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Neutrophil to lymphocyte with monocyte to lymphocyte ratio and white blood cell count in prediction of lung cancer

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RESEARCH

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ABSTRACT

Background
Lung cancer is the most common cause of cancer deaths in both sexes, while it is very difficult for screenings and early detection.

Aims
This study aims to clarify the role of systematic inflammation markers, including white blood cell (WBC), neutrophil (NEU), monocyte (MONO), platelet (PLT), neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR) and platelet to lymphocyte ratio (PLR) in prediction of lung cancer.

Methods
A case-control study was conducted on 1,315 primary lung cancer patients and 1,315 healthy adults with matched age and gender at Cho Ray hospital. NLR, MLR and PLR were calculated by using neutrophil, lymphocyte, monocyte and platelet count which were recalled from laboratory database. With 600 cases in the derivation set, the logistic regression with univariate analysis was used to identify the impacted marker, then developing the optimal prediction model for lung cancer by logistic regression with multivariate method. The diagnostic values of optimal model consisting of sensitivity (Sen), specificity (Spe), positive predictive value (PPV), negative predictive value (NPV) and the area under the ROC curve (AUC) value were extracted and verified on all data, in validation set.

Results
The median values of WBC, NEU, MONO, PLT, NLR, MLR and PLR in lung cancer were not significantly difference between histological subtypes and clinical stages (p>0.05), but higher than the values in control group (p<0.01). Multivariates analysis shows that NLR, MLR and WBC were three parameters that have the significant impact of the optimal prediction model (p<0.01). The AUC value, sensitivity and specificity of the optimal model for lung cancer detection were 0.881, 73.5 per cent (95 per cent CI:70.3–76.6) and 87.7 per cent (95 per centCI:85.2–89.9), respectively. Whereas, the PPV and NPV values of prediction model were 85.7 per cent (95 per cent CI:82.8–88.2) and 76.8 (95 per centCI:73.9–79.5), respectively. Among three biomarkers, the AUC values of NLR (0.853) and MLR (0.842) were higher than the value of WBC (0.752) (p<0.01).

Conclusion
The results of this study show that NLR with MLR and WBC in optimal prediction model are promising biomarkers for lung cancer screening that could be applied in clinical practice with the advantage of convenience and low cost.

Key Words
NLR, MLR, lung cancer diagnosis

What this study adds:

1. What is known about this subject?

High elevated level of inflammatory markers such as NLR, MLR or PLR were correlated with various types of solid tumours.

2. What new information is offered in this study?

An investigation on large number of patients shows that NLR, MLR and WBC are promising markers in screening for lung cancer with high diagnostic values.

3. What are the implications for research, policy, or practice?

Further research on added groups such as benign polyp or pneumonia patients should be made, and NLR or MLR should be used in combination with conventional serum biomarkers.

Background

Lung cancer is a leading cause of cancer deaths worldwide.[1] This cancer develops silently with no specific symptoms, while it is difficult for screening and early detection. Several serum biomarkers such as cyfra 21-1 (cytokeratin 19), CA12-5 (cancer antigen 125), carcinoembryonic antigen (CEA) or neurone specific enolase (NSE) are used frequently in diagnosis and treatment monitoring for lung cancer but with limited sensitivity and specificity.[2] Beside, the imaging diagnostic tools as CT-Scanner (computerised tomography), PET-CT (positron emission tomography - computed tomography) or MRI (magnetic resonance imaging) are also used frequently, but they have quite high cost and potential risks from radioactive rays. Many studies recently show that systematic inflammation index such as neutrophil absolute count (NEU), monocyte absolute count (MONO), neutrophil to lymphocyte ratio (NLR), monocyte to lymphocyte ratio (MLR), platelet absolute count (PLT) or platelet to lymphocyte ratio (PLR) are seem to be the promising markers in solid tumours including lung cancer.[3-7] This study aims to identify the valuable factors from above markers which help in discrimination lung cancer from healthy adults.

