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Multiplication of neutrophil and monocyte counts (MNM) as an easily obtainable tumour marker for cervical cancer

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Abstract

Simple, non-aversive methods to identify cervical neoplasia are needed. The purpose of this study was to investigate the clinical value of differential white blood cell (WBC) counts as a biomarker for cervical neoplasia. We performed a retrospective review of laboratory results in 407 cervical cancers, 495 cervical intraepithelial neoplasias (CIN) and 916 healthy controls. Because pretreatment neutrophil and monocyte counts showed the potential as a biomarker, we combined these parameters and designated this combined marker MNM (multiplication of neutrophil and monocyte counts). MNM showed a sensitivity of 53.1% and a specificity of 78.1%, which are much higher than those of SCC-Ag. On Cox multivariate analysis, MNM positivity (hazard ratio = 2.82, $p = 0.042$), stage and tumour size were independent predictors of poor prognosis. Our findings suggest that pretreatment MNM could be a candidate as a simple and cost-effective biomarker in cervical cancer.

Keywords: *White blood cell; neutrophil; monocyte; cervical cancer; tumour marker*

Introduction

Cervical cancer continues to be one of the leading female genital cancers worldwide (Parkin et al. 2005). New and promising vaccines to prevent human papillomavirus (HPV) infection have been developed but they do not protect against all HPV subtypes and are primarily effective in uninfected women (Bosch & Munoz 2002, Koutsky & Harper 2006). Cervical cancer will, for decades to come, remain a serious health problem, and improvements in both early detection and prediction of prognosis and new adjuvant treatments are needed.

The current standard for cervical disease diagnosis is based upon the morphological assessment of cells with abnormal appearance in a cervical cytology specimen. The incidence and mortality of cervical cancer have decreased substantially in all western countries as result of cytological screening (Gustafsson et al. 1997). At the same time, the impact of screening has plateaued due to the limited accuracy of the tests and the choice of some women not to participate (Petignat et al. 2006). Although non-participation is the main reason that women develop cervical cancer in regions with widespread screening, there is clearly a need for a more sensitive method of finding premalignant lesions. Moreover, cytological screening is generally not yet available in developing nations, where cervical cancer remains a major public health problem for young women. In addition, most women feel uncomfortable with gynaecological pelvic examination.

An ideal tumour marker should have a high sensitivity and specificity and should also provide information related to tumour burden and activity. No validated tumour marker is currently available for the diagnosis, prognostic evaluation, treatment monitoring and follow-up of patients with cervical cancer. Some tumour-associated antigens have been measured in the sera from patients with cervical cancer and correlated with the clinical course of disease. For instance, serum squamous cell carcinoma (SCC) antigen levels

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are elevated in 28–80% of patients with squamous cell cervical cancer and correlate with tumour stage, tumour size, cervical stromal invasion, lymph-vascular space involvement and lymph node status (Duk et al. 1996, Molina et al. 2005). However, the clinical relevance of pretreatment serum SCC levels is still under debate (Strauss et al. 2002, Yuan et al. 2002).

The recognition of a functional relationship between inflammation and cancer is not new. In 1863, Rudolf Virchow speculated that the chronic inflammatory infiltrates observed in tumours reflect the origins of cancer (Balkwill & Mantovani 2001). More recently, the role that the immune system plays in disease cessation or progression has been examined and haematological markers including white blood cell (WBC) subtypes and the neutrophil to lymphocyte ratio (NLR) have been proposed as both diagnostic and prognostic factors in a variety of cancers (Cho et al. 2009, Schmidt et al. 2005, Ueno et al. 2007, Walsh et al. 2005). Because cervical cancer is preconditioned by a viral infection, the immune response towards the cancer is potentially important to overcome the disease by clearing or controlling the virus. We hypothesized, therefore, that changes in the relative levels of circulating WBC would also be found in cervical cancer patients and could be used as an additive diagnostic and prognostic marker for cervical cancer. Moreover, assessment of the inflammatory response to the tumour may be easier and more cost-effective in clinical practice than current methods.

In this study, we evaluated the correlation between pretreatment differential WBC counts (neutrophils, lymphocytes, monocytes, eosinophils and basophils) and clinicopathological characteristics in cervical cancer, and investigated whether these markers, either alone or in combination, could be used as diagnostic and prognostic markers for cervical cancer.

Materials and methods

Study subjects

We conducted a retrospective review of the medical records of 407 newly diagnosed and previously untreated cervical cancer and 495 cervical intraepithelial neoplasia (CIN) patients at the Department of Obstetrics and Gynecology, Yongdong Severance Hospital and Severance Hospital between January 2000 and December 2007. All patients had a histological diagnosis of cervical carcinoma or CIN, and the cervical cancer patients were clinically staged according to the International Federation of Gynecology and Obstetrics (FIGO) staging system. If operable, patients underwent hysterectomy with pelvic lymph node dissection, and in cases of increased risk of relapse (assessed from depth of invasion, lymphovascular space invasion, spread to lymph node, invasion of parametrium and cancer close to resection margins) cisplatin-based concurrent chemoradiation was added. In bulky tumours of stage IB and IIA, neoadjuvant chemotherapy was performed before surgery. Inoperable patients were treated by radiotherapy (both external radiotherapy and brachytherapy) or both cisplatin-based chemotherapy and radiotherapy. Patients were not included in the study if they were more than 70 years old at the time of diagnosis or if they had previous malignant diseases. The healthy control group ($n = 916$) consisted of health check examinees at Yongdong Severance Hospital with no history of cancer or gynaecological disease and no abnormalities in laboratory examinations or gynaecological sonography. This study was approved by the Institutional Review Boards (IRBs) of Yongdong Severance and Severance Hospital.

