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The Pretreatment Tumor Infiltrating T Lymphocytes (CD8+, CD4+, FOXP3+) and Systemic Neutrophil-Lymphocytes Ratio in Definitively Treated Cervical Cancer Patients: The correlation to clinicopathological factors and Survival

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Abstract

Purpose: To link pretreatment tumor infiltrating T-lymphocytes (TILs) (CD8+, CD4+, FOXP3+) and systemic neutrophil to lymphocyte ratio (NLR) to different clinical/pathological elements. Consequently, emphasizing their impact in predicting the outcome in definitively treated cervical cancer patients.

Methods: The most relevant clinical/pathological factors were used to establish a link with pre-treatment NLR and densities of TILs (CD8+, CD4+, FOXP3+) in cervical biopsies. The predictive significance of pre-treatment TILs and NLR, both for disease free survival (DFS) and overall survival (OS) were evaluated using Log rank alongside Cox regression analysis.

Results: Radical hysterectomies followed by adjuvant radiation with or without chemotherapy were offered to 28 patients, while the remaining twenty eligible patients received curative concurrent chemo-radiation. Augmented levels of CD8+, CD8+/CD4+, while reduced levels of FOXP33+ and NLR were linked to node negative, radical hysterectomies and early stages. Cox-regression demonstrated that augmented levels for NLR and nodal disease were individually correlated to dismal prognosis with HR 3.06 (95%confidence interval [CI], 3.45-9.24), 5.63 (95% CI, 2.61-9.32) for OS and (HR 8.21 (95% CI, 4.21-16.53) and 5.32 (95% CI, 2.37-10.24)for DFS, respectively. On the contrary, FOXP3+≥19 and CD8+/CD4+< 2 had a substantial link to reduced OS (HR 4.37(95% CI, 2.48-12.37), 2.31(95% CI, 2.34-9.32) and worsened DFS (HR 3.61 (95% CI , 1.38-9.32), 4.32(95%CI, 3.12-8.34).

Conclusion: The pretreatment NLR, CD8+, FOXP3+and C8+/CD4+showed a substantial link to various clinical/pathological prognostic characteristics for curatively treated cervical cancer patients. Furthermore, the prognostic prospective of the tested indicators could be emphasized.

Keywords: Tumour infiltrating lymphocytes; Neutrophil lymphocytes ratio; Cervical cancer patients; Haemoglobin; Antitumor immune capacity

Introduction

Cervical cancer claims the lives of around nine percent of all women plagued with cancer annually [1]. Platinum salts were established as the core regimen of curative chemo-radiation for locally advanced cervical cancer (stage IIB to IV) with an approximate 6% enhancement in 5-year overall survival [2]. The devised conventional clinical-pathological indicators of outcome in cervical cancer patients as lymph node, tumor size and pretreatment hemoglobin level, were proved to be incompetent due to unreliable specificity and sensitivity [3-6]. Accordingly, supplementary prognostic variables are considered indispensable to augment accuracy in clinical outcome predictions in definitively treated cervical cancer patients.

Of late cancer pathogenesis have developed greatly with substantial focus on the imperative power of the host immune system in tumor milieu. Emergent data showed that Neutrophil to lymphocyte ratio (NLR) possessed prognostic implication for patients with different types of cancers [7–13]. The (NLR) is an indicator for evaluating the systemic equilibrium between neutrophil-derived tumor induced inflammation and host lymphocyte-associated tumoricidal effect [14,15]. A higher level of NLR may highlight a possible trend towards augmented tumor induced inflammation alongside reduced capacity for tumor cell killing governed by host immunity. In addition, the immune response guarding against carcinogenesis, defined as cancer immunomodulation is showcased by tumor-infiltrating lymphocytes (TILs), permeating either the tumor associated epithelium or the surrounding stroma [16]. The Fork head box P3- positive (Foxp3+) regulatory T cells (Tregs), CD8+ T cells, and CD4+T cells are considered as essentials for immune tolerance and" surveillance [17]. "CD8+ T cells are cytotoxic inducing direct tumor cell killing [18]. The Substantial improvement in survival outcomes of many cancer patients had been directly associated with augmented levels of cytotoxic CD8+T cells [19-25]. Furthermore, CD4+ T cells can hold innumerable effector

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tasks, including promoting tumor-directed cytotoxic T cells involved in tumor cell lysis [18]. Conversely, regulatory T (Treg) cells reign a considerable oppressive impact on direct tumor cell killing modulated by host immune response. Moreover, Treg cells are further sub classified according to surface marker expression such as cluster of differentiation CD4+, the interleukin (IL)-2 receptor alpha chain CD25+, and the fork-head family transcription factor FOXP3+ [26,28]. The FOXP3 gene exerts dominant effect on CD4+ CD25+ T cells by reverting them toward a suppressive T regulatory modulation as confirmed in FoxP3+ Tregs cells retrieved from large cohort of cancer patients [29-32]. Consequently, FOXP3 is at present recognized as the most relevant indicator of Treg cells, and its identification provides a deeper comprehension of the control exerted by Treg cells on exacerbation of autoimmune diseases, alongside transplantation and immunity against carcinogenesis. Further to this, the amplified expression of suppressive Treg cells (CD4+CD25+FOXP3+), is significantly associated to worse clinical outcomes in certain cancers [33,34]. However, the precise role that each TILs exerts in optimizing host immune response in cervical cancer patients necessitates additional elaboration."

As a result, our work reasoning revolved around correlating pretreatment of NLR and TILs (CD8+, CD4+ and FOXP3+) clinicopathological factors, so that their prognostic prospects could be highlighted in the context of definitively treated patients with cervical cancer.

Material and Methods

A retrospective review of the patients getting treatment or getting referred to the Radiation Oncology Department at Clinical Oncology Department of the Alexandria University and King Fahad Specialist Hospital Al Dammam, during the time period between Jan 2013 and Dec 2016 was conducted once IRB approval have been acquired. The patients also consented to their participation. The medical records were examined to pick patients with non-metastatic cervical cancer and had undergone cervical biopsies before surgical procedure or radiation therapy. Age, grade, stage, histology, response to chemo, were included as prognostic variables. NLR, measured through the pretreatment peripheral blood cell count. We selectively included histologically proven, localized uterine cervix cancer FIGO stages IA1, IA2, IB1 and IIA1 who had radical hysterectomy and pelvic lymph nodes dissection. Additionally, adjuvant radiation was implemented based on risk factors (>1/3 stromal invasion, lympho-vascular invasion or tumor size >4 cm). While adjuvant chemo-radiation was offered to patients with positive pelvic nodes & surgical margins, alongside microscopic parametrial involvement. Whole pelvic irradiation 45-50.4 Gy in 1.8 Gy /25-28 fractions ± weekly cisplatin 40 mg/m2. We also included FIGO Stages IB2, IIA2, IIB, IIIA, IIIB and IVA patients who had definitive chemo-radiation 50.4 Gy in 1.8 Gy /28 fractions given to the tumor and pelvic lymph nodes given concurrently with weekly cisplatin 40 mg/m2 to be followed by Image guided. High Dose Rate Intra-cavitary Brachytherapy 7Gy x 4 fractions to deliver total dose of 85–90 Gy to high risk CTV. We excluded patients who demonstrated evidence of distant dissemination at initial inclusion or during treatment.