Method

Study populations and parameters

The study was conducted following case-control method which consists of 1,315 primary lung cancer patients and 1,315 healthy adults with matched age and gender. Lung cancer was confirmed by Hematoxylin-Eosinophil staining on biopsy sample at the Pathology Department, Cho Ray hospital from July 2014 to December 2016. All healthy adults was classified as Class I following to Annual Examination Criteria of Cho Ray hospital. The number of white blood cell, neutrophil, lymphocyte, monocyte and platelet of healthy adults and lung cancer patients at diagnosis were recalled from the laboratory database and calculated the inflammation index of NLR, MLR and PLR. The blood cell analysis was performed on UniCel DxH 800 (Beckman Coulter, CA, USA), and CELL-DYN Sapphire system (Abbott, Illinois, USA).

Statistical analysis

The data was checked for probability of normal distribution by Skewness and Kurtosis test. The Kruskal-Wallis rank test was used to compare the median value of each marker between groups. To develop the prediction model for lung cancer, a group of 600 cases consisting of 300 male and 300 female patients, and 600 control subjects with matched age and gender was chosen randomly for analysis in derivation set. The logistic regression with univariate analysis was used to identify the marker which has the significant impact of prediction model (p<0.2). Then, the logistic regression with multivariate analysis was used for developing the optimal model. The diagnostic values of optimal model consisting of sensitivity (Sen), specificity (Spe), positive predictive value (PPV), negative predictive value (NPV) and the area under the ROC curve (AUC) value were extracted and then verified in validation set with all data. For further analysis, the ROC curve of each markers was built for defining the optimal cut-off point, together with diagnostic values, and for comparing the AUC values between biomarkers. All data analysis was performed on STATA statistical software v.14.0 (Lakeway Drive College Station, Texas, USA). P<0.05 was regarded as significant statistic.

Results

Clinical and paraclinical characteristics

The median age of patients in this study was 54 (from 18 to 69 years old). Among 1,315 cases, 822 were male patients (account for 62.6 per cent), which is more than the number of female patients (37.4 per cent). Most of patients was classified as adenocarcinoma (1,132 cases; 86.1 per cent), and as stage IV (1,017 cases; 77.3 per cent) according to TNM classification system (Table 1). There were 149 cases (11.3 per cent) with poor differentiated tumour tissue.

The results of Skewness and Kurtosis test (p<0.05) shown that the data of this study was not normally distributed. Therefore, we chose the Kruskal-Wallis rank test to compare and seek any significant difeerences of parameters between groups. The results were presented as median value with 95 per cent confidence interval, and shown in Table 1.

Adeno: Adenocarcinoma; Larg: Larg cell carcinoma; Small: Small cell carcinoma; Squa: Squamous cell carcinoma; Mod: moderate; NC: Non-classified; ǂ: lung cancer compared to control group; ¥: between histological types; ↿: between grades of differentiation; £: stage IV compared to stage I-III.

The results demonstrated that the median values of WBC, NEU, MONO, PLT, NLR, MLR and PLR of lung cancer patients are significantly higher than the values of control group (p<0.01). Meanwhile, the median value of lymphocyte in patient group is significantly lower than the value in the control group (p<0.01). In lung cancer patients, the median values of study parameters are not statistically significant difference between group of histological types, grades of differentiation, and clinical stages (p>0.05).

Developing the prediction model for lung cancer

The prediction model for lung cancer was developed in derivation set with 600 lung cancer patients and 600 control subjects. By univariate analysis, we recorded five parameters that have the impact of prediction model consisting of WBC, NEU, MONO, NLR and MLR. Continuing with multivariate analysis, we noted that the optimal prediction model consist of three parameters including NLR, MLR and WBC. The Akaike Information Criterion value (AIC) of optimal prediction model (1315.7) was lowest of analysed values. The Odds ratio of three parameters were estimated and presented in Table 2. We noted that all three Odds ratios of parameters is higher than 1.0, with all low levels of 95 per cent confident interval is higher than 1.0 (p<0.01).