Clinical and laboratory data collection

For study subjects, differential WBC counts and SCC antigen levels were recorded from full blood counts routinely performed the day before surgery. For healthy controls, laboratory data were obtained from full blood counts performed as part of their health-check examination. Differential WBC counts were analyzed by an ADVIA 120/2120 Hematology system (Bayer HealthCare, Diagnostics Division, Tarrytown, NY, USA) and SCC antigen levels were measured by the IMx SCC-Ag microparticle enzyme immunoassay (Abbott Diagnostics, Chicago, IL, USA). The upper limit of 1.5 ng ml^{-1} for SCC antigen was used to define normal values as recommended by the manufacturer. To determine the HPV infection rate in the study group, Hybrid Capture® 2 test results were also reviewed. The following variables were assessed in all patients: age, FIGO stage, tumour size, pathological results, tumour status at the time of follow-up (no evident disease, tumour recurrence, progressive disease) and time lapse between initial treatment and follow-up.

Statistical analysis

Differential WBC counts and serum SCC antigen levels were expressed as the mean (95% confidence interval) of each group. The differences in means between groups were compared using one-way analysis of variance, *post hoc* tests, or Student's *t*-test where appropriate, and an analysis of the percentage of patients with abnormally elevated values was performed using the χ^2 test. The sensitivity and specificity of each marker was assessed using receiver operating characteristic (ROC) curves. The area under the ROC curve was calculated as a measure of the ability of each potential marker to discriminate between cervical cancer cases and healthy controls. An

area under the curve (AUC) of 0.5 indicates classifications assigned by chance. The ROC analysis was plotted to investigate the optimal cut-off values that maximized the sum of sensitivity and specificity.

Kaplan–Meier survival analysis was used to determine the univariate relationship of tumour markers with overall and disease-free survival times. The log rank test was used to examine the significance of the differences of survival distributions between groups. Subsequently, multivariate analysis with Cox proportional hazards regression was performed to determine which biomarkers predicted disease-free and overall survival after having adjusted for the effects of known prognostic factors. A p-value <0.05 was considered significant for all analyses unless otherwise stated. All analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

The clinical characteristics of the patients are listed in Table 1. The overall mean age was 47.1 ± 10.8 years for cervical cancer patients and 46.1 ± 10.3 years for healthy controls. No significant age differences were noted between the groups. FIGO staging was available for all 407 cases, and 136 cases were stage IA, 212 cases were stage IB, 27 cases were stage IIA, 23 cases were stage IIB, two cases were stage III and seven cases were stage IV. The following histological types were assigned according to World Health Organization (WHO) criteria: 312 SCCs (including 165 non-keratinizing type and 147 keratinizing type), 73 pure adenocarcinomas, 15 adenosquamous carcinomas, three small-cell carcinomas, two clear-cell carcinomas and two papillary serous carcinomas. Approximately 60% of the patients with cervical cancer received only primary treatment surgery. Of the individuals with CIN (n = 495), 115 (23.2%) had CIN1, 114 (23.0%) had CIN2 and 266 (53.7%) had CIN3. The HPV DNA test positive rate was 95.8% for cervical cancer, 78.1% for CIN and 10.3% for healthy controls.

Differential WBC counts and NLR

Differential WBC counts were analyzed in 407 patients with cervical cancer, 495 patients with CIN and 916 healthy controls according to subtypes (Table 2). Serum SCC antigen did not correlate with differential WBC counts, except for lymphocyte count (Spearman $r = -0.12$, $p = 0.024$). The mean neutrophil and monocyte counts in the cervical cancer group were significantly higher than in the other groups ($p < 0.001$).

The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count and calculated from differential WBC counts (Zahorec 2001). Pretreatment NLR value was also evaluated in the study subjects. The mean value for NLR was significantly elevated in the cervical cancer group ($p < 0.001$), but there was no significant difference in the mean value for NLR between the CIN and healthy control groups.

The cervical cancer group was also evaluated according to clinicopathological characteristics (Table 3). Only neutrophil and monocyte counts and NLR, which showed significant differences between the three study groups, were included in this further evaluation. Within cervical cancer patients, neutrophil counts were positively correlated with lymphovascular space invasion ($p = 0.043$) and tumour size ($p = 0.034$), while monocyte counts were positively correlated with tumour stage ($p = 0.020$). There were no significant correlations between NLR and clinicopathological characteristics.

