RESEARCH ARTICLE

May the Neutrophil/Lymphocyte Ratio Be a Predictor in the Differentiation of Different Thyroid Disorders?

Derya Kocer[1]*, Cigdem Karakukcu[1], Hatice Karaman[2], Ferhat Gokay[3], Fahri Bayram[4]

Abstract

Background: The neutrophil/lymphocyte ratio (NLR) is a simple index of systemic inflammatory response, and has been shown to be a prognostic indicator in some types of cancer. Inflammation has been implicated in the initiation and progression of thyroid cancer. The aim of this study was to examine the relationship of NLR with papillary thyroid cancer (PTC) and different benign thyroid pathologies like multinodular goiter (MNG) and lymphocytic thyroiditis (LT). Materials and Methods: We retrospectively evaluated the neutrophil, lymphocyte counts and NLR calculated from these parameters of 232 patients with histologically confirmed as multinodular goiter (group MNG) (n=70), lymphocytic thyroiditis (group LT) (n=97), LT with PTC (group LT-PTC) (n=25) and PTC (group PTC) (n=40). The optimal cut-off value for NLR was determined. Results: NLR level was significantly higher in groups LT-PTC and PTC as compared to groups MNG and LT (p<0.05). NLR of LT subgroups according to TSH levels were not different (p>0.05). When we grouped the patients as benign and malignant according to PTC presence, the optimum NLR cut-off point obtained from ROC analysis was 1.91 (sensitivity 89.0% and specificity 54.5%). Conclusions: Since NLR was significantly elevated in group LT-PTC and group PTC, NLR value may give an opinion as a potential marker in differentiation of benign and malign thyroid disorders. For this purpose a cut-off value of 1.91 for NLR may be accepted.

Keywords: Cancer - goiter - neutrophil/lymphocyte ratio - thyroid

Introduction

Thyroid nodules are a very common finding, present in at least 4% of the population in iodine-sufficient countries upon palpation, and in over 50% of patients using ultrasonography (Polyzos et al:,2007; Luo et al., 2012). Multinodular goiters (MNG) are defined as an enlarged thyroid gland with multiple nodules, have historically been thought of as a benign condition with a low risk of associated malignancy (Pinchera et al.,1996). Recent studies have suggested that the incidence of malignancy in patients with MNG approaches that of patients with a solitary thyroid nodule (Gandolfi et al., 2004). However, fine needle aspiration of a MNG is limited due to the presence of multiple nodules (Rios et al., 2004). Thyroid cancer is the most common endocrine malignancy in the USA in 2009 (Cunha et al., 2011), especially papillary thyroid carcinoma (PTC) is the most common form of thyroidal malignancy. The incidence rate of PTC has been increased during the past few decades (Lloyd et al., 2011). Lymphocytic thyroiditis (LT), is one of the common thyroid pathologies resulting from the autoimmune process. Cooccurrences of LT and thyroid cancer, mainly

PTC, have been repeatedly reported (Cipolla et al., 2005; Tamimi et al., 2002). Radiological and histopathological evaluations are essential in differentiating these thyroid disorders (Luo et al., 2012).

It is clear that the response of the immune system plays a vital role in the control and progression of many disease states including cancer. Simple measures of immune responsiveness include simple routine haematological markers such as total and differential leukocyte counts, which have been proposed as diagnostic and prognostic factors for a variety of cancers (Coussens et al., 2002; Baykan et al., 2015; Duzlu et al., 2015; Karaman et al., 2013; Ozaksit et al., 2015).

Unlike other diseases, little is known and published about Neutrophil/Lymphocyte ratio (NLR) and its relationship with thyroid disorders. Therefore, the current study was conducted to investigate the potential relationship between NLR and different thyroid pathologies.