Table 2: Odds ratio of NLR, MLR and WBC in multivariate analysis

Parameter	OR	95%CI	P-value
WBC	1.17	1.09-1.24	<0.01
NLR	1.98	1.69-2.33	<0.01
MLR	764.3	147.3-3961.5	<0.01

We also recorded the Pseudo R^2 value of optimal model was 0.394 that fitting with expected value >0.05. However, the variance inflation factor (VIF) value of optimal model which was higher than 2 (3.55) indicated the possibility of existence of the multicollinearity in prediction model. This phenomenon might be due to the co-existance of WBC, NEU and MONO in a same diagnostic test, the complete blood count.

The diagnostic values of prediction model in derivation set were extracted and presented in Table 3. The area under the ROC curve of prediction model was quite high (0.895) whereas the sensitivity reached at 74.8 per cent, and specificity reached at 87.1 per cent.

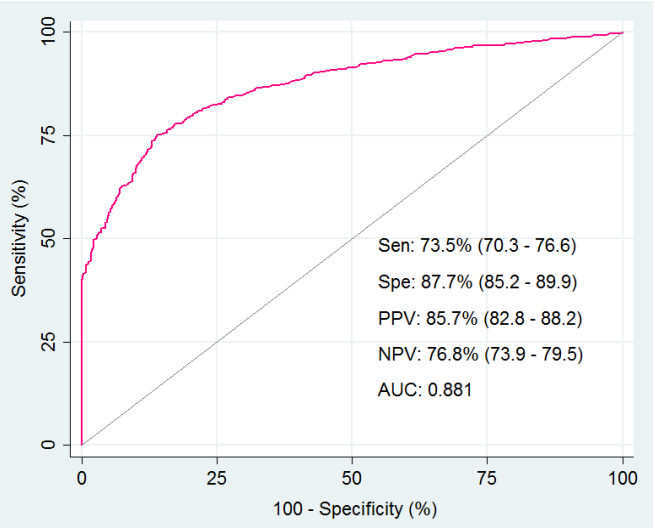
Table 3: The diagnostic values of prediction model in derivation set

Diagnostic value	Results (95%CI)
Sensitivity, %	74.8 (70.0-79.2)
Specificity, %	87.1 (83.2-90.3)
Positive predictive value, %	85.2 (80.8-88.9)
Negative predictive value, %	77.6 (73.3-81.5)
AUC	0.895

The diagnostic values of optimal prediction model and each inflammatory markers

To verify the performance of optimal prediction model, all data (1,315 cases) was used for analysis in validation set. The diagnostic values were extracted and shown in Figure 1. We noted that the diagnostic values in validation set are equal to the values in derivation set. The combination of NLR, MLR and WBC can help to discriminate lung cancer from healthy adults with correctly classified rate as 80.5 per cent (AUC: 0.881), whereas the sensitivity and specificity reached at 73.5 per cent and 87.7 per cent, respectively. The PPV and NPV values of prediction model were also high (85.7 per cent and 76.8 per cent, respectively).

Figure 1: The area under the ROC curve and diagnostic values of prediction model in validation set



For comparing the diagnostic values between three biomarkers, the ROC curve of each markers was built for

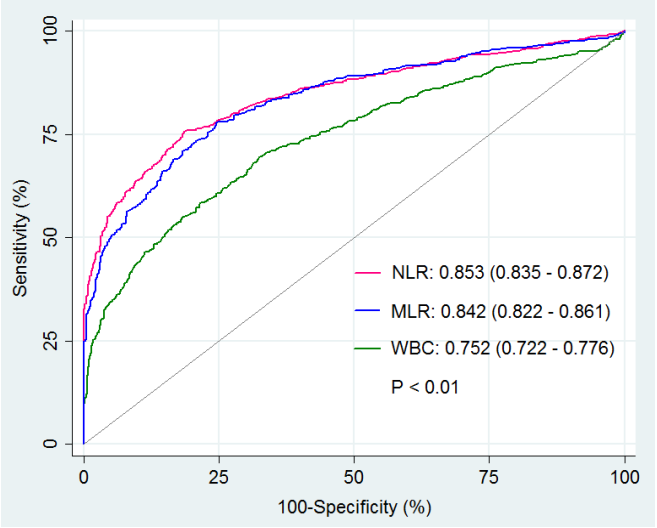
defining the optimal cut-off point, together with sensitivity, specificity, PPV, NPV and AUC values.