Multiplication of neutrophil and monocyte counts (MNM)

Because the neutrophil and monocyte counts in cervical cancer patients were significantly different from those of healthy controls and showed correlation with some clinicopathological characteristics, we designed a combined marker by multiplying neutrophil counts by monocyte counts and then dividing by 10 000. We designated this combined marker MNM (multiplication of neutrophil and monocyte counts) and compared pretreatment SCC antigen level and MNM in all study groups (Figure 1). The mean SCC antigen level and MNM value in cervical

Table 1. Clinicopathological characteristics of 407 cervical cancer patients.

Variables	n	%
Age (years), mean	47.1	
FIGO stage		
I	348	85.5
II	50	12.3
III	2	0.5
IV	7	1.7
Histological type		
Non-keratinizing	165	40.6
Keratinizing	147	36.1
Adenocarcinoma	73	17.9
Adenosquamous	15	3.7
Others	7	1.7
Tumour size (cm), mean		
<1.0	211	51.8
1.0–2.0	64	15.7
2.0–3.0	50	12.3
>3.0	82	20.2
Treatment		
Primary treatment surgery	241	59.2
Postoperative adjuvant therapy	106	26.0
Neoadjuvant therapy before surgery	38	9.4
Primary radiotherapy ± concurrent chemotherapy	22	5.4

Table 2. Mean counts of WBC subtypes and NLR in study subjects.

	Healthy controls ($n=916$)	CIN ($n=495$)	Cervical cancer ($n=407$)	p-Value
Neutrophil (mm^{-3})	3401.0 (3315.0–3486.9)	3749.2 (3622.9–3875.5)	4656.4 (4409.1–4903.6)	<0.001
Lymphocyte (mm^{-3})	1847.5 (1814.3–1880.7)	1887.1 (1838.1–1936.1)	1912.2 (1838.6–1985.8)	0.146
Monocyte (mm^{-3})	253.6 (248.2–259.0)	296.0 (286.5–305.6)	332.0 (319.2–344.8)	<0.001
Eosinophil (mm^{-3})	144.1[a,b] (135.9–152.4)	133.9[a] (123.0–144.7)	157.3[b] (140.4–174.2)	0.040
Basophil (mm^{-3})	37.6[a] (36.3–38.9)	32.3 (30.6–34.0)	36.6[a] (33.7–39.4)	<0.001
NLR	1.95[a] (1.89–2.01)	2.22[a] (2.05–2.38)	3.31 (2.81–3.82)	<0.001

Data are presented as mean counts (95% confidence interval).
CIN, cervical intraepithelial neoplasia; NLR, neutrophil to lymphocyte ratio (neutrophil count/lymphocyte count).
[a,b]There was no statistically significant difference in differential counts between groups with the same symbol.

Table 3. Clinicopathological characteristics associated with neutrophil count, monocyte count, and NLR.

	Neutrophil (mm^{-3})	Monocyte (mm^{-3})	NLR
Stage	$p=0.104$	$p=0.020$	$p=0.925$
IA ($n=136$)	5071.7 (4588.5–5554.9)	327.1 (304.7–350.7)	3.57 (2.88–4.26)
IB ($n=212$)	4417.1 (4092.2–4742.0)	330.0 (312.0–348.0)	3.20 (2.35–4.05)
IIA ($n=27$)	4102.9 (3142.0–5063.8)	315.5 (291.6–339.5)	3.20 (1.79–4.60)
IIB ($n=23$)	4604.3 (3948.0–5260.6)	359.5 (314.2–404.9)	2.56 (2.03–3.08)
III ($n=2$)	4785.0 (1012.1–8472.1)	415.0 (29.7–859.7)	3.74 (0.92–6.56)
IV ($n=7$)	6102.8 (4188.5–8017.1)	440.0 (255.1–624.8)	4.65 (2.71–6.60)
Lymph node metastasis	$p=0.852$	$p=0.149$	$p=0.921$
No ($n=335$)	4638.1 (4356.5–4919.8)	328.4 (314.9–341.8)	3.32 (2.72–3.91)
Yes ($n=41$)	4557.8 (3784.8–5330.7)	298.5 (260.0–337.0)	3.40 (2.16–4.65)
Parametrium invasion	$p=0.948$	$p=0.124$	$p=0.582$
No ($n=358$)	4627.4 (4354.1–4900.6)	322.9 (310.1–335.7)	3.36 (2.79–3.93)
Yes ($n=18$)	4668.3 (3682.7–5653.9)	369.4 (292.6–446.1)	2.64 (1.92–3.46)
Lymphovascular space invasion	$p=0.043$	$p=0.120$	$p=0.204$
No ($n=258$)	4445.8 (4143.3–4748.4)	318.3 (303.8–332.8)	3.02 (2.56–3.49)
Yes ($n=118$)	5030.5 (4513.5–5547.6)	340.0 (314.7–365.2)	3.99 (2.57–5.41)
Histological type	$p=0.283$	$p=0.500$	$p=0.409$
Non-keratinizing ($n=166$)	4700.1 (4266.6–5133.6)	321.8 (301.3–342.4)	3.63 (2.54–4.72)
Keratinizing ($n=147$)	4816.0 (4391.6–5240.4)	344.4 (322.9–365.9)	3.44 (2.83–4.05)
Adenocarcinoma ($n=87$)	4446.3 (4057.0–4835.5)	333.5 (306.0–361.0)	2.63 (2.14–3.13)
Others ($n=7$)	3111.4 (2186.2–4036.5)	318.5 (240.0–397.1)	1.66 (1.20–2.11)
Tumour size (cm)	$p=0.034$	$p=0.204$	$p=0.270$
<1.0 ($n=211$)	4561.1 (4159.2–4963.0)	339.8 (321.9–357.7)	3.82 (2.88–4.75)
1.0–2.0 ($n=64$)	4472.0 (4105.2–4838.8)	301.0 (269.2–332.9)	2.88 (1.97–3.79)
2.0–3.0 ($n=50$)	4843.0 (4228.0–5457.9)	342.0 (301.7–382.2)	2.52 (1.72–3.32)
>3.0 ($n=82$)	4936.4 (4486.9–5385.9)	330.2 (302.6–357.8)	2.97 (2.45–3.49)