Materials and Methods

We retrospectively evaluated neutrophil, lymphocyte and platelet counts and thyroid hormone levels in

[1]*Clinical Biochemistry,* [2]*Clinical Pathology,* [3]*Endocrinology and Metabolism, Training and Research Hospital,* [4]*Endocrinology and Metabolism, Erciyes University, Faculty of Medicine , Kayseri, Turkey*   **For correspondence: ayder78@yahoo.com*

232 patients with thyroid pathologies, who applied to Training and Research Hospital, Kayseri, Turkey, between January 2012 and January 2014. Of 232 patients, 70 were histologically confirmed as MNG (mean age, 49.25±13.15 years) (Group MNG), 97 were confirmed as LT (mean age, 48.1±12.36 years) (Group LT), 25 were confirmed as LT with PTC (mean age, 50.72±10.98 years) (Group LT-PTC), and 40 were confirmed as PTC (mean age, 53.57±13.32 years) (Group PTC). All patients in group LT-PTC and group PTC received surgical treatment for papillary carcinoma. The diagnosis of patients in group MNG and group LT were made by fine needle aspiration. Demographic, clinical, and laboratory data, including patient age, sex, white cell and differential counts, thyroid hormone, thyrotropin levels were determined by examining the records of Clinical Biochemistry Laboratory. The pathological information was obtained from the records of Department of Clinical Pathology.

Group LT were divided into two groups according to their thyroid-stimulating hormone (TSH) levels [TSH> 4 IU/mL (n:30) and TSH< 4 IU/mL (n:67)] to assign the effect of thyroid hormones on white blood cell (WBC) counts.

Patients with known any systematic disease, such as diabetes mellitus, hypertension, using drugs which have effects on both platelets and thyroid function tests according to data about patient information were excluded from the study.

The neutrophil, lymphocyte and platelet counts and thyroid hormone levels of patients those were analyzed at the day of pathological sampling were recorded. This was a retrospective study in which the data were obtained from a computerized patient registry database. To standardize the known effect of circulating hormones on white blood cell indices, we recorded the morning levels of these parameters. Neutrophil, lymphocyte counts were measured with Mindray BC-6800 (Senzhen Mindray Biomedical Electronics, Nanshan, P.R.China). NLR was calculated as the ratio of the total count of neutrophils divided by the total count of lymphocytes. Thyroid hormone levels of patients were analyzed by Beckman Coulter DXI800 immunoassay analyzer (Beckman Coulter Inc, CA, USA).

The Institution Review Board of Kayseri Training and Research Hospital approved the study protocol. The study was performed in accordance with the Declaration of Helsinki.

Statistical analysis

Statistical evaluation was carried out with the SPSS® 20.0 (Statistical Packages for Social Sciences; SPSS Inc, Chicago, Illinois, USA). The Kolmogorov-Smirnov test was used to assess the normality of the data. Intergroup comparisons were made by the independent sample t-test. To compare the parameters of all groups the one way ANOVA test was used. Data were presented as "mean with their standard deviation" (mean ± SD). p value less than 0.05 was considered as statistically significant. Receiver

Table 1. Demographic and Laboratory Characteristics of all Patient Groups

	Group MNG (n:70)	Group LT (n:97)	Group LT-PTC (n:25)	Group PTC (n:40)
Age (year)	49.25±13.15	48.10±12.36	50.72±10.98	53.57±13.32
Gender (F/M)	38/32	55/42	22/3	28/12
White blood cell ($10^3/\mu L$)	7.27±1.80	7.24±1.61	7.12±1.50	8.05±1.72
Neutrophil ($10^3/\mu L$)	4.24±0.95	4.39±1.12	4.61±0.92	5.14±1.16 a
Lymphocyte ($10^3/\mu L$)	2.57±0.72 [b]	2.23±0.59	1.96±0.58	2.10±0.68
Platelet count ($10^3/\mu L$)	295.81±73.8	280.57±55.19	297.22±62.33	266.72±63.87
NLR	1.74±0.53 [b]	2.05±0.57 [c]	2.47±0.64 [d]	2.57±0.60 [e]
fT3 (pg/mL)	3.19±1.29	3.14±0.72	3.18±0.47	3.18±0.51
fT4 (ng/dL)	1.13±0.29	0.93±0.37 [f]	1.12±0.25	1.09±0.19
TSH (mIU/L)	1.10±0.93	5.51 (0.02-79.8) [f]	2.14±1.66	1.71±1.51