Immunohistochemistry

This staining was implemented on 4-5-mm paraffin sections. The main antibody consisted of mouse monoclonal antihuman anti-CD8 (DakoCytomation, Glostrup, Denmark; 1:100 dilution), anti-CD4 (Novocastra; 1:30), anti-FOXP3 (Abcam, Cambridge, UK; 1: 50). Tonsil tissue was used as a positive control for all the antibodies. T- lympocytes positive for CD4/CD8 exhibited cellular membranous immunostaining while FOXP3 positive T cells exhibited nuclear staining. The TILs were counted using ocular grid and a light microscope. Mean scoring of intraepithelial FOXP3+, CD8+ and CD4+ T cells in addition to positive cells within the immediate peri-tumoral stroma (within the same high-powered field; HPF) were manually counted twice for 3HPFs (x400) after which each marker's positive cells mean was estimated.

Patient Follow-Up

All the follow ups took place at regular intervals till December 2016, or till the time of a participant's death. This follow-up in essence took place every four months. The process included lab tests and imaging alongside physical exams. The period varied between a timespan of three to 48 months, while the median stood at 26 months. The OS was calculated from the diagnosis till the patient's demise date. The patients that dropped out had their last follow-up dates were registered as the end point of follow up. DFS was recorded from surgery till relapse or the day of last visit.

Statistical consideration

The "link between the aforementioned TILs, NLR, and other clinic-pathological factors were analyzed with the Spearman test. The influences of the studied variables (TILs and NLR) on studied patients related clinical characteristics were evaluated by Mann-Whitney U test (between 2 groups) or Kruskal- Wallis test (≥3 groups). The receiver operator curves (ROC) were implemented to define cutoffs values of the tested variables. A 1.0 area under of the curve (AUC) meant the test was perfect, while 0.5 value" meant the test was non-informative. The variables for prognosis, as outlined through the univariate analysis at a value of $P<0.1$ were examined through the Cox model. The statistical significance was decided to be $P<0.05$. The OS and DFS was measured through the "Kaplan Meier method. Log-rank and Cox regression were conducted to link survival outcomes to different clinical and pathological factors. All these processes took place with the help of SPSS 16.0."

Results

Forty-eight cervical cancer patients were eligible as per the defined inclusion criteria. Of this, around 41.7% or 20 patients had curative chemo-radiation, while another 28 or 58.3% had hysterectomy. This was then followed with adjuvant radiation for 12 or 25% of the sample, or chemo-radiation for 33.3% or 16 patients, as per the postoperative risks (Table 1). The age range for the patients registered between 35 and 72. In terms of histology, 79.2% or 38 patients in the study had squamous cell carcinoma, while 20.8% or 10 patients had uterine cervix adenocarcinoma.

NLR's median value was 1.95 furthermore, the mean for CD8+, CD4+ and FOXP3+ CD4+ positive T lymphocytes were 58, 30 and 18 Cells/HPF. Furthermore, TILs ratio CD8+/ CD4+ was 1.9. Finally, the characteristics for all other baseline elements can be reviewed in (Table 1)."

ROC showed NLR baseline cutoff of 2, where the AUC was 0.873, for forecasting DFS with a specificity level of 82.9% and sensitivity level of 97.4%. Furthermore, CD8+, FOXP3+, CD8+/CD4+ and CD4+ cutoffs stood at 64,19, 2 and 32 with AUC that mounted up to 0.794, 0.854, 0.859 and 0.721 correspondingly. The estimated sensitivities for CD8+, FOXP3+, CD8+/CD4+ and CD4+were 93.3%, 94%, 92.3% and 85.1%, in that order. While their projected specificities were 84.3%, 84.5%, 81.5% and 75.3%, respectively in forecasting DFS (Figure 1 and 2). The sample was split into two based on the optimum cutoffs. It

was observed that those with poorly differentiated histology, regional nodal disease, stage III-IV, and definitive chemo-radiation had an accentuated NLR value as opposed to others (Table 2). In addition, elevated expression for CD8+and CD8+/CD4+" were linked to early disease stages, node negative disease and radical hysterectomies. CD4+T lymphocytes had no link to any clinical-pathological factors (Table 2). Moreover, decreased expression of FOXP3+ T lymphocytes were associated with surgically treated patients who had limited tumor burden without nodal involvement (Table 2). More importantly, the baseline NLR was determined by regression analysis to be substantially linked with pretreatment FOXP3+ and CD8+/CD4+ ratio, as decreased values of NLR were linked with inferior expressions of FOXP3+ and augmented CD8+/CD4+ ratio (OR2.4, 95% CI 1.6-6.8, OR 2.1, 95%CI 1.1-7.8 respectively) (Table .3)."

Twenty patients who presented with stageIB2-IVA, were offered concurrent chemo-radiation. Pathological complete resolution was encountered in 12/20 (60%) of the patients. However, 20% or four of the 20 patients went through only a partial regression, while another four patients demonstrated stable progressive disease. Histopathological type (P=0.002), N Stage (P=0.001) and baseline T-stage (P=0.01) on top of the low cutoffs NLR (P=0.001) had a substantial link to path CR, which can be reviewed in (Table 3), alongside any pathological response. Furthermore, the higher cutoffs for CD8+/CD4+Ratio (0.002), while lower cutoffs of FOXP3+ (P=0.003) were thought to be

