patients (26.7%) who had no response. Five (62.5%) of them, had a CD4/CD8 ratio before 3rd series of NAC ≥ 1.485. Patients with CD4/CD8 ratio before 3rd series of NAC ≥ 1.485 had 2.4 times higher risk of having no response, but it was insignificant (OR = 2.407, 95% CI = 0.456-12.720, p=0.417).

Table 5. CD4/CD8 ratio before 3rd series of NAC and therapeutic response.

| Characteristics (cells/mm$^3$) | Therapeutic Response | Total | OR (95% CI) | p value |
|-------------------------------|----------------------|-------|-------------|---------|
| CD4/CD8 ≥ 1.485               | Negative             | 5     | 9           | 14      | 2.407   |
| CD4/CD8 < 1.485               | Positive             | 13    |             |         | 16      |
| Total                         |                      | 8     | 22          |         | 30      |

Figure 5. (a) ROC curve, (b) Cut-off point of CD4/CD8 ratio before 3rd series of NAC and therapeutic response.
Table 6. Therapeutic response.

| Therapeutic Response | Total (n) | Percentage (%) |
|----------------------|-----------|----------------|
| Complete             | 13        | 43.3           |
| Partial              | 9         | 30.0           |
| No response/progressive | 8     | 26.7           |
| Total                | 30        | 100.0          |

In our study, based on therapeutic response towards neoadjuvant chemotherapy, we obtained that as many as 13 patients (43.3%) of 30 cases had a complete response, 9 patients (30.0%) had a partial response, and 8 patients (26.7%) had no response/progression.

4. Discussion

In our study, the mean of CD4 in control and case group were not significantly different, namely 747.17 ± 212.09 cells/mm³ and 744.84 ± 401.72 cells/mm³ respectively. Besides that, the CD8 value in the control group (608.53 ± 204.86 cells/mm³) was approximately 10% higher than in the case group (550.02 ± 329.74 cells/mm³). There was a slight difference with the study from Bosire et al. who conducted a study assessing CD3, CD4 and CD8 lymphocytes levels of normal people. They obtained the assessment result namely in women aged between 16-60 years is CD4+ 1010 (422-1572 cells/μL), CD8+ 659 (187-1180 cells/μL), where the CD4+ CD8+ ratio is 1.69 (0.055-2.95) [7].

Our study showed that the CD4/CD8 ratio in the control group was 1.34 ± 0.53 and in the case group 1.51 ± 0.71. Then, the CD4/CD8 ratio had increased (1.53 ± 0.49) and then decreased (1.49 ± 0.50) after 1st and 2nd series of NAC, respectively. Our study was similar to Zhao et al. who conducted a Randomized Controlled Trial (RCT) in 62 patients with different stages of renal cell carcinoma. After 6 cycles of Ag-DC-CIK (Autologous tumor lysate-pulsed dendritic cells co-culture with cytokine induce killer) treatment, the rate of CD4+ T lymphocytes (37.88 ± 1.353 to 42.62 ± 1.468) and CD4/CD8 in peripheral blood significantly increased (1.2 ± 0.086 to 1.63 ± 0.97) (p=0.021 and p=0.002), while the rate of CD8+ T lymphocytes significantly decreased (34.51 ± 1.946 to 28.72 ± 1.841) (p=0.035). After 6 cycles of CIK (Cytokine Induced Killer) treatment, the rates of peripheral blood CD4+ T Lymphocytes (35.88 ± 1.488 to 41.43 ± 1.745) and CD4/CD8 significantly increased (1.25 ± 0.094 to 1.53 ± 0.101) (p=0.019 and p=0.047) [8],[9].

A study by Yang et al. (2017) which showed an elevated CD4/CD8 ratio in breast cancer patients was found to be associated with tumor progression and poor survival rate [2]. In contrast, Shah W. et al. (2011) suggested that patients with a high CD4/CD8 ratio had significantly higher 5-year survival rate, compared with those with a low CD4/CD8 ratio (82.4% vs 44.4%, p=0.029). The clinical outcome of patients with cervical cancer was influenced by the decreased proportions of tumor-infiltrating CD4+ T cells with a high percentage of Tregs and reversed CD4/CD8 ratios [10]. We got the fluctuating cut-off point of CD4/CD8 ratio before therapy, 2nd, and 3rd series of NAC and therapeutic response which were 1.325, 1.505, and 1.485 respectively. This fluctuating pattern can be caused by many factors, such as the number of samples, patients’ characteristics, and response to the treatment.