Table 4: The optimal cut-off point and diagnostic values of NLR, MLR and WBC separately

Diagnostic value	WBC, 10^9/L	NLR	MLR
Cut-off	8.9	2.94	0.32
Sen, % (95%CI)	60.3 (56.8-63.8)	67.7 (64.3-71.0)	68.1 (64.7-71.4)
Spe, % (95%CI)	78.6 (75.6-81.4)	88.9 (86.4-91.0)	84.9 (82.2-87.3)
PPV, % (95%CI)	73.8 (70.2-77.2)	85.9 (82.9-88.5)	81.8 (78.7-84.7)
NPV, % (95%CI)	66.5 (63.3-68.5)	73.4 (70.4-76.2)	72.7 (69.7-75.6)

At the optimal cut-off point of 2.94, NLR can help in screening lung cancer with moderate sensitivity (67.7 per cent) but high specificity (88.9 per cent) (Table 4). These values of MLR were 68.1 per cent and 84.9 per cent equivalent to the cut-off point of 0.32, and of WBC were 60.3 per cent and 78.6 per cent equivalent to the cut-off point of 8.9, respectively. Among three biomarkers, the AUC values of NLR (0.853) and MLR (0.842) were higher than the value of WBC (0.752) (p<0.01) (Figure 2).

Figure 2: Comparison of the AUC values of NLR, MLR and WBC in lung cancer screening



Discussion

In this study we noted that the median values of WBC, NEU, MONO, PLT, NLR, MLR and PLR in lung cancer patients were not difference between histological subtypes and clinical stages, but higher than the values of control group. These results are consistent with the results of other study.[4] The high level of inflammation markers in lung cancer patients compared to healthy controls is because of the increased secretion of some cytokines and chemokines for granulocyte and platelet differentiation by tumour cells, such as GM-CSF (granulocyte-macrophage colony stimulating marker), GCSF (granulocyte colony stimulating marker), IL1 (interleukin 1), IL6 (interleukin 6), ADP (adenosine diphosphate), Thrombin, TXA_2 (Thromboxane A_2) or Mucin.[8,9] By this way, the tumour cells can attract, control and utilize neutrophil, monocyte or platelet in a manner benefiting to the development.[8,9] Based on this principle, the increase of absolute count of white blood cell, platelet or inflammatory indexes as NLR, MLR or PLR are indicators that help in predicting lung cancer.

From previous studies, there are many diagnostic and prognostic models that are useful for lung cancer as combination of NLR with PLR,[3,4,10,11] NLR with PLT,[12] or NEU with MONO.[13] In this study, we noted that the optimal prediction model for lung cancer consisted of three biomarkers, NLR, MLR and WBC. With results of high diagnostic values, our study once again confirmed the finding of previous studies.[3,4] These results also indicated that this model could be applied in clinical practice for lung cancer screening, with advantages of convenience and low cost compared to the conventional biomarkers as CEA, CYFRA 21-1 or CA19-9. However, it is better that the use of this model in combination with conventional markers might help to increase the diagnostic values. This is also recorded in other studies.[14] With limitation of this study, we suggested a further research which conducting on added groups such as benign polyp patients or pneumonia patients. And in that study, the inflammation markers as NLR or MLR should be used in combination with conventional serum biomarkers.

Conclusion

The results of this study show that NLR with MLR and WBC in optimal prediction model are promising biomarkers for lung cancer screening and that could be applied in clinical practice with the advantage of convenience and low cost.

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PEER REVIEW

Not commissioned. Externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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ETHICS COMMITTEE APPROVAL

Ethics committees of Cho Ray hospital.