Characteristics	No. of Patients	%
Age, years		
Median	52	---
Range	(35-72)	--
<52	22	46%
≥ 52	26	54%
Zubrod performance scale		
0	29	60.4%
1	15	31.3%
2	4	8.3%
Histopathological type		
Squamous cell carcinoma	38	79.2%
Well differentiated- Grade 1	1	2.6 %
Moderately differentiated - Grade 2	9	23.7%
Poorly differentiated – Grade 3	28	73.7%
Adenocarcinoma	10	20.8%
Well differentiated- Grade 1	0	
Moderately differentiated- Grade 2	4	40%
Poorly differentiated- Grade 3	6	60%
Stage group		
IB1	12	25%
IB2	11	23%
IIA1	5	10.4%
IIA2	4	8.3%
IIB	8	16.6%
IIIA	4	8.3%
IIIB	2	4.2%
IVA	2	4.2%
N stage		
N0	25	52.1%
N1	23	47.9%
Definitive treatment		
I-Radical hysterectomy	28	58.3%
II-Concurrent chemoradiation	20	41.7%
A-Radical hysterectomy and adjuvant radiation	12	25%
Tumor>4 cm	7	14.6%
>1/3 stromal invasion	3	6.3%
Lymphovascular invasion	2	4.1%
B-Radical hysterectomy and adjuvant chemoradiation	16	33.3%
Positive pelvic lymph nodes	3	6.3%
Parametrical involvement	7	14.5%
Positive margin	6	12.5%
C- Definitive concurrent chemoradiation	20	41.7%
IB2	5	10.4%
IIA2	4	8.3%
IIB	3	6.3%
IIIA	4	8.3%
IIIB	2	4.2%
IVA	2	4.2%
Inflammatory Response biomarkers (I-NLR)		
Median	1.95	--
Mean ± SD	1.98 ± 6.7	--
Range	(0.6-24)	
ROC Cut off	2	--
<2	26	54.2%
≥2	22	45.8%
II-Tumor Infiltrating Lymphocytes (TILs)		
1- $CD8^+$TILs - Cells/HPF		
Median	58	--
Mean ± SD	59 ± 31.3	--
Range	6.55-190.52	--
ROC Cutoff	64	--
<64	21	43.7%
≥64	27	56.3%
2-$CD4^+$TILs - Cells/HPF		
Median	30	--
Mean ±SD	31.7 ± 31.5	--
Range	8.7-220.5	--
ROC Cutoff	32	--
<32	23	47.9%
≥ 32	25	52.1%
3- $CD4^+$ $FOXP3^+$TILs - Cells/HPF		
Median	18	--
Mean ± SD	17.3 ± 8.31	--
Range	0.98-32.4	--
ROC Cutoff	19	--
<19	28	58.3%
≥ 19	20	41.7 %
4-$CD8^+$/$CD4^+$Ratio		
Median	1.9	--
ROC Cutoff	2	--
<2	19	39.6%
≥ 2	29	60.4%
Radiological /pathological response in Definitive concurrent chemoradiation patients	20	--
Complete response	12	60%
Partial response≥30%	4	20%
Stable disease	2	10%
Progressive disease	2	10%

Table 1: Patient characteristics at baseline (N=48).

substantially linked with CR path (Table 4) alongside any pathological response. Whereas CD4+ had no reportable link to any pathologic response (Table 4)."

The metrics for surgically treated 28 participants were illustrated in (Table 5). It was concluded from regression analysis that nodal disease was isolated as the unique prognosticator substantially connected to accentuated expressions of pretreatment NLR (P=0.02), FOXP3+ (P=0.001) and lower thresholds for CD8+ (P=0.01) ,CD8+/CD4+ratio (P=0.02), respectively CD4+ had no link to a single clinical/pathological factor".

The median follow-up stood at a period of around 26 months wherein 13 patients, who account for 27.1% of the sample, either developed distant dissemination or local relapse. From this group, around 20.8% or 10 patients succumbed secondary to cancer related complications. Further, the four-year DFS and OS stood at 73.7% and 81.2%. (Figure 3 and 4). With regards to survival analyses, the regional nodal spread, higher stages, poorly differentiated histology, gross residual disease following chemo-radiation and primary definitive chemo-radiation, were substantially linked to inferior DFS alongside worsened OS "(Table 6). Furthermore, patients that demonstrated decreased NLR <2 (Figure 5 and 6) and augmented TILs [CD8+≥ 64 (Figure 7 and 8), CD8+/CD4+≥ 2 (Fig 9 and 10) and reduced FOXP3+<19 (Figure 11 and 12) experienced substantially extended OS and DFS (Table 6). Conversely, CD4+ values had no effect on either of the two (Figure 13 and 14). More interestingly, the worst OS and DFS were robustly linked to accentuated expression of pretreatment NLR and nodal disease, with a hazard ratio "(HR 3.06 (95% confidence interval [CI], 3.45-9.24), 5.63 (95% CI, 2.61-9.32) for OS and hazard ratio (HR 8.21 (95% CI, 4.21-16.53) and 5.32 (95% CI, 2.37-10.24) for DFS, respectively. Furthermore, the augmented pretreatment thresholds of FOXP3+≥19 and lower CD8+/CD4+< 2 were seen as significantly related to reduced OS (HR 4.37 (95% CI, 2.48-12.37), 2.31(95%CI, 2.34-9.32) and worsened DFS (HR 3.61 (at 95% CI, 1.38-9.32), 4.32(95%CI, 3.12-8.34), respectively."

Discussion

A mounting volume of data has emphasized the link of TILs with an enhanced clinical result [35]. This study at hand contains a comprehensive evaluation of the performance of baseline intraepithelial "TILs i.e. CD8+, CD4+ and FOXP3+, in 48 cancer cervix patients and their prognosis as per FIGO stage IA-IVA, who were given treatment either with definitive concurrent chemo-radiation or through radical hysterectomies followed by adjuvant .chemo/radiation.

Our analysis illustrated that higher intensities of TILs ratios were linked to node negative disease "(P=0.0126, 0.003), radical hysterectomies (P=0.003, 0.003), and earlier stages of the disease (P=0.0161, 0.0167). Whereas CD4+T lymphocytes were linked to no clinical-pathological elements. Concordantly, Piersma stated that patients with nodal negative disease enjoyed the best clinical outcomes and they possessed as well a significantly greater densities of "CD8+ T cells(P<0.01), an augmented CD8+/CD4+ T-cell ratio(P=0.01), and increased CD8+/regulatory T-cell ratio" when compared to others with regional nodal involvements [25]. However, low pretreatment FOXP3+ T lymphocytes had a link to early stage disease(P=0.011) and node negative patients(P=0.0112). Wu had a similar result wherein they showed that regional nodal disease had substantially augmented densities for FOXP3+ T cells as opposed to those negative regional nodes (P= 0.045) [36]. Shah highlighted that FOXP3+was substantially augmented for higher clinical stages as opposed to lower stages (P=0.023) [37]."

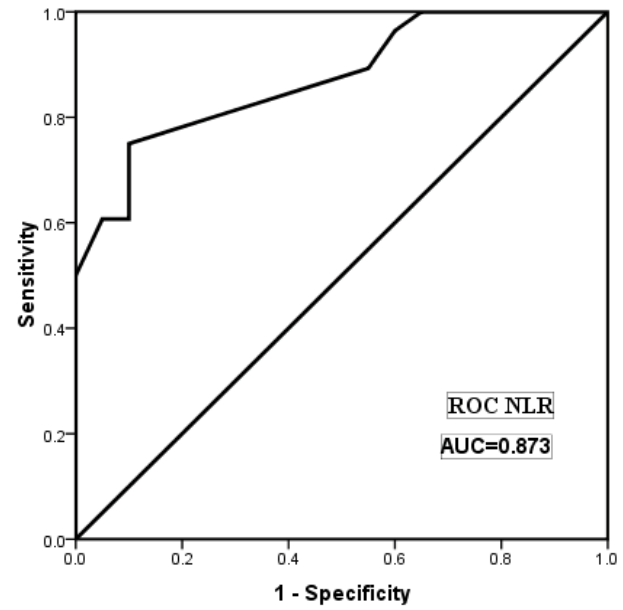

Figure 1: The receiver operator curve (ROC) of pretreatment NLR in cervical cancer patients.

