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Correlation of Leukocyte Subtypes, Neutrophil-to-Lymphocyte Ratio, and Functional Outcome in Brain Metastasis

Kiking Ritarwan[1], Irina Keumala Nasution[1], Iswandi Erwin[1], Nerdy Nerdy[2*]

[1]Department of Neurology, Faculty of Medicine, Universitas Sumatera Utara, Padang Bulan, Medan Baru, Medan, Sumatera Utara, Indonesia; [2]Department of Pharmaceutical Chemistry, Academy of Pharmacy Yayasan Tenaga Pembangunan Arjuna, Pintubosi, Laguboti, Toba Samosir, Sumatera Utara, Indonesia

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***Correspondence:** Nerdy Nerdy. Department of Pharmaceutical Chemistry, Academy of Pharmacy Yayasan Tenaga Pembangunan Arjuna, Pintubosi, Laguboti, Toba Samosir, Sumatera Utara, Indonesia. E-mail: nerdy190690@gmail.com

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Abstract

BACKGROUND: As the most cause of death in patients with solid extracranial malignancy, brain metastasis (BM) nowadays being studied extensively especially on how to find a reliable laboratory marker that can correlate with its clinical outcome. Leukocyte subtypes, primarily neutrophils and lymphocytes and its ratio known as Neutrophils-Lymphocyte Ratio (NLR) have been known before its relationship with progressivity of BM from other solid tumours.

AIM: The objectives of this research to study the correlation of leukocyte subtypes, neutrophil-lymphocyte ratio & functional outcome in brain metastasis.

METHODS: The study subjects were recruited consecutively from the study population. Venous blood was taken 5 ml of venous blood samples done in the first day of admission on emergency department and neurology clinic of Neurology Department of Adam Malik General Hospital before any drug injections. Samples were kept in vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) and sent to Department of Clinical Pathology laboratory of Adam Malik General Hospital, immediately centrifuged at 3100 rpm for 10 minutes in -20°C temperature and analysed using Sysmex XT-2000i. Functional outcome of the patient assessed using Karnofsky performance scale (KPS) in a cross-sectional manner with laboratory analysis.

RESULTS: We conduct a mean differences and correlational leukocytes and its subsets analysis of 72 BM patients resulting on significant positive correlation on lymphocyte percentage ($r = 0.383$, $p = 0.001$) and lymphocyte absolute ($r = 0.265$, $p = 0.024$), also significant negative correlation on neutrophils ($r = -0.240$, $p = 0.042$) and NLR ($r = -0.432$, $p < 0.001$) with Karnofsky Performance Scale (KPS).

CONCLUSION: Increased lymphocyte absolute and lymphocyte percentage correlated significantly ($p < 0.05$) with better KPS, while elevated neutrophils percentage and increased NLR show significant correlation with worse outcome of BM patients.

Introduction

Brain metastasis (BM) is one of the central nervous system consequences of primary extracranial malignancy that mainly happen on 20-40% in adult cancer patients. As one of the main cause of death for these population, its progression is determined by several condition such tumour cell migration (intravasation, dissemination and extravasation) and colonisation also microenvironment inflammation that correlates in the severity of the disease. The basic mechanism of BM begin with Paget's "seed and soil" hypothesis included three main principles: the heterogeneous population of a tumour with different characteristics, specific traits of metastasis and secondary tumoral microenvironment [1], [2], [3], [4].

The human immune system, in general, have several myeloid cells especially leukocyte subtypes which are responsible in cancer metastasis regulation, such neutrophils and monocytes; both considered immunocytes that could secrete vascular endothelial growth factor (VEGF) contributing to increased tumour size and development. While regulation of aspartic proteinase cathepsin E, which act as tumour suppressor activated by lymphocytes, macrophage and a dendritic cell which and apoptosis induction [5], [6].

Neutrophil-to-lymphocyte ratio (NLR) is the ratio of the absolute neutrophil count to the absolute lymphocyte count and used as one of a simple, rapid, cost-effective and indicative marker of inflammatory process and stress of human body as it can be obtained on complete blood count (CBC) analysis.

While normal immune system would prevent the dissemination of cancer cells and prevent proliferation, about 0.01% escaping circulating tumor cells (CTC) survival from primary sites resulting from shear stress, mechanical detachment even cell-mediated cytotoxicity which provoked epithelial to mesenchymal transition (EMT) regulated by tumor necrosis factor α (TNF- α) and nuclear factor κβ (NF-κβ). Individual immunocompetence capability could also be estimated by NLR and correlated with disease progression and functional outcome in malignancy measured by Karnofsky performance scale (KPS) [5]. On this basis, elevated NLR ratio could be consequences of either neutrophilia which promotes tumour granulocyte colony-stimulating factor (GM-CSF) and/or lymphopenia that can cause circulating tumour cell escape systemic immunosurveillance in the human body [5], [6].