Table 1: The median values of study parameters in lung cancer patients and healthy controls

Group	WBC, 10^9/L	NEU, 10^9/L	LYM, 10^9/L	MONO, 10^9/L	PLT, 10^9/L	NLR	MLR	PLR
Control	7.0 (6.9-7.1)	3.74 (3.62-3.84)	2.33 (2.28-2.38)	0.44 (0.43-0.45)	233 (228-237)	1.62 (1.57-1.65)	0.19 (0.18-0.20)	101.6 (98.6-103.8)
Lung cancer	9.2 (8.9-9.4)	6.10 (5.76-6.38)	1.75 (1.69-1.82)	0.62 (0.60-0.64)	274 (264-283)	3.46 (3.23-3.77)	0.36 (0.34-0.38)	151.9 (146.4-158.7)
Histological type								
Small, n=42	8.9 (7.5-12.7)	7.12 (4.95-8.11)	1.71 (1.07-2.33)	0.51 (0.44-0.80)	243 (201-425)	3.66 (2.69-6.61)	0.31 (0.21-0.62)	150.1 (103.7-219.7)
Adeno, n=1132	9.3 (9.0-9.6)	6.24 (5.85-6.63)	1.73 (1.68-1.81)	0.63 (0.60-0.65)	274 (265-285)	3.58 (3.31-3.85)	0.37 (0.35-0.39)	150.9 (144.8-158.8)
Squa, n=84	9.0 (7.5-10.2)	5.82 (4.77-6.70)	1.86 (1.55-2.20)	0.63 (0.56-0.68)	292 (242-318)	3.48 (2.39-4.22)	0.34 (0.30-0.39)	162.2 (130.7-182.1)
Larg, n=14	7.6 (5.8-14.5)	4.83 (3.72-12.91)	1.73 (1.31-3.17)	0.45 (0.19-1.18)	246 (98-474)	3.07 (0.98-8.12)	0.28 (0.11-1.05)	143.2 (68.8-300.3)
NC, n=43	8.5 (7.9-9.4)	6.62 (5.11-7.23)	1.91 (1.67-2.03)	0.58 (0.52-0.65)	265 (242-310)	3.26 (2.71-3.79)	0.34 (0.28-0.38)	155.2 (127.9-182.5)
Grade of differentiation								
Poor, n=149	8.5 (7.5-9.6)	5.68 (4.92-6.43)	1.77 (1.56-1.97)	0.56 (0.51-0.63)	284 (252-311)	3.33 (2.55-3.94)	0.32 (0.28-0.37)	155.4 (144.9-170.7)
Mod, n=41	8.5 (7.3-11.2)	5.59 (4.85-8.80)	1.52 (1.12-1.71)	0.62 (0.48-0.73)	258 (185-301)	4.29 (2.66-6.11)	0.42 (0.34-0.57)	152.0 (131.8-221.5)
NC, n=1125	9.2 (8.9-9.5)	6.18 (5.81-6.46)	1.78 (1.71-1.85)	0.62 (0.60-0.65)	274 (265-283)	3.47 (3.21-3.77)	0.36 (0.34-0.38)	151.5 (144.9-158.6)
Clinical stage								
I-III, n=298	9.1 (8.7-9.4)	5.90 (5.69-6.28)	1.77 (1.68-1.83)	0.61 (0.57-0.63)	278 (260-291)	3.42 (3.16-3.69)	0.36 (0.33-0.38)	150.2 (143.1-156.8)
IV, n=1017	9.4 (9.1-10.0)	6.47 (6.96-7.03)	1.68 (1.59-1.80)	0.62 (0.59-0.66)	282 (264-290)	3.59 (3.21-4.31)	0.39 (0.35-0.42)	158.3 (148.4-173.3)
P-value	<0.01‡ 0.33¥ 0.27[1] 0.26£	<0.01‡ 0.19¥ 0.27[1] 0.21£	<0.01‡ 0.58¥ 0.06[1] 0.23£	<0.01‡ 0.37¥ 0.14[1] 0.44£	<0.01‡ 0.66¥ 0.22[1] 0.21£	<0.01‡ 0.22¥ 0.12[1] 0.20£	<0.01‡ 0.25¥ 0.07[1] 0.09£	<0.01‡ 0.95¥ 0.64[1] 0.05£