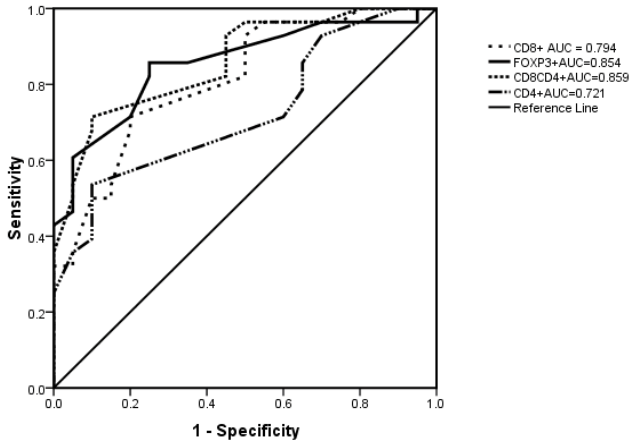

Figure 2: The receiver operator curve (ROC) of pretreatment tumor infiltrating lymphocytes (CD8+, CD4+, FOXP3+ CD4+ and CD8+ / CD4+ ratio) in cervical cancer patients.

It has also concluded from the current work, that amplified NLR was explicitly encountered in grade III cervical cancer (P=0.0311), lymph node involvement (P=0.0132) and advanced stage III-IV(P=0.0132) normally had an amplified NLR. Huang also showed in their meta-analysis that NLR had a substantial correlation with advanced FIGO stage (OR 2.12, 95% CI1 28–3.49) and regional nodal disease (OR 2.24, 95% CI 1.65–3.04) [38]. Furthermore, through regression scrutiny it was found that baseline NLR was substantially linked to pretreatment FOXP3+ and CD8+/CD4+ ratio, as reduced expressions of NLR were linked to the lower intensities of FOXP3+ and elevated ranks of CD8+/CD4+ ratio (OR2.4, 95% CI 1.6-6.8, OR 2.1, 95%CI 1.1-7.8). It is pertinent to mention that this is the first study to outline the correlative link between baseline systemic bio variable. NLR and pretreatment. TILs (CD8+, CD4+ and FOXP3+) in tumor microenvironment. ROC" more so highlighted that baseline NLR and TILs could predict

Clinico- pathological parameters	No. of Patients	%	Baseline NLR		CD8+TILs - Cells/ HPF		CD4+TILs - Cells/ HPF		CD4+FOXP3+ TILs - Cells/HPF		CD8+/CD4+	
			Median	*P* value	Median	*P* value	Median	*P* value	Median	*P* value	Median	*P* value
Age, years												
<52	22	46%	1.8	0.656	56	0.538	30	0.457	18	0.673	1.7	0.765
≥ 52	26	54%	1.96		54	--	29	--	21	--	1.9	--
Histopathological type												
Squamous cell carcinoma	38	--	1.8	--	51	--	27	--	19	--	1.8	--
Well differentiated- Grade 1	1	2.6 %	2.3		48	--	30		15	--	1.6	
Moderately differentiated - Grade 2	9	23.7%	4.5	**0.0311***	52	0.212	32	**0. 121**	16	0. 136	1.63	**0.546**
Poorly differentiated – Grade 3	28	73.7%	3.6		56	--	39		18	--	1.44	
Adenocarcinoma	10	--	3.9	--	47	--	27	--	17	--	1.7	--
Well differentiated- Grade 1	0	--	--		--	--	--		--	--	1.43	
Moderately differentiated- Grade 2	4	40%	1.9	**0.0216***	58	--	42	**0. 316**	15	--	1.4	**0.535**
Poorly differentiated- Grade 3	6	60%	2.6		51	0.315	37		16	0. 126	1.37	
N stage												
N0	25	52.1%	1.8	**0.0124***	78	**0.0126***	34	**0.324**	18	**0.0112***	2.8	**0.0132***
N1	23	47.9%	2.9		35		20		29		1.3	
Stage group												
IB1	12	25%	2.5		68		27		14		2.5	
IB2	11	23%	3.7		64		28		18		2.4	
IIA1	5	10.4%	3.9		65		26		17		2.5	
IIA2	4	8.3%	3.8	**0.0145***	50		29	**0.141**	16	**0.011***	1.7	**0.0167***
IIB	8	16.6%	4.1		49	**0. 0161***	34		25		1.4	
IIIA	4	8.3%	4.4		48		36		26		1.3	
IIIB	2	4.2%	4.9		52		35		29		1.48	
IVA	2	4.2%	5.1		48		33		28		1.45	
Definitive treatment												
I-Radical hysterectomy	28	58.3%	2.3		68	**0.003***	34		17		2.83	**0.003***
II-Concurrent chemoradiation	20	41.7%	5.6	**0.0132***	48		24	0.111	28	**0.002***	1.41	

Table 2: Association between baseline inflammatory biomarkers (NLR, subtypes of TILs) and different clinico-pathological parameters.

DFS in the group of patients suffering from cervical cancer. The 48 participants of the study were then segmented as per the definitive treatment given. Of this sample, 20 patients were ones who had locally advanced stages and were administered concurrent chemo-radiation. Several other clinical elements were seen to be linked with pathologic response as stage, grade, and lymph node contribution. "Furthermore, the accentuated cutoffs for pretreatment CD8+ (P=0.002) and CD8+/CD4+Ratio (P=0.002) while a reducedcutoffs for FOXP3+ (P=0.003) were found to be considerably linked with path CR and any pathologic response." "Whereas pretreatment CD4+ had no link to any kind of pathologic response. Draghiciu et al also outlined that decreased intensities CD8+ were observed in locally advanced cervical cancer that is dealt with using definitive concurrent chemo-radiation and achieved minimal response to treatment (OR=0.562; 95%CI=0.319-0.991; P=0.046) [39]. For the patients remaining, 28 in number, earlier stage were given radical hysterectomy after which adjuvant radiation and chemotherapy was implemented as per the postoperative pathological risk factor. For patients that had been through surgery, lymph nodal contribution was seen through a regression investigation, and was outlined as the single element most profoundly linked to accentuated intensities of pretreatment NLR (P=0.02), FOXP3+ (P=0.001) and reduced densities of pretreatment CD8+ (P=0.01), cutoffs of CD8+/CD4+ratio (P=0.02), respectively.