*p: Group differences computed by one way ANOVA test; a: Group 4 is different from group 1 and 2 (p<0.05); b: Group 1 is different from group 2, 3 and 4 (p<0.05); c: Group 2 is different from group 1, 3 and 4 (p<0.05); d: Group 3 is different from group 1 and 2 (p<0.05); e: Group 4 is different from group 1 and 2 (p<0.05); f: Group 2 is different from group 1 and 4 (p<0.05)

Table 2. Comparison of LT Subgroups According to their TSH Levels

	Group LT (n:97)		
	TSH>4 IU/mL (n:30)	TSH<4 IU/mL (n:67)	p
1Age (year)	47.20±10.65	48.52±13.11	0.60
Gender (F/M)	19/11	36/31	0.43
White blood cell ($10^3/\mu L$)	7.01±1.94	7.34±1.45	0.41
Neutrophil ($10^3/\mu L$)	4.40±1.32	4.39±1.02	0.99
Lymphocyte ($10^3/\mu L$)	2.06±0.65	2.30±0.55	0.08
Platelet count ($10^3/\mu L$)	268.8±51.82	285.85±56.21	0.15
NLR	2.11±0.42	1.97±0.49	0.22
fT3 (pg/mL)	3.05±0.70	3.18±0.73	0.41
fT4 (ng/dL)	0.82±0.31	0.98±0.38	0.05
TSH (mIU/L)	4.92-79.81	1.36±1.06	< 0.05

p: Group differences computed by Independent Sample-t test
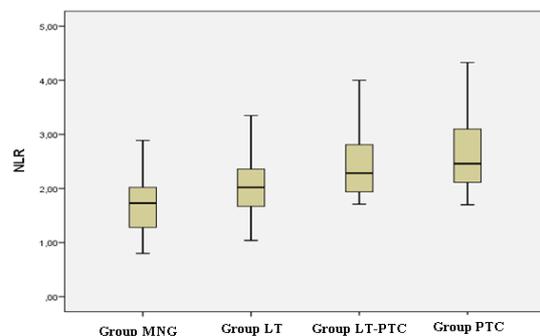
Figure 1. Mean NLR Levels. Statistical significance was obtained when comparing group MNG and LT (p=0.004), group MNG and group LT-PTC (p<0.001), group MNG and group PTC (p<0.001), group LT and group LT-PTC (p=0.011), group LT and group PTC (p<0.001). There was no statistical difference between group LT-PTC and group PTC (p= 0.921)

May the Neutrophil/Lymphocyte Ratio Be a Predictor in the Differentiation of Different Thyroid Disorders?

Table 3. Diagnostic Validity of NLR at Different Cut-off Values for Discrimination of Benign and Malign Thyroid Pathologies

Cut-off values for NLR	Sensitivity, (%)	Spesificity, (%)	PPV, (%)	NPV, (%)	Accuracy Rate, (%)
1.85 [a]	90	47	38	93	58.3
1.91 [b]	89	54.5	41	92	63.1
2.13 [c]	69	69	45	86	69.2
2.55 [d]	40	90	69	85.7	81.5

*[a]Threshold with diagnostic sensitivity of 90%; [b]Threshold with the highest diagnostic sensitivity and spesificity; [c]Threshold at the point with similar sensitivity and spesificity; [d]Threshold with diagnostic spesificity of 90%
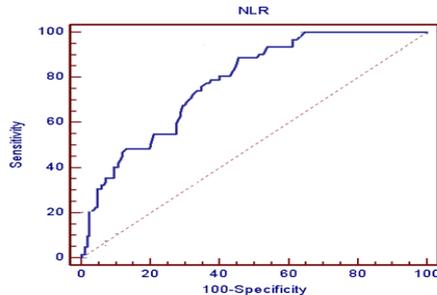
Figure 2. Receiver Operating Characteristic Curves for NLR at Cut-off value of 1.91

operating characteristic (ROC) curve was constructed for NLR and the area under the ROC curve (AUC) value with 95% CI was calculated. Optimal cut-off value for NLR was determined; sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy rate were calculated.