Growing interest of NLR count on has been established today as several studies tried to elaborate between this ratio and progression of the disease that implicated by even slight process of inflammation, for examples malignancy in various primary organ (e.g.: small cell lung cancer, renal cell carcinoma, pancreatic cancer, primary liver ca, breast cancer, glioblastoma multiforme), arterial disease (e.g: acute coronary syndrome, cerebral infarction, Takayasu arteritis), degenerative disease (e.g: ankylosing spondylitis) also in some autoimmune disorders (e.g: psoriasis, systemic lupus erythematosus [SLE]) [7], [8], [9], [10], [11], [12], [13], [14], [15], [16], [17], [18], [19].

Methods

Ethical approval received from the Health Research Ethical Committee, Faculty of Medicine Universitas Sumatera Utara, Medan-Indonesia. The research was conducted from January 2016 to December 2016 at Adam Malik General Hospital recruiting 72 consecutive sample of patients diagnosed with BM. Confirmation of BM diagnosis was done by means if there are any clinical neurologic deficits and/or any suspicious prove of in any established modalities (laboratory tumour markers, chest radiography, ultrasonography, computed tomography (CT) scan, radionucleotide tracing and/or another diagnostic test) supporting central nervous system (CNS) involvement from other extracranial primary malignancy [20].

Exclusion criteria in this study including sepsis, septicaemia, bacteremia, septic shock and other systemic inflammatory response syndrome (SIRS)-like condition, leukemia and leukemoid reaction, prior dexamethasone, methylprednisolone and/or any intravenous steroid injection, autoimmune disease (e.g: SLE, scleroderma) or immunodeficient syndrome (e.g: HIV/AIDS) essentials heart,liver and/or kidney disorders, pneumonia, seizure at the beginning or during hospitalization, massive upper and/or lower GI bleeding which all of the above could modify, primarily suppress the baseline immunological condition of the patients [21], [22], [23].

Informed consent was asked from the patients (or their legal responders in any means the patients cannot give consent) and collection of 5 ml of venous blood samples done in the first day of admission on emergency department and/or neurology clinic of Neurology Department of Adam Malik General Hospital before any drug injections. Samples were kept in vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) and sent to Department of Clinical Pathology laboratory of Adam Malik General Hospital, immediately centrifuged at 3100 rpm for 10 minutes in -20o C temperature and analysed using Sysmex XT-2000i. Functional outcome of the patient assessed using Karnofsky performance scale (KPS) in a cross-sectional manner with laboratory analysis.

Data were collected and calculated using IBM SPSS Statistic for Windows, Version 24.0 and test of normality was conducted using Kolmogorov-Smirnov on more than 50 study sample. Variables characteristics were shown in Table 1 while mean differences were compared between groups using one-way analysis of variant (ANOVA) (Table 2), and each variable was correlated with KPS using Pearson's correlation test on the nature of normal distribution of the samples (Table 3).

Result

We divided subjects into 3 categorised KPS groups (KPS 80-100 as symptomatic, KPS 50-70 as symptomatic with assistance and KPS 0-40 as symptomatic with bed confinement). Test of normality was conducted using Kolmogorov-Smirnov test on more than 50 study sample. Variables characteristics of the patients were shown in Table 1.

Table 1: Characteristics of BM Subjects

Variable Characteristics (n = 72)	Mean (Percentage)
Age	51.74 ± 11.17 years old
Minimum	22 years old
Maximum	74 years old
Sex	
Male	30 (41.7%)
Female	42 (58.3%)
Origins of Primary Tumor	
Pulmonary	37 (51.4%)
Breast	17 (23.6%)
Cervix-genito-urinary	10 (13.9%)
Others	4 (5.55%)
Unknown	4(5.55%)
KPS	
80-100	10 (13.89%)
50-70	34 (47.22%)
0-40	28 (38.89%)

Mean differences were also compared between groups using analysis of variant (ANOVA) (Table 2).