Data are presented as mean counts (95% confidence interval).
NLR, neutrophil to lymphocyte ratio (neutrophil count/lymphocyte count).

cancer patients were significantly higher than those of healthy controls ($p<0.001$), but there was no significant difference in SCC antigen level between the CIN and control groups ($p=0.741$). MNM was weakly correlated with the serum SCC antigen level of study subjects (Spearman $r=0.09$, $p=0.030$).

Diagnostic significance of MNM along with SCC antigen in cervical cancer

Statistical tests of SCC antigen level, NLR and MNM between cervical cancer and healthy control group were performed according to the severity of disease (Table 4).

To categorize patients as NLR and MNM positive or negative, an optimal cut-off value that maximized the sum of sensitivity and specificity in the ROC curve was used. In the analysis of all cervical cancer patients ($n=407$), the AUC for SCC antigen was 0.565 (95% CI, 0.516–0.613) with a sensitivity of 17.1% and specificity of 96.1%. For MNM, the AUC was 0.703 (95% CI, 0.672–0.734) and the cut-off value was 123.9 with a 53.1% sensitivity and 78.1% specificity. In early-stage cervical cancer (≤ FIGO stage IIA, $n=375$) at a cut-off value of 123.9, the AUC for MNM was 0.694 (95% CI, 0.661–0.726) with a sensitivity of 51.4% and a specificity of 78.0%. At a cut-off value of 2.43, NLR yielded a sensitivity of 41.4% and 40.8% and

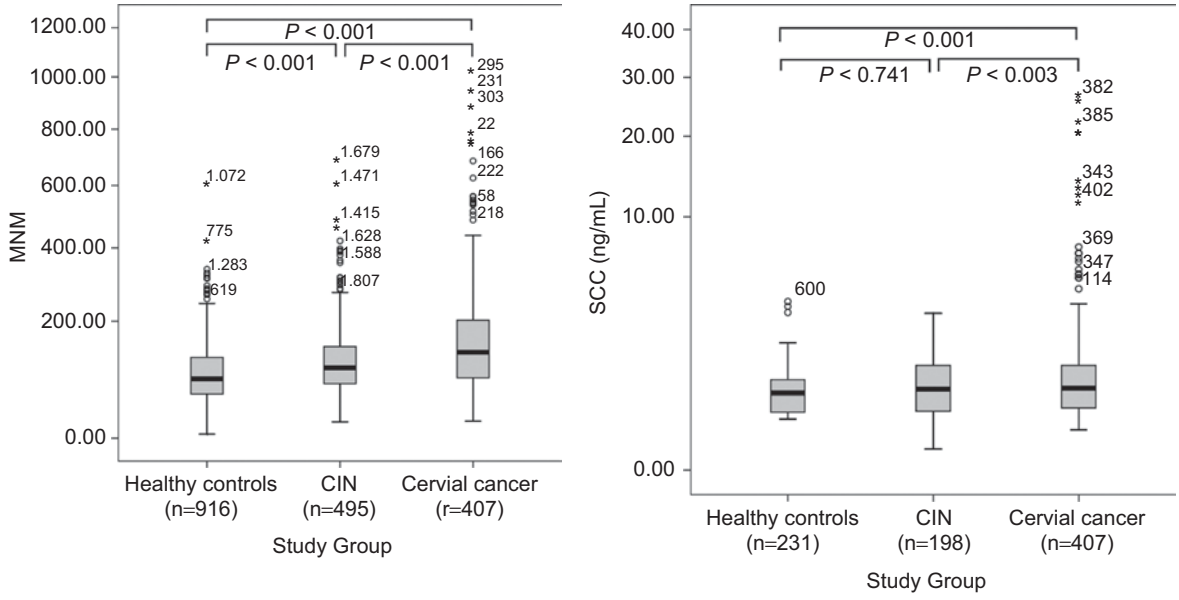

Figure 1. Squamous cell carcinoma (SCC) antigen levels and MNM (multiplication of neutrophil and monocyte counts) in healthy controls and patients with cervical cancer and cervical intraepithelial neoplasia (CIN). MNM was significantly higher in cervical cancer patients than in CIN patients or healthy controls, but there was no significant difference between the CIN and control group in mean SCC antigen levels.