Results

As shown in Table 1, WBC and platelet counts were not significantly different between groups (p>0.05). Neutrophil counts were significantly higher in group PTC than group MNG and group LT (p<0.05). Lymphocyte counts were significantly higher in group MNG than the other groups (p<0.05). There was no significant difference between group LT-PTC and group PTC according to neutrophil and lymphocyte counts. Statistically significant differences were observed in NLR between groups (p<0.05). NLR levels were significantly higher especially in group 3 and 4 than group 1 and 2. There was no significant difference between group LT-PTC and group PTC according to NLR levels (p=0.921). Mean NLR of group LT-PTC and group PTC were 2.57±0.60 and 2.47±0.64 respectively (Table 1). Comparison of NLR levels of all groups were shown in Figure 1.

According to TSH levels, group LT was significantly higher than group MNG and Group PTC (p<0.05). Since TSH levels showed a wide distribution in patients with LT, group LT were divided into two subgroups in respect to their TSH levels (as TSH>4 IU/mL and TSH<4 IU/mL). We observed no significant effect of TSH on NLR levels of LT subgroups (p>0.05). Comparison of LT subgroups was shown in Table 2. As shown in Table 1, fT4 levels were significantly lower in group LT than group MNG and Group PTC (p<0.05).

The ROC curve for NLR was shown in Figure 2, in which histopathological diagnosis was taken as the reference for presence of malignancy. According to PTC presence, group 1 and 2 were thought as benign, group 3 and 4 were thought as malign group. ROC curve analysis suggested that the optimum NLR cut-off point for PTC presence was 1.91, with sensitivity, specificity, PPV, and NPV of 89%, 54.5%, 41%, and 92%, respectively. The overall accuracy of the NLR in determination of PTC presence was 63.1%. The AUC value for NLR was 0.78 (95%CI: 0.72-0.83). ROC curve for NLR at cut-off value of 1.91 is shown in Figure 2. The diagnostic validity criteria sensitivity and specificity of NLR at different decision limits of the ROC curves are shown in Table 3.

Discussion

Studies showed a link between the inflammatory microenvironment of a cancer and systemic responses induced by the tumour. Many cancer survival studies have suggested that NLR is a significant predictor of overall and disease specific survival of patients. Patients with elevated NLR have a relative lymphocytopenia and neutrophil leukocytosis in favor of protumor inflammatory response, in different types of cancer (Guzel et al., 2014; He et al., 2014; Kum et al. 2014; Ozyalvacli et al., 2014; Yildirim et al., 2014).

It has been shown thyroid cancer is closely related to inflammation (Liu et al., 2013). Several authors reported there is an increased incidence of PTC in patients with thyroiditis (Cipolla et al., 2005; Tamimi et al., 2002). Indeed, several articles have reported that papillary thyroid cancer with thyroiditis is associated with a lower tumor stage and better prognosis (Singh et al.,1999). The intratumoral lymphocyte infiltrate is importantly reduced in poorly differentiated and undifferentiated thyroid cancer (Ugolini et al., 2007). According to these data, it is likely that inflammatory cells and mediators can exhibit different functions (cancer promoting or inhibiting) in different conditions. But, it is not clear how the presence of inflammation alter the biological effect of PTC; its mechanism has not been well elucidated.

There are characteristic morphologic features for different thyroid lesions, but many studies indicated that differentiation of PTC from thyroid gland benign lesions based on their morphology is often questionable (DeVita et al., 2008). The aim of our study was to investigate the possible application of NLR, simple, cheaper and determined from routine automated hemogram analysis, as an indirect marker of PTC presence in patients with thyroidal goiters. Our results demonstrate that NLR is higher in group LT than group MNG. Lymphocyte count was significantly lower, while neutrophil count was higher in group LT than group MNG. The increased NLR in group LT confirms the key role of neutrophils and lymphocytes

in the inflammation process. We can obtain information about two different immune pathways from the NLR. First of all about the neutrophils that are responsible for lasting inflammation and the second about the lymphocytes that demonstrate the regulatory pathway (Avanzas et al., 2004; Ommen et al.,1998). Elevated TSH levels are seen in LT patients that it was in group LT in our study. We divided group LT into two subgroups according to TSH levels to investigate the relation of TSH with neutrophil, lymphocyte counts and NLR, but we did not observe any effects of TSH levels on NLR values in group LT (Table 2).