Table 2: Means Difference of Leukocyte Subtypes

Variables		Karnofsky Performance Scale (KPS) Groups			P
	Total Subjects	KPS 80-100 (n = 10)	KPS 50-70 (n = 34)	KPS 0-40 (n = 28)	
Mean Leukocytes (x10^3/mm^3)	11.93 ± 5.96	11.48 ± 5.69	11.14 ± 5.38	13.05 ± 6.70	0.446
Mean Neutrophil Percentage (%)	78.28 ± 9.74	73.21 ± 10.34	77.75 ± 8.86	80.74 ± 10.09	0.099
Mean Neutrophil Absolute (x 10^3/µL)	9.57 ± 5.29	8.71 ± 5.01	8.83 ± 4.62	10.79 ± 6.06	0.303
Mean Lymphocyte Percentage (%)	13.18 ± 7.68	17.03 ± 9.39	14.81± 7.89	9.84 ± 5.38	0.008*
Mean Lymphocyte Absolute (x 10^3/µL)	1.40 ± 0.74	1.66 ± 0.74	1.47 ± 0.67	1.23 ± 0.81	0.217
Mean Monocyte Percentage (%)	7.14 ± 3.21	7.04 ± 2.38	7.45 ± 3.42	6.81 ± 3.28	0.739
Mean Monocyte Absolute (x 10^3/µL)	0.90 ± 0.63	0.92 ± 0.66	0.94 ± 0.67	0.84 ± 0.60	0.827
Mean Basophil Percentage (%)	0.31 ± 0.36	0.40 ± 0.36	0.36 ± 0.42	0.21 ± 0.28	0.202
Mean Basophil Absolute (x 10^3/µL)	0.03 ± 0.03	0.04 ± 0.05	0.03 ± 0.03	0.03 ± 0.03	0.424
Mean Neutrophil-to-Lymphocyte Ratio (NLR)	8.23 ± 5.05	5.62 ± 2.94	6.87 ± 4.02	10.82 ± 5.72	0.001*

*significant p-value (< 0.05).

Each variable was then correlated with KPS using Pearson's correlation test on the nature of normal distribution of the samples as seen in Table 3.

Table 3: Pearson's Correlation of Leukocytes Subtypes and KPS

Leukocytes Subtype	Karnofsky Performance Scale	
	r (Pearson's Correlation)	p (Significance)
Leukocytes (x10^3/mm^3)	-0.569	0.625
Neutrophil Percentage (%)	-0.240	0.042*
Neutrophil Absolute (x 10^3/µL)	-0.106	0.375
Lymphocyte Percentage (%)	0.383	0.001*
Lymphocyte Absolute (x 10^3/µL)	0.265	0.024*
Monocyte Percentage (%)	0.100	0.405
Monocyte Absolute (x 10^3/µL)	0.121	0.311
Basophil Percentage (%)	0.188	0.115
Basophil Absolute (x 10^3/µL)	0.153	0.200
Neutrophil-to- Lymphocyte Ratio (NLR)	-0.432	< 0.001*

*significant p-value (< 0.05).

Discussion

On the mean differences and correlational analysis, we found significant differences on mean lymphocyte percentage between symptomatic group (KPS 80-100), symptomatic with assistance group (KPS 50-70) and symptomatic with bed confinement group (KPS 0-40), (17.03 ± 9.39% vs 14.81 ± 7.89% vs 9.84 ± 5.38 %, respectively) with Pearson correlation product moment of lymphocyte percentage and KPS show rather weak but significant positive correlation (r = 0.383; p = 0.001) [24].

Although we were not getting any ANOVA's significant differences on mean neutrophile percentage and meant lymphocyte absolute, there are weak significant negative correlation on neutrophile percentage and KPS (r = -0.240, p = 0.042) also on lymphocyte absolute and KPS show weak significant positive correlation on lymphocyte absolute and KPS (r = 0.265, p = 0.024). This view was in line with my review on multiple tumour progression correlated with tumour-infiltrating neutrophils, as elevated blood neutrophils proven to be a poor prognostic factor for functional outcome [25] also stated that circulating tumour-infiltrating-lymphocyte number elevation also has correlated with favourable clinical outcomes in subsets of human cancer [26].

On NLR variable, we found significant mean differences percentage between symptomatic group (KPS 80-100), symptomatic with assistance group (KPS 50-70) and symptomatic with bed confinement group (KPS 0-40) (5.62 ± 2.94 vs 6.87 ± 4.02 vs 10.82 ± 5.72, respectively) and also significant moderate negative correlation between NLR and KPS (r = -0.432, p < 0.001). Although applied on different population (Glioblastoma Multiforme/ GBM), NLR also has been studied by Alexiou et al., resulting on NLR > 4.7 was associated with decreased survival time (11 vs 18.7 months, p = 0.01) [7].