The study results indicate that nodal involvement, advanced stage, adenocarcinoma, definitive concurrent chemo-radiation, poorly differentiated tumors, along with partial response to chemo-radiation

Pretreatment TILs	Pretreatment NLR		
	Standardized Coefficient	OR (95%CI)	*P-value*
$CD8^+$	-0.234	0.85 (0.41-3.2)	0.358
$CD4^+$	-0.134	0.95 (0.44-2.9)	0.245
$FOXP3^+$	+0.764*	2.4 (1.6-6.8)	0.001*
$CD8/CD4^+$	-0.663*	2.1 (1.1-7.8)	0.024*

Table 3: Correlation between pretreatment NLR and TILs ($CD8^+$, $CD4^+$ and $FOXP3^+$ $CD4^+$ T lymphocytes).

can be considerably linked to reduced OS and DFS. Further, patients with reduced "NLR<2 and FOXP3+<19 while accentuated TILS. (CD8+≥64, CD8+/CD4+≥2) went through considerably extended DFS and OS. Cox regression analysis for survival demonstrated that augmented NLR and nodal involvement were individually linked to abysmal OS with a hazard ratio." (HR 3.06 (95% confidence interval [CI], 3.45-9.24), 5.63 (95% CI, 2.61-9.32) and worsened DFS (HR 8.21 (95% CI, 4.21-16.53), 5.32 (95% CI, 2.37-10.24). Furthermore, the augmented FOXP3+≥19 and reduced CD8+/CD4+<2 were substantially linked to abysmal OS (HR 4.37 (95% CI, 2.48-12.37), 2.31(95%CI, 2.34-9.32) and much worse DFS (HR 3.61 (95% CI, 1.38-9.32), 4.32(95%CI, 3.12-8.34) respectively. In the same vein, Huang demonstrated that high pretreatment NLR levels was substantially linked to dismal OS (HR:1.88, 95% CI 1.30–2.73) and shortened PFR (HR 1.65, 95% CI 1.18–2.29) [38]. Further to this, Draghiciu outlined that accentuated expression CD8+ was linked to augmented

Characteristics	Complete pathologic response Total CR =12Pts % CR (12/20) =60%		P Value	Partial pathologic response Total PR=4 Pts % PR (4/20) =20%		P Value	Stable /Progressive disease Total=4Pts %SD+PD (4/20) =20%		P Value
	No	%CR		No	%PR		No	%SD+PD	
Age, years									
<52	7	35%	0.64	2	10%	0.89	2	10%	0.89
≥52	5	25%		2	10%		2	10%	
Histopathological type									
Squamous cell carcinoma	12	--	**0.002***	2	--	**0.036***	2	--	**0.012***
Well differentiated- Grade 1	0	0%		0	--		0	--	
Moderately differentiated - Grade 2	9	45%		0	--		**0**	**--**	
Poorly differentiated – Grade 3	3	15%		2	10%		2	10%	
Adenocarcinoma	0	0%		2	--		2	--	
Well differentiated- Grade 1	0	0%		0	0%	**0.031***	--	--	**0.011***
Moderately differentiated- Grade 2	0	0%		2	10%		--	--	
Poorly differentiated- Grade 3	0	0%		0	0		2	10%	
N stage									
N1 (1-2) positive LNs	12	60%	**0.001***	1	5%	**0.01***	1	5%	**0.01***
N1>2LNs	0	0%		3	15%		3	15%	
Stage group									
IB2	5	25%	**0.01***			**0.013***			0.89
IIA2	4	20%							
IIB	3	15%							
IIIA				4	20%				
IIIB				0	0%		2		
IVA							2		
Inflammatory Response biomarkers									
I-NLR									
Median	5.6								
<4.3	11	55%	**0.001***	3	15%	**0.01***	0	--	**0.013***
≥ 4.3	1	5%		1	5%		4	20%	
II-Tumor infiltrating lymphocytes (TILs)									
1- CD8+TILs - Cells/HPF									
Median ROC Cutoff	48 46	--	--	--	--	**--**	**--**	**--**	**--**
<46	2	10%	**0.002***	1	5%	**0.01***	1	5%	**0.01***
≥ 46	10	50%		3	15%		3	15%	
2-CD4+TILs - Cells/HPF									
Median ROC Cutoff	34 32	--	**--**	--	--	**--**	**--**	**--**	**--**
<32	7	35%	0.113	2	10%	0.215	2	10%	0.215
≥ 32	5	25%		2	10%		2	10%	
3- CD4+ FOXP3+TILs - Cells/HPF									
Median ROC Cutoff	29 26	--	**--**	--	--	**--**	**--**	**--**	**--**
<26	10	50%		3	15%		4	20%	--
≥ 26	2	10%		1	5%		0	0%	--
4-CD8+/CD4+Ratio									
Median ROC Cutoff	1.41 1.8	--	--	--	--	--	--	--	--
<1.8	3	15%	**0.002***	1	5%	**0.01***	0	0%	**0.013***
≥ 1.8	9	45%		3	15%		4	20%	

Table 4: Association between pathological response and different clinicopathological parameters in patients treated with definitive concurrent chemoradiation (N=20).

Characteristics	No. of Patients	%
Age, years		
Median Range	52 (35-72)	--
<52	16	57%
≥52	12	43%
Histopathological type		
Squamous cell carcinoma	22	78.6%
Well differentiated- Grade 1	1	3.5 %
Moderately differentiated - Grade 2	9	32.1%
Poorly differentiated – Grade 3	12	43%
Adenocarcinoma	6	21.4%
Well differentiated- Grade 1	0	
Moderately differentiated- Grade 2	4	14.3%
Poorly differentiated- Grade 3	2	7.1%
Stage group		
IB1	12	42.8%
IB2	3	10.7%
IIA1	5	17.9%
IIA2	4	14.3%
IIB	4	14.3%
N stage		
N0	25	89.3%%
N1	3	10.7%
Adjuvant treatment		
A-Radical hysterectomy and adjuvant radiation:	12	42.9%
1-Tumor > 4 cm	7	25%
2-> 1/3 stromal invasion	3	10.7%
3-Lymphovascular invasion	2	7.1%
B-Radical hysterectomy and adjuvant chemoradiation:	16	57.1%
2. Positive pelvic lymph nodes	3	10.7%
3. Parametrical involvement	7	25%
4. Positive margin	6	21.4%
Inflammatory Response biomarkers		
I-NLR		
Median Range ROC Cutoff	2.3 (0.6-23) 2	
<2	19	67.9%
≥ 2	9	32.1%
II-Tumor infiltrating lymphocytes (TILs)		
1- CD8+TILs - Cells/HPF		
Median Mean ±SD Range ROC Cutoff	68 58 ± 30.3 6.45-189.52 62	
<62	21	75%
≥ 62	7	25%
2-CD4+TILs - Cells/HPF		
Median Mean ±SD Range ROC Cutoff	24 30.2 ± 32.5 8.9-215.5 22	
<22	19	67.9%
≥ 22	9	32.1%
3- CD4+ FOXP3+TILs - Cells/HPF		
Median Mean ±SD Range ROC Cutoff	14 17.8 ± 9.31 0.97-31.6 16	--
<16	20	71.4 %
≥ 16	8	28.6%
4-CD8+/CD4+Ratio		
Median ROC Cutoff	2.83 2.6	--
<2.6	18	64.3%
≥ 2.6	10	35.7%

Table 5: Clinicopathological parameters of patients treated with radical hysterectomy +/-adjuvant radiation or concurrent chemoradiation (N=28).