Our results also demonstrate that NLR is higher in group LT-PTC and group PTC than the other two groups. Since the significant elevation of NLR in malign groups, it may be used for be aware of presence of malignity. As in our study, high NLR reflects an increased neutrophil count and/or a decreased lymphocyte count. Low lymphocyte counts have been associated with generalized suppression of the immune systems of patients with cancer. Neutrophilia could represent a consequence of ectopic production of myeloid growth factors as part of a paraneoplastic syndrome or, more likely, a nonspecific response to cancer-related inflammation secondary to tissue destruction and cytokine releases (Wenger et al.,1999; Vassilatou et al., 2006). Experimental data indicate that activated neutrophils may directly and indirectly stimulate tumor growth. An elevated NLR may be either the result of an excessive but ineffective immune response to the tumor load, or it may be a marker of imbalanced inflammatory state which facilitates tumor growth (Liu et al., 2013).

ROC analysis of our data suggested that use of cut-off value of 1.91 for NLR would be optimum for clinical use to identify patients with PTC as sensitivity of 89% and specificity of 54.5% can be achieved. At this cut-off point NPV was 92%. We determined different cut-off values with different sensitivity and specificity as seen in Table 3. Considering the sensitivity, specificity and NPV values of cut-off 1.91, its utilization may permit a simple estimate of inflammatory response to PTC which is easily assessed in everyday clinical practice.

The study has some limitations including, (i) the use of a retrospective analysis, and (ii) our study was not designed to elucidate the mechanistic pathways that lead to higher NLR in patients with PTC.

In summary, the results of current study indicate that due to significant elevation of NLR in group LT-PTC and PTC than benign thyroid lesions it might be used in making distinction between benign thyroid lesions and PTC. Since NLR does not have specificity and histopathologic evaluation is gold standard on evaluation of the thyroidal disorders, this test alone should not be considered as a determining factor, NLR value only may give an opinion. Unlike many other noninvasive markers the NLR is inexpensive and readily available. Although NLR is of little value in the diagnosis of thyroid cancer, a possible relationship existing in the reciprocal interaction between systemic inflammatory response and thyroid tumor formation deserves further investigation.

References

Avanzas P, Quiles J, Lopez de Sa E, et al (2004). Neutrophil count and infarct size in patients with acute myocardial infarction. *Int J Cardiol*, **97**, 155-6.

Baykan H, Cihan YB, Ozyurt K (2015). Roles of white blood cells and subtypes as inflammatory markers in skin cancer. *Asian Pac J Cancer Prev*, **16**, 2303-6.

Cipolla C, Sandonato L, Graceffa G, et al (2005). Hashimoto thyroiditis coexistent with papillary thyroid carcinoma. *Am Surg*, **71**, 874-8.

Coussens LM, Werb Z (2002). Inflammation and cancer. *Nature*, **420**, 860-7.

Cunha LL, Ferreira RC, Marcello MA, Vassallo J, Ward LS (2011). Clinical and pathological implications of concurrent autoimmune thyroid disorders and papillary thyroid cancer. *J Thyroid Res*, **1**, 1-13.

DeVita VT, Hellman JS, Rosenberg SA (2008). Cancer: Principles and Practice of Oncology. Philadelphia, Pa: Lippincott Williams and Wilkins, pp. 1674-99.

Duzlu M, Karamert R, Tutar H, et al (2015). Neutrophil-lymphocyte ratio findings and larynx carcinoma: a preliminary study in Turkey. *Asian Pac J Cancer Prev*, **16**, 351-4.

Gandolfi PP, Frisina A, Raffa M, et al (2004). The incidence of thyroid carcinoma in multinodular goiter: retrospective analysis. *Acta Biomed*, **75**, 114-7.

Guzel AI, Kokanali MK, Erkilinc S, et al (2014). Predictive role of the neutrophil lymphocyte ratio for invasion with gestational trophoblastic disease. *Asian Pac J Cancer Prev*, **15**, 4203-6.