Table 4. Diagnostic sensitivity and specificity of SCC antigen, NLR and MNM in cervical cancer.

	SCC[a]	NLR	MNM
Cervical cancer ($n=407$)			
AUC	0.565	0.616	0.703
Sensitivity	0.171	0.417	0.531
Specificity	0.961	0.792	0.781
PPV	0.852	0.472	0.518
NPV	0.468	0.754	0.789
Cut-off[b]	1.5 ng ml^{-1}[c]	2.43	123.9
Early-stage cervical cancer ($n=375$)			
AUC	0.547	0.608	0.694
Sensitivity	0.139	0.408	0.514
Specificity	0.961	0.791	0.780
PPV	0.809	0.446	0.490
NPV	0.485	0.766	0.797
Cut-off[b]	1.5 ng ml^{-1}[c]	2.43	123.9

MNM, multiplication of neutrophil and monocyte counts (neutrophil count × monocyte count/10 000); NLR, neutrophil to lymphocyte ratio (neutrophil count/lymphocyte count); AUC, area under the curve.
[a]Serum squamous cell carcinoma (SCC) antigen levels were available for all cervical cancer patients and 231 healthy controls. [b]Cut-off value that maximized the sum of sensitivity and specificity in the ROC curve. [c]A fixed cut-off value of 1.5 ng ml^{-1} was used for SCC antigen.

a corresponding specificity of 79.2% and 79.1% in all-cervical cancer and early-stage cervical cancer cases, respectively.

We next assessed the diagnostic significance of MNM along with SCC antigen for predicting cervical cancer. In cervical cancer patients, SCC antigen was not elevated in 337 (82.9%) out of 407 patients. Of the 337 patients who were false negative for SCC antigen, 188 patients (55.8%) were MNM positive (Figure 2). Subgroup analysis was also conducted for various stages of cervical cancer (Table 5). The results were similar in all subgroups according to disease severity. More than 50% of patients across all subgroups had an MNM level above the cut-off (123.9), while approximately 10% of patients had SCC levels greater than the cut-off (1.5 ng ml^{-1}). In FIGO stage IA patients in particular, MNM was above the cut-off level in 81 out of 136 patients (59.6%). In contrast, SCC antigen level was only elevated above the cut-off value in 14 patients (10.3%). Of the remaining 122 patients, 79 (64.8%) were MNM positive.

Prognostic significance of MNM in cervical cancer

Clinicopathological and outcome information and marker values for SCC antigen and MNM were available for 407 cervical cancer patients who were monitored for survival and recurrence. The mean follow-up time was 37.8 months. Seven patients (1.7%) died within this period, 32 (7.9%) survived but suffered recurrence and 368 (90.7%) showed no evidence of disease after treatment. In the recurrent disease group ($n=38$), the mean time to recurrence after initial treatment was 19.2 months.

Kaplan–Meier estimates of survival for patients with different MNM levels are shown in Figure 3. The disease-free survival rates for MNM-positive patients (>123.9) were significantly lower than the survival rates of NLR-negative patients ($p<0.001$). A Cox univariate proportional hazards analysis showed that MNM positive, SCC positive, age, stage, lymph node metastasis, parametrium

Table 5. Diagnostic significance of MNM along with SCC antigen in various stages of cervical cancer.

	MNM positive	SCC positive	SCC negative + MNM positive
Stage I+II+III+IV ($n=407$)	216 (53.1)	70 (17.1)	188/337 (55.8)
Stage IA+IB+IIA ($n=375$)	193 (51.5)	56 (14.9)	175/319 (54.8)
Stage IA+IB ($n=348$)	183 (52.5)	45 (12.9)	171/303 (56.4)
Stage IA ($n=136$)	81 (59.6)	14 (10.3)	79/122 (64.8)

Data are expressed as number of patients with percentages in parentheses.
MNM, multiplication of neutrophil and monocyte counts (neutrophil count × monocyte count/10,000); SCC, squamous cell carcinoma.