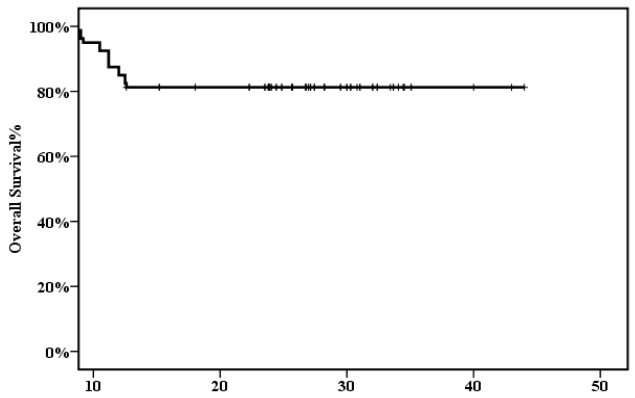

Figure 3: The overall survival of cervical cancer patients in months.

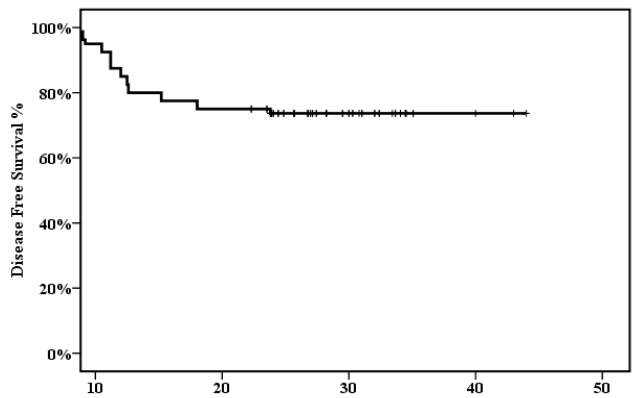

Figure 4: The disease-free survival of cervical cancer patients in months.

OS (HR=0.642; 95%CI=0.448-0.919; P=0.016) and DSS (HR=0.607; 95%CI=0.403-0.915; P=0.017) [39]."Jordanova also explained that augmented expressions of Treg (FoxP3+) "and decreased cutoffs of CD8+/regulatory T-cell ratio were linked to inferior survival (P=0.034 and0.02), respectively. In later Cox regression investigation, reduced CD8+/Treg ratio (P=0.047), decreased CD8+/Treg ratio (P=0.002) were seen as independent uncomplimentary predictive prognosticators in cervical carcinoma [40]. In the same vein, Piersma outlined a substantially more robust intratumoral densities of CD8+ and a greater CD8+/CD4+ T-cell ratio in localized cervical cancer without regional nodal involvement and were significantly correlated with superior prognoses [25]." Additionally, Shah stated that a substantially inferior survival rate was observed for patients with augmented expressions of CD4+FOXP3+ Tregs, as opposed to those with inferior expressions (35.3% versus 88.9%, P=0.001) [37]."

The study at hand has a potential input as it scrutinized on the role that TIL types (CD8+, FOXP3+, CD8 +/CD4+) and NLR exert as individual prognostic indicators. It is also confirmed the competencies of pretreatment TILs and NLR in forecasting prognoses of curatively

Characteristics	No. of Patients	%	Overall survival		Disease free survival	
			No (%) No (%) of patients alive 38	P-value	No (%) of patients 35	P-value
Age, years						
<52	22	46%	18 (47.4%)	0.112	17 (48.6%)	0.345
≥52	26	54%	20 (52.6%)		18 (47.4%)	
Histopathological type						
Squamous cell carcinoma	38	--	36 (94.7%)		33 (94.3%)	
Well differentiated- Grade 1	1	2.6 %	1 (2.6%)		1 (2.9 %)	
Moderately differentiated - Grade 2	9	23.7%	9 (23.7%)		9 (25.7%)	
Poorly differentiated – Grade 3	28	73.7%	26 (68.4%)	**0.003***	23 (65.7%)	**0.012***
Adenocarcinoma	10	--	2	--	2	--
Well differentiated- Grade 1	0	--	--	**--**	--	**--**
Moderately differentiated- Grade 2	4	40%	2 (5.3%)	--	2 (5.7%)	--
Poorly differentiated- Grade 3	6	60%	--	--	--	--
Stage group	--	--	1 (1.7%)	--	0	--
IB1	12	25%	12 (31.6%)		12	
IB2	11	23%	11 (28.9%)		10 (34.6%)	
IIA1	5	10.4%	5 (13.2%)		4 (61.5%)	
IIA2	4	8.3%	4 (10.5%)		3 (3.9%)	**0.031***
IIB	8	16.6%	6 (15.8%)		5	
IIIA	4	8.3%	0	**0.02***	0 (15.4%)	
IIIB	2	4.2%	0		0	
IVA	2	4.2%	0		0 (84.6%)	
N stage						
N0	25	52.1%	25 (65.8%)		25 (71.4%)	**0.003***
N1	23	47.9%	13 (34.2%)	**0. 001***	10 (28.6%)	
Definitive treatment						
I-Radical hysterectomy	28	58.3%	26/28 (92.6%)	**0. 002***	25/28 (89.3%)	**0.001***
A-Radical hysterectomy and adjuvant radiation:	12	25%	12/28 (42.9%)		12 (34.3%)	
1-Tumor > 4 cm	7	14.6%	7/28 (25%)		7 (20%)	
2->1/3 stromal invasion	3	6.3%	3/28 (10.7%)	0. 13	3 (8.6%)	0.65
3-Lymphovascular invasion	2	4.1%	2/28 (7.2%)		2 (5.7%)	
B-Radical hysterectomy and adjuvant chemoradiation:	16	33.3%	14/28 (50%)		13/28 (46.5 %)	
1-Positive pelvic lymph nodes	3	6.3%	1/28 (3.5%)		0 (0%)	
2-Parametrial involvement	7	14.5%	7/28 (25%)	**0.016***	7 /28 (25%)	**0.011***
3-Positive margin	6	12.5%	6/28 (21.5%)		6/28 (21.5%)	
II- Definitive concurrent chemoradiation	20	41.7%	12/20 (60%)	**0. 002***	10/20 (50%)	**0.001***
IB2	5	10.4%	5/20 (25%)		5/20 (25%)	
IIA2	4	8.3%	3/20 (15%)		3/20 (15%)	
IIB	3	6.3%	3/20 (15%)	**0. 014***	2/20 (10%)	**0. 021***
IIIA	4	8.3%	1/20 (5%)		0	
IIIB	2	4.2%	0		0	
IVA	2	4.2%	0		0	
Radiological response in Definitive concurrent chemoradiation patients	20	--	--	--	--	--
Complete response	12/20	60%	12/20 (60%)		12/20 (60%)	
Partial response ≥ 30%	4/20	20%	4/20 (20%)		1 (5%)	
Stable disease	2/20	10%	0	**0. 024***	0	**0.011***
Progressive disease	2/20	10%	0		0	
Inflammatory Response biomarkers						
I-NLR						
Median	1.95					
<2	26	54.2%	26 (68.4%)		24 (68.6%)	
≥ 2	22	45.8%	12 (31.6%)	**0.001***	11 (31.4%)	**0.007***
II-Tumor infiltrating lymphocytes (TILs)						
1- CD8+TILs - Cells/HPF						