He WZ, Jiang C, Yin CX, et al (2014). Prognostic model built on blood-based biomarkers in patients with metastatic colorectal cancer. *Asian Pac J Cancer Prev*, **15**, 7327-31.

Karaman H, Karaman A, Erden A, et al (2013). Relationship between colonic polyp type and the neutrophil/ lymphocyte ratio as a biomarker. *Asian Pac J Cancer Prev*, **14**, 3159-61.

Kum RO, Ozcan M, Baklaci D, et al (2014). Elevated neutrophil-to-lymphocyte ratio in squamous cell carcinoma of larynx compared to benign and precancerous laryngeal lesions. *Asian Pac J Cancer Prev*, **15**, 7351-5.

Liu CL, Lee JJ, Liu TP, et al (2013). Blood neutrophil-to-lymphocyte ratio correlates with tumor size in patients with differentiated thyroid cancer. *J Surg Oncol*, **107**, 493-7.

Lloyd RV, Buehler D, Khanafshar E (2011). Papillary thyroid carcinoma variants. *Head Neck Pathol*, **5**, 51-6.

Luo J, McManus C, Chen H, Sippel RS (2012). Are there predictors of malignancy in patients with multinodular goiter? *J Surg Res*, **174**, 207-10.

Ommen SR, Hodge DO, Rodeheffer RJ, et al (1998). Predictive power of the relative lymphocyte concentration in patients with advanced heart failure. *Circulation*, **97**, 19-22.

Ozaksit G, Tokmak A, Kalkan H, Yesilyurt H (2015). Value of the platelet to lymphocyte ratio in the diagnosis of ovarian neoplasms in adolescents. *Asian Pac J Cancer Prev*, **16**, 2037-41.

Ozyalvacli G, Yesil C, Kargi E, Kizildag B, Kilitci A, Yilmaz F (2014). Diagnostic and prognostic importance of the neutrophil lymphocyte ratio in breast cancer. *Asian Pac J Cancer Prev*, **15**, 10363-6.

Pinchera A, Aghini-Lombardi F, Antonangeli L, Vitti P (1996). Multinodular goiter. Epidemiology and prevention. *Ann Ital Chir*, **67**, 317-25.

Polyzos SA, Kita M, Avramidis A (2007). Thyroid nodules-stepwise diagnosis and management. *Hormones* **6**, 101-19.

Rios A, Rodriguez JM, Galindo PJ, et al (2004). Utility of fine-needle aspiration for diagnosis of carcinoma associated

with multinodular goitre. *Clin Endocrinol (Oxf)*, **61**, 732-7.

Singh B, Shaha AR, Trivedi H, Carew JF, Poluri A, Shah JP (1999). Coexistent Hashimoto's thyroiditis with papillary thyroid carcinoma: impact on presentation, management, and outcome. *Surgery*, **126**, 1070-6.

Tamimi DM (2002). The association between chronic lymphocytic thyroiditis and thyroid tumors. *Int J Surg Pathol*, **10**, 141-6.

Ugolini C, Basolo F, Proietti A, et al (2007). Lymphocyte and immature dendritic cell infiltrates in differentiated, poorly differentiated, and undifferentiated thyroid carcinoma. *Thyroid*, **17**, 389-93.

Vassilatou E, Fisfis M, Morphopoulos G, et al (2006). Papillary thyroid carcinoma producing granulocyte-macrophage colony-stimulating factor is associated with neutrophilia and eosinophilia. *Hormones (Athens)*, **5**, 303-9.

Wenger FA, Jacohi CA, Zieren J, et al (1999). Tumor size and lymph-node status in pancreatic carcinoma is there a correlation to the preoperative immune function? *Langenbecks Arch Surg*, **384**, 473-8.

Yildirim MA, Seckin KD, Togrul C, et al (2014). Roles of neutrophil/lymphocyte and platelet/lymphocyte ratios in the early diagnosis of malignant ovarian masses. *Asian Pac J Cancer Prev*, **15**, 6881-5.