Recently, the rapid development of cancer immunotherapies, such as Tumor Infiltrating Lymphocytes (TILs), Natural Killer Cells (NK), Cytotoxic T-Lymphocytes (CIKs) and other immune cells, give a new probability for cancer treatment. Compared with other immune cells, CIKs exhibit a higher proliferation rate, stronger anti-tumor activity, and broader anti-tumor spectrum. CIKs induce the apoptosis of tumor cells and kill them by contacting directly and secreting cytokines such as IL-2 and IFN-γ. DCs are the most potent antigen-presenting cells in the body and they present tumor antigen to T lymphocytes and also induce anti-tumor immune responses. Besides that, their roles are as stimulators of effector T cells and promoter of the helper and cytotoxic T cells’ generation. Furthermore, DCs can promote the gathering of effector T cells around the tumor site and pathogenic tissue by secretion of chemokines [11][12].

CD4+ T cells act in initiating and maintaining anticancer immune responses. During the primary antigen-specific response, CD4+ T-cell is needed to produce CD8+ T cells which can develop into long-lived functional memory cells. CD4 regulatory T cells can suppress anti-tumor immunity in both
self and foreign antigens encoded by tumor viruses [13]. The suppressive effect of HPV-specific CD4+ T cells was affected by their activation by cognate HPV antigen and on interaction with responder T cells [10].

The CD4/CD8 ratio in our study was lower in control group than case group. However, based on the study of Shah W. et al. (2011), patients who had high Tregs percentage had significantly lower 5-year survival rate, compared to those with a lower Tregs percentage (35.3% vs 88.9%, p=0.001). They also found that the CD4/CD8 ratio was significantly lower in the deceased group, compared to the surviving group (0.60±0.25 vs 1.17±1.02, p = 0.019) [10]. This difference can be influenced by the difference in the sum of samples and the distribution of data.

The high number of tumor-infiltrating CD8+ T lymphocytes could be considered as a favorable prognostic factor in some cancer studies, such as endometrial cancer, ovarian cancer, etc. [10]. CD8+ T cells need the CD4+ T cells to function maximally. In our study, we found that the mean of CD4+ and CD8+ value before therapy, before 2nd and 3rd series of NAC had an increasing pattern. It can be interpreted that NAC treatment for cervical cancer patients had a favorable prognosis.

Based on the therapeutic response, the patients in Iwata et al.’s study (2016) who underwent NCS (Neoadjuvant chemotherapy followed by surgery) would have a better outcome compared to radiation therapy alone. In contrast, NCS would give a similar or better outcome than CCRT (Concurrent Chemoradiotherapy) [14]. Our study showed the similar result (73.3% for both complete and partial response) with an RCT conducted by Eddy et al. (2007) which found that the success rate of NAC (complete response and partial response) was 69.4%. The antitumor effect of NAC is by reducing lymph node metastasis and tumor size before surgery so it can reduce the probability of having postoperative radiation therapy.

A meta-analysis conducted by Kim et al. (2013) showed that although NAC reduced the need of adjuvant radiotherapy by decreasing tumor size and lymph node metastasis, and distant metastasis, it failed to improve survival when compared with primary surgical treatment in cervical cancer patients with FIGO stage IB1 to IIA [3].

5. Conclusion
Before therapy, most patients had lower CD4 and CD8 value compared to control. There was a similar risk of having no response towards NAC in both CD4/CD8 ratios before 2nd series of NAC above and below the cut-off point. The higher the CD4/CD8 ratio before the 3rd series of NAC was, the higher the risk of having no response towards NAC treatment in advanced cervical cancer patients. Moreover, most of our patients had either complete or partial response.

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Conflict of interest statement
The author declares that there are no conflicts of interest.