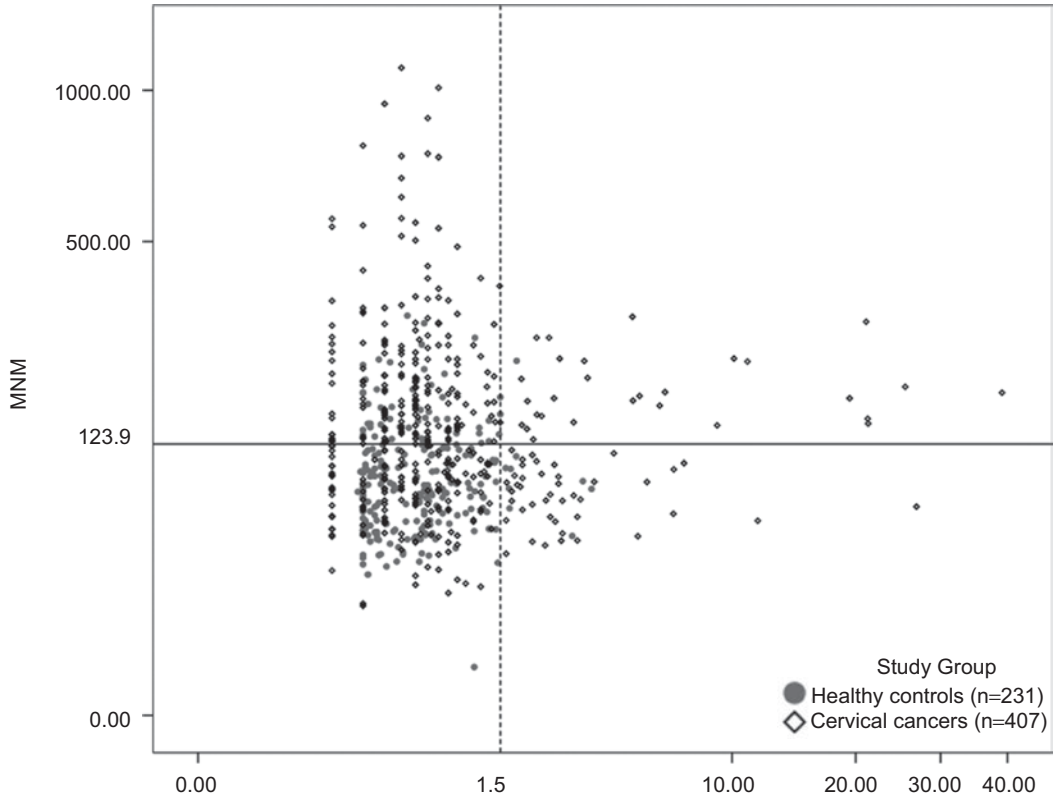

Figure 2. Composite analysis of MNM (multiplication of neutrophil and monocyte counts) (y-axis) and squamous cell carcinoma (SCC) (x-axis) levels in patients with cervical cancer (◊) and healthy controls (•). The dotted line indicates the optimal cutoff value (123.9) of MNM that maximizes the sum of sensitivity and specificity, and the solid line indicates the cutoff value (1.5 ng/mL) of SCC.

invasion, lymphovascular space invasion and tumour size were related to poor disease-free survival. On multivariate analysis, MNM positive (hazard ratio = 2.82 (95% CI, 0.97–8.16), $p=0.042$), stage (hazard ratio = 4.57 (95% CI, 1.59–13.15), $p=0.005$), lymph node metastasis (hazard ratio = 2.22 (95% CI, 0.75–6.60), = 0.047) and tumour size (hazard ratio = 1.48 (95% CI, 1.77–20.71), $p=0.001$) were independent predictors of poor prognosis (Table 6).

Discussion

In the present study, we found that pretreatment serum neutrophil and monocyte counts were elevated in cervical cancer patients. Combining these two parameters into the marker MNM yielded a higher sensitivity than SCC antigen in detecting cervical cancer, with an overall sensitivity and specificity of 53.1% and 78.1%, respectively, based on a cut-off value of 123.9. In addition, patients with an elevated MNM at diagnosis had significantly worse disease-free survival rates.

Chronic inflammation has become a recognized risk factor for epithelial-derived malignancies (Brower 2005). We have only begun in the past decade, however, to understand the complexities of the tumour inflammatory microenvironment and the host's response to tumour-induced inflammatory pathways, resulting in an improved ability to prevent and treat malignancy. The immune response towards cancer is very complex, as the different cell types interact and influence each