Templeton et al., allege that high NLR has an adverse effect on overall survival (OS) in many solid tumours [27], and this result was also agreeable with the previous study by Serdarevic et al., [28] on BM and non-BM population of non-small-cell lung cancer (NSCLC) which conclude higher NLR mean on BM group and more progressive condition (6.05 vs 4.6, p = 0.023) [28]. This was also confirmed by another prognostic study of NLR that elevated preoperative NLR is a predictor of worse survival after BM resection (OS 14 month for NLR < 5 and 5 months for NLR ≥ 5, p = 0.001) [27], [29].

In conclusion, increased lymphocyte absolute and lymphocyte percentage correlated significantly with better KPS, while elevated neutrophils percentage and increased NLR show significant correlation with worse outcome of BM patients.

References

1. Berghoff AS, Preusser M. The inflammatory microenvironment in brain metastases: potential treatment target? Chinese clinical oncology. 2015; 4(2). PMid:26112807

2. Maman S, Witz IP. The metastatic microenvironment. The tumour immune environment. Springer: Dordrecht, 2013:15-38. https://doi.org/10.1007/978-94-007-6217-6_2

3. Singh M, Manoranjan B, Mahendram S, McFarlane N, Venugopal C, Singh S. Brain metastasis-initiating cells: survival of the fittest. International journal of molecular sciences. 2014; 15(5):9117-33. https://doi.org/10.3390/ijms15059117 PMid:24857921 PMCid:PMC4057778

4. Svokos K, Salhia B, Toms S. Molecular biology of brain metastasis. International journal of molecular sciences. 2014; 15(6):9519-30. https://doi.org/10.3390/ijms15069519 PMid:24879524 PMCid:PMC4100107

5. Erez N, Coussens LM. Leukocytes as paracrine regulators of

metastasis and determinants of organ-specific colonization. International journal of cancer. 2011; 128(11):2536-44. https://doi.org/10.1002/ijc.26032 PMid:21387299 PMCid:PMC3084629

6. Faria SS, Fernandes Jr PC, Silva MJ, Lima VC, Fontes W, Freitas-Junior R, Eterovic AK, Forget P. The neutrophil-to-lymphocyte ratio: a narrative review. Ecancermedicalscience. 2016; 10.

7. Alexiou G, Vartholomatos E, Zagorianakou P, Voulgaris S. Prognostic significance of neutrophil-to-lymphocyte ratio in glioblastoma. Neuroimmunology and Neuroinflammation. 2014; 1(3):131-5. https://doi.org/10.4103/2347-8659.143666

8. Ataseven A, Bilgin AU, Kurtipek GS. The importance of neutrophil lymphocyte ratio in patients with psoriasis. Materia socio-medica. 2014; 26(4):231-3. https://doi.org/10.5455/msm.2014.231-233 PMid:25395882 PMCid:PMC4214808

9. Ayna, AB, Ermurat S, Coşkun BH, Harman H, Pehlîvan Y. Neutrophil to Lymphocyte Ratio and Mean Platelet Volume as Inflammatory Indicators in Systemic Lupus Erythematosus Nephritis. Archives Rheumatology. 2016; 32(1): 21-25. https://doi.org/10.5606/ArchRheumatol.2017.5886 PMid:30375538 PMCid:PMC6190939

10. Azab B, Bhatt VR, Phookan J, Murukutla S, Kohn N, Terjanian T, Widmann WD. Usefulness of the neutrophil-to-lymphocyte ratio in predicting short-and long-term mortality in breast cancer patients. Annals of surgical oncology. 2012; 19(1):217-24. https://doi.org/10.1245/s10434-011-1814-0 PMid:21638095

11. Chrom P, Stec R, Semeniuk-Wojtas A, Bodnar L, Spencer NJ, Szczylik C. Fuhrman grade and neutrophil-to-lymphocyte ratio influence on survival in patients with metastatic renal cell carcinoma treated with first-line tyrosine kinase inhibitors. Clinical genitourinary cancer. 2016; 14(5):457-64. https://doi.org/10.1016/j.clgc.2016.02.005 PMid:26980234

12. Coşkun BN, Öksüz MF, Ermurat S, Tufan AN, Oruçoğlu N, Doğan A, Dalkılıç E, Pehlivan Y. Neutrophil lymphocyte ratio can be a valuable marker in defining disease activity in patients who have started anti-tumor necrosis factor (TNF) drugs for ankylosing spondylitis. European journal of rheumatology. 2014; 1(3):101-103. https://doi.org/10.5152/eurjrheumatol.2014.034 PMid:27708888 PMCid:PMC5042217

13. Gürol G, Ciftci IH, Terizi HA, Atasoy AR, Ozbek A, Köroğlu M. Are there standardized cutoff values for neutrophil-lymphocyte ratios in bacteremia or sepsis. J Microbiol Biotechnol. 2015; 25(4):521-524. https://doi.org/10.4014/jmb.1408.08060 PMid:25341467