Median	58	--	--	--	--	--
<64	21	43.7%	13 (34.2%)	**0.002***	11 (31.4%)	**0.001***
≥ 64	27	56.3%	25 (65.8%)		24 (68.6%)	
2-CD4+TILs - Cells/HPF						
Median	30	--	--	--	--	--
<32	23	47.9%	18 (47.4%)	**0.064**	16 (45.7%)	0. 426
≥ 32	25	52.1%	20 (52.6%)		19 (54.3%)	
3- CD4+ FOXP3+TILs - Cells/HPF						
Median	18	--	--	--	--	--
< 19	28	58.3%	24 (63.2%)	**0.04***	23 (65.7%)	**0.001***
≥ 19	20	41.7 %	14 (36.8%)		12 (34.3%)	
4-CD8+/CD4+Ratio						
Median	1.9	--	--	--	--	--
<2	19	39.6%	13 (34.2%)	**0.007***	11 (31.4%)	**0.001***
≥ 2	29	60.4%	25 (65.8%)		24 (68.6%)	

Table 6: Association between different clinico-pathological parameters and clinical prognosis.

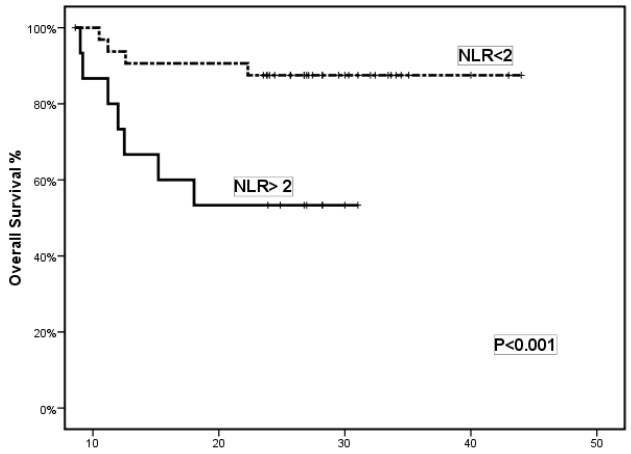

Figure 5: The impact of NLR on overall survival of all cervical cancer patients in months.

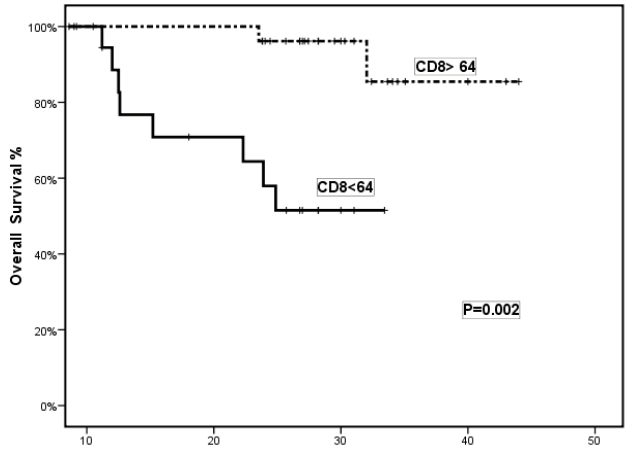

Figure 7: The impact of CD8+TILs on overall survival of all cervical cancer patients in months.

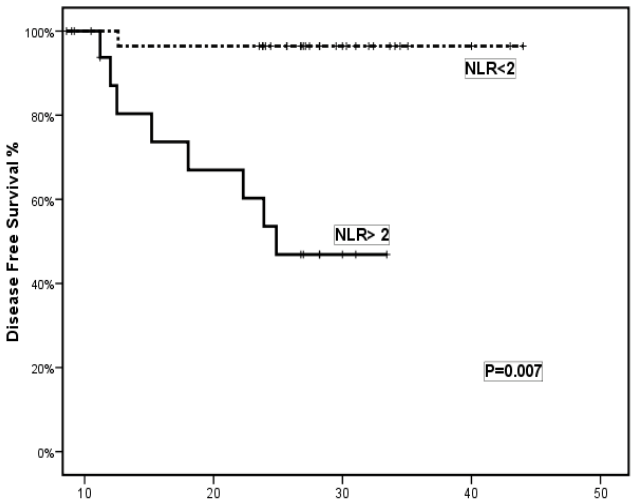

Figure 6: The impact of NLR on Disease Free survival of all cervical cancer patients in months.

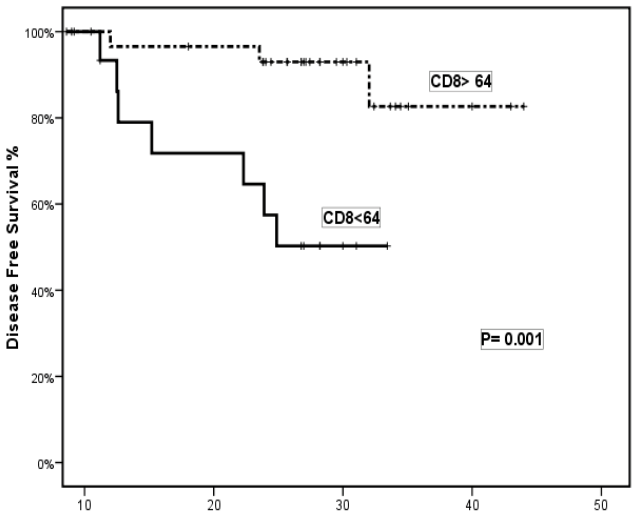

Figure 8: The impact of CD8+TILs on Disease Free survival of all cervical cancer patients in months.