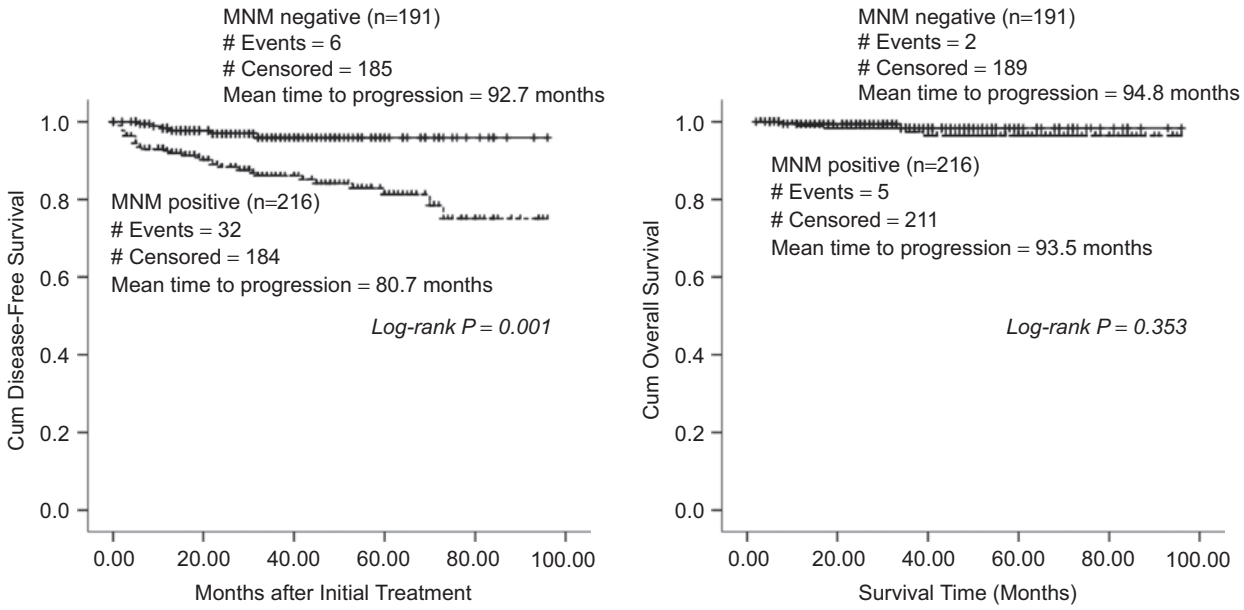

Figure 3. Kaplan-Meier disease-free survival curves (P = 0.001) and overall survival (P = 0.353) for patients with cervical cancer. MNM positive (> 123.9), n = 216, dotted line; MNM negative (≤ 123.9), n = 191, broken line.

Table 6. Univariate and multivariate analyses of the associations between prognostic variables and disease-free survival in 407 cases of cervical cancer.

	Univariate analysis	Multivariate analysis
SCC positive (>1.5 ng ml^{-1})	6.05 (3.04–12.05), <0.001	NS
NLR positive (>2.43)	NS	NS
MNM positive (>123.9)	4.49 (1.87–10.75), 0.001	2.82 (0.97–8.18), 0.042
Age	1.03 (1.00–1.07), 0.014	NS
Stage	12.19 (6.32–23.51), <0.001	4.57 (1.59–13.15), 0.005
Lymph node metastasis	5.65 (2.50–12.8), <0.001	2.22 (0.75–6.60), 0.047
Parametrium invasion	18.04 (7.42–43.90), <0.001	NS
Lymphovascular space invasion	2.84 (1.30–6.17), 0.008	NS
Histological type	NS	NS
Tumour size	1.94 (1.67–2.25), <0.001	1.48 (1.17–1.87), 0.001

Data are presented as disease-free survival hazard ratio (95% confidence interval), *p*-Value; MNM, multiplication of neutrophil and monocyte counts (neutrophil count × monocyte count/10,000); NLR, neutrophil to lymphocyte ratio (neutrophil count/lymphocyte count); NS, not significant.

other (Eiben et al. 2002). In our study, analyses of WBC subtypes in cervical cancer revealed significantly higher neutrophil counts, monocyte counts and NLR compared with the CIN and control groups. However there was no significant difference in pretreatment serum lymphocyte, eosinophil and basophil counts between cervical cancer patients and healthy controls.

The explanation for the association between high neutrophil counts and CIN or cervical cancer remains uncertain. Neutrophils express membrane receptors necessary for the recognition and elimination of microorganisms and tumour cells. Additionally, an elevated neutrophil count may aid in the development and progression of the neoplasm by providing a supportive environment for growth. Circulating neutrophils have been shown to contain and secrete the vast majority of circulating vascular endothelial growth factor (VEGF), a proangiogenic factor that is thought to play an integral role in tumour development (Kusumanto et al. 2003). The association between elevated monocyte counts and cervical carcinogenesis is probably complex and also largely undefined, but a possible explanation does exist. Circulating blood monocytes supply peripheral tissues with macrophage and dendritic cell (DC) precursors and, in the setting of infection, also contribute directly to immune defence against microbial pathogens. The HPV infectious cycle is confined to the epithelial compartment, and virus particles are shed from mucosal surfaces far from vascular and lymphoid channels. As a result, virus particles and viral proteins seldom reach the lymph nodes where adaptive immune responses are initiated. In contrast, stromal dendritic cell are activated

by virus-like particles (VLPs) in HPV infection (Da Silva et al. 2007). Therefore, we suspect that macrophage chemoattractants could be derived from dysplastic cervical cells or the surrounding stroma, and these may be responsible for the high monocyte counts in CIN and cervical cancer. Punnonen et al. (1998) identified high concentrations of CSF-1 (also known as macrophage-colony stimulating factor (M-CSF)), which is responsible for promoting differentiation, proliferation and activation of mononuclear phagocytes, in the peritoneal fluid and serum of patients with cervical cancer. Adam et al. (1999) also associated a single infection by HPV with an increase of CSF-1 serum levels.

In our previous study, we observed that pretreatment serum neutrophil counts were significantly higher and lymphocyte counts were significantly lower in ovarian cancer patients than in other groups, and we found NLR to be the strongest marker for detecting ovarian cancer and predicting poor survival (Cho et al. 2009). In the present study, however, NLR was diagnostically inferior to MNM or monocyte counts. NLR was significantly elevated in cervical cancer patients compared with CIN or healthy controls, but it did not correlate with poor disease-free survival on Cox multivariate analyses. The reasons for the discrepancy in the diagnostic and prognostic value of NLR between the two studies may be explained by the fact that the malignant neoplasm examined in our previous study was located in a different organ and was of mostly advanced stages (approximately 70% of ovarian cancer cases were FIGO stage III, IV or recurrent). It is possible that lymphocyte counts tend to decrease with disease progression, with lower levels in advanced-stage disease than in early-stage disease. Moreover, the biological behaviours of malignant tumours may differ according to the anatomic site of the tumour.