14. Hong X, Cui B, Wang M, Yang Z, Wang L, Xu Q. Systemic immune-inflammation index, based on platelet counts and neutrophil-lymphocyte ratio, is useful for predicting prognosis in small cell lung cancer. The Tohoku journal of experimental medicine. 2015; 236(4):297-304. https://doi.org/10.1620/tjem.236.297 PMid:26250537

15. Liu X, Shen Y, Wang H, Ge Q, Fei A, Pan S. Prognostic significance of neutrophil-to-lymphocyte ratio in patients with sepsis: a prospective observational study. Mediators of inflammation. 2016; 2016:1-3. https://doi.org/10.1155/2016/7432845 PMid:28100936 PMCid:PMC5215628

16. Alexander NI. Reference Values of Neutrophil-Lymphocyte Ratio, Platelet-Lymphocyte Ratio and Mean Platelet Forum in Healthy Adults in North Central Nigeria. Journal of Blood & Lymph. 2016; 6(1):1-4.

17. Pan L, Du J, Li T, Liao H. Platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio associated with disease activity in patients with Takayasu's arteritis: a case-control study. BMJ open. 2017; 7(4):e014451. https://doi.org/10.1136/bmjopen-2016-014451 PMid:28473512 PMCid:PMC5623399

18. Piciucchi M, Stigliano S, Archibugi L, Zerboni G, Signoretti M, Barucca V, Valente R, Fave GD, Capurso G. The neutrophil/lymphocyte ratio at diagnosis is significantly associated with survival in metastatic pancreatic cancer patients. International journal of molecular sciences. 2017; 18(4):730. https://doi.org/10.3390/ijms18040730 PMid:28353661 PMCid:PMC5412316

19. Tokgoz S, Keskin S, Kayrak M, Seyithanoglu A, Ogmegul A. Is neutrophil/lymphocyte ratio predict to short-term mortality in acute cerebral infarct independently from infarct volume? Journal of Stroke and Cerebrovascular Diseases. 2014; 23(8):2163-8. https://doi.org/10.1016/j.jstrokecerebrovasdis.2014.04.007 PMid:25106834

20. Bartelt S, Lutterbach J. Brain metastases in patients with cancer of unknown primary. Journal of neuro-oncology. 2003; 64(3):249-53. https://doi.org/10.1023/A:1025621819250 PMid:14558600

21. László I, Trásy D, Molnár Z, Fazakas J. Sepsis: from pathophysiology to individualized patient care. Journal of immunology research. 2015; 2015.

22. Saez-Cirion A, Jacquelin B, Barré-Sinoussi F, Müller-Trutwin M. Immune responses during spontaneous control of HIV and AIDS: what is the hope for a cure? Phil Trans R Soc B. 2014; 369(1645):20130436. https://doi.org/10.1098/rstb.2013.0436 PMid:24821922 PMCid:PMC4024229

23. Son Y, Kim BY, Eo SK, Park YC, Kim K. Dexamethasone suppresses oxysterol-induced differentiation of monocytic cells. Oxidative medicine and cellular longevity. 2016; 2016.

24. Evans JD. Straightforward statistics for the behavioral sciences. Brooks/Cole, 1996.

25. Donskov F. Immunomonitoring and prognostic relevance of neutrophils in clinical trials. Seminars in cancer biology. 2013; 23(3):200-207. https://doi.org/10.1016/j.semcancer.2013.02.001 PMid:23403174

26. Dunn GP, Dunn IF, Curry WT. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human glioma. Cancer Immunity Archive. 2007; 7(1):12.

27. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Oca-a A, Leibowitz-Amit R, Sonpavde G, Knox JJ, Tran B, Tannock IF. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. JNCI: Journal of the National Cancer Institute. 2014; 106(6). https://doi.org/10.1093/jnci/dju124

28. Serdarevic M, Kukulj S, Nikolic I, Taradi I, Romic Z, Samarzija M. 203P: Could neutrophil-to-lymphocyte ratio be predictor of brain metastases in non small cell lung cancer? Journal of thoracic oncology. 2016; 11(4):S145. https://doi.org/10.1016/S1556-0864(16)30311-2

29. Mitsuya K, Nakasu Y, Kurakane T, Hayashi N, Harada H, Nozaki K. Elevated preoperative neutrophil-to-lymphocyte ratio as a predictor of worse survival after resection in patients with brain metastasis. Journal of neurosurgery. 2017; 127(2):433-7. https://doi.org/10.3171/2016.8.JNS16899 PMid:27911233