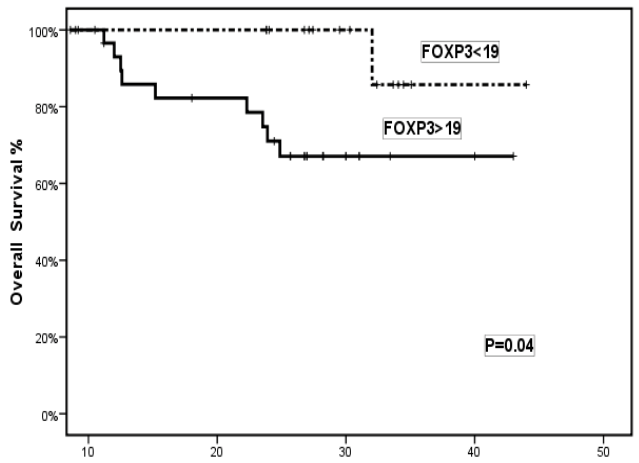

Figure 9: The impact of FOXP3+ TILs on overall survival of all cervical cancer patients in months.

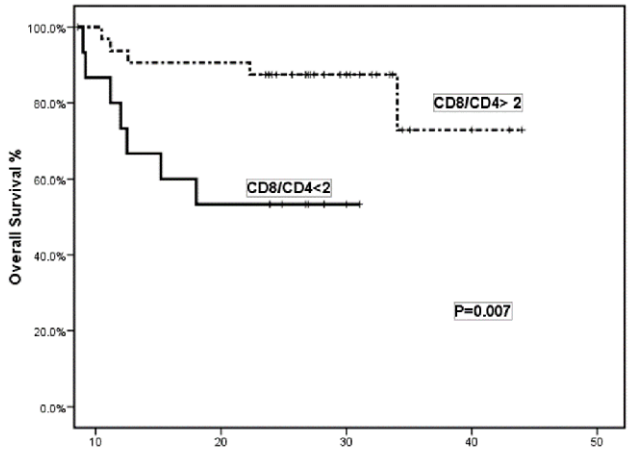

Figure 12: The impact of $CD8^+$ /$CD4^+$ TILs ratio on disease free survival of all cervical cancer patients in months.

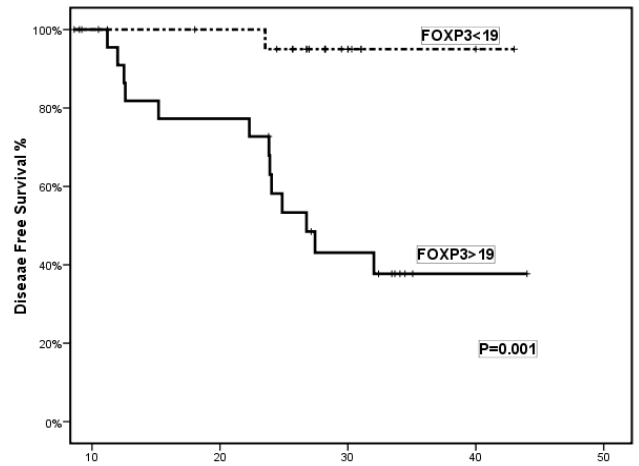

Figure10: The impact of $FOXP3^+$ TILs on Disease Free survival of all cervical cancer patients in months.

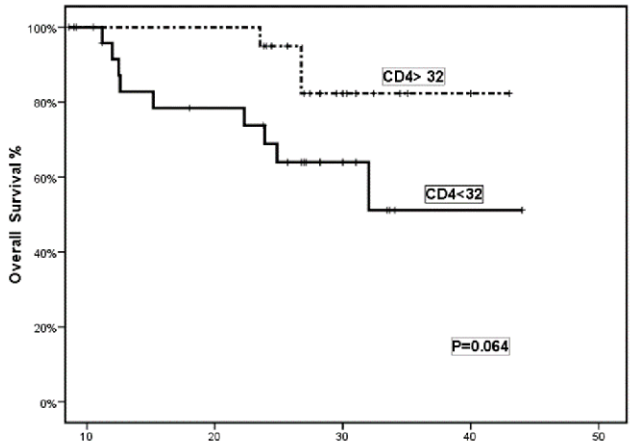

Figure 13: The impact of $CD4^+$TILs on overall survival of all cervical cancer patients in months.

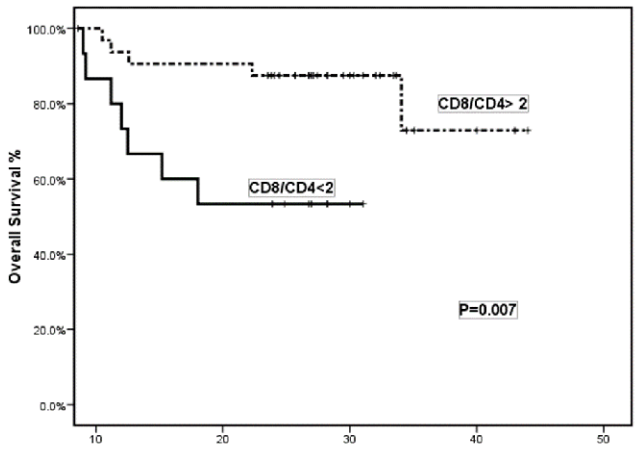

Figure 11: The impact of CD8+ /CD4+ TILs Ratio on Overall survival of all cervical cancer patients in months.

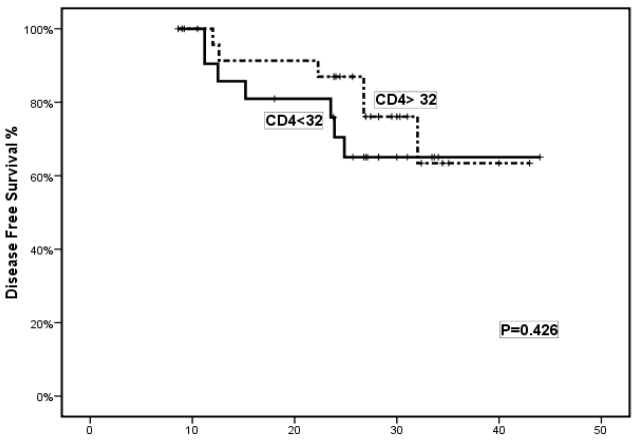

Figure 14: The impact of $CD4^+$TILs on disease free survival of all cervical cancer patients in months.

treated patients who received either concurrent chemo-radiation or radical hysterectomies. However, the study confronted some restrictions because of its retrospective nature. The limited sample size alongside its heterogeneity, negatively impacted the generalizability of our results. Moreover, we did not delve into the details of how different TILs optimize and modulate each other's activities.

Conclusion

The tested pretreatment TILs and NLR demonstrated a considerable link to various clinical-pathological prognostic variables in terms of definitively treated patients with cervical cancer. Furthermore, they could be perceived as independent prognostic forecasters of clinical outcomes.

Conflict of Interest

The authors ascertain that they did not receive any financial support or funding from any institution or entity or any commercial operation. The authors further confirm that the study posed no conflicts of interest for them.

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