In the current study, we compared the utility of several serum markers, including SCC antigen, MNM, NLR and neutrophil and monocyte counts in diagnosing various stages of cervical cancer. Although serum SCC antigen is often elevated in advanced cervical cancer, the low sensitivity of the SCC antigen assay limits its usefulness for detecting early-stage disease. The sensitivity and specificity of SCC antigen with a cut-off level of 1.5 ng ml^{-1} in this study was 17.1% and 96.1%, respectively. As expected, the sensitivity dropped to 10.3% for FIGO stage IA patients. In previous research with various proportions of disease states, elevated SCC antigen levels have been found in 28–88% of patients with squamous cell carcinoma of the cervix, and the different positivity rates among the studies reflect differences in the selected cut-off value for the antigen (ranging from 1.5 to 2.5 ng ml^{-1}) and in patient characteristics (Chou et al. 1994, Massuger et al. 1997). Our cervical cancer populations consisted of 348 (85.5%) FIGO stage I and 50 (12.3%) FIGO stage II patients and we suspect that the main difference between our results and the previous studies may lie in the proportions of early-stage disease.

In contrast to SCC antigen, pretreatment serum MNM was a useful discriminative marker for early-stage cervical cancer in this study. The neutrophil and monocyte counts in cervical cancer patients were significantly higher than those of CIN and healthy controls and were correlated with tumour stage and tumour size, leading us to combine them into the marker MNM. Compared with SCC antigen, NLR and other leukocyte subsets, MNM showed the highest AUC in both the all-cervical cancer and early-stage cervical cancer groups. MNM was particularly effective in detecting FIGO stage IA patients. These results indicate that the combined marker, MNM, has better diagnostic value for patients in all stages of disease than the other serum markers tested in this study, and it has the best value for patients in early-stage disease. MNM was also able to discriminate between patients with cervical cancer and CIN and those with CIN and healthy controls. The mean MNM was highest in the cervical cancer group, followed by the CIN group and the healthy control group. MNM was superior to WBC subtypes, in particular NLR, and SCC antigen in predicting cervical cancer at different tumour stages.

There are several well-known prognostic factors in cervical cancer, including clinical stage, spread of the cancer to lymph nodes, tumour size and depth of invasion (Kristensen et al. 1999, Singh & Arif 2004). With respect to serum tumour markers, several roles for serum SCC antigen in the clinical management of cervical cancer patients have been suggested. Some authors have shown that it has no prognostic value (Abe et al. 1999, Bolger et al. 1997) whereas others found that higher pretreatment serum SCC antigen level is a strong predictor of shorter disease-free and overall survival (Avall-Lundqvist et al. 1992, Duk et al. 1996, Strauss et al. 2002).

In the present study, elevated MNM was shown to be a prognostic indicator of poor outcome in cervical cancer. There was, however, no significant relationship between NLR and recurrence of cervical cancer. It may be that an elevated NLR reflects a compromised acquired immunity, as higher NLR was associated with an increase in lymphopenia. In contrast, an elevated MNM may reflect an altered innate immunity, as it represents increased neutrophil and monocyte counts. Our results, therefore, suggest that activation of the innate immune system, rather than downregulation of the acquired immune system, is the most important factor in determining poor outcome in cervical cancer patients. Irrespective of the mechanisms involved, we believe that the presence or absence of a systemic inflammatory response as measured by MNM should be evaluated prior to treatment in patients with cervical cancer and should be used in the

risk stratification of such patients. The ability to successfully predict poor prognosis in cervical cancer patients using MNM would be valuable in directing both pre- and postoperative therapies to improve outcome. These results may also offer insight into the nature of the relationship between the systemic inflammatory response and survival in patients with cervical cancer.

Our sample size was large, making our results statistically valid. In addition to MNM serum levels, commonly accepted prognostic factors such as clinical stage, lymph node metastasis and tumour size were found to be independently associated with disease-free survival. However, there were only nine patients (2.2%) with FIGO stage III or IV disease, and so the effect of FIGO stage on overall survival did not reach statistical significance in our analysis (data not shown).

In summary, neutrophil and monocyte counts in cervical cancer patients were significantly higher than in CIN patients or healthy controls. A combination marker, MNM, was a superior serum marker for detecting early-stage cervical cancer compared with SCC antigen and other components of the systemic inflammatory response such as NLR or WBC subtypes. In addition, patients with an elevated MNM at diagnosis had significantly worse disease-free survival rates. Although MNM cannot provide all of the necessary information for optimal cervical cancer diagnosis and prognosis, pretreatment serum MNM measurement should become part of the routine diagnosis of early-stage cervical cancer. MNM has a major advantage in that it can be easily calculated from data that are already routinely available with no additional costs or testing.

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