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The value of blood cell markers in patients with thyroid nodules including atypia of undetermined significance/follicular lesion of undetermined significance cytology

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Abstract

Atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) is a heterogeneous that cannot be definitively diagnosed as benign, malignancy-suspect, or malignant. This study was conducted to evaluate the ability of mean platelet volume (MPV) and neutrophil-to-lymphocyte ratio (NLR) to predict the malignant potential of nodules diagnosed as AUS/FLUS. We retrospectively analyzed 101 patients for whom thyroid fine needle aspiration biopsy (FNAB) analysis indicated AUS/FLUS and who underwent surgery between 2011 and 2015. Demographic, laboratory, and histopathological data were obtained from a database. The patients were categorized into two groups: malignant or benign and comparisons between groups were performed. Of the 101 patients, 26 (25.7%) had solitary nodules and 75 (74.3%) had multinodular goiter. The malignancy rate was 33.7%, and there were no differences between the two groups in terms of age, gender and thyroid function tests. Median preoperative red cell distribution width (RDW) level was 13.4 in the benign group, while it was 14.4 in patients with malignancy, demonstrating a significant correlation. Both MPV and NLR were elevated in malignant nodules. The malignancy risk of AUS/FLUS evident upon thyroid FNAB was higher than anticipated by the Bethesda system (BS). MPV, NLR and RDW are useful for estimating the malignancy risks of these diseases.

Keywords: Malignancy, thyroid nodule, MPV, NLR, RDW

Introduction

Currently, FNAB (fine needle aspiration biopsy) is the gold standard for diagnosing thyroid nodules and identifying patients needing surgery [1]. Bethesda System (BS) used to evaluate FNAB results and revised six-tiered reporting system that also explores the risk of malignancy associated with each disease category. Bethesda Category 3 disease, termed AUS/FLUS, is a gray zone. Although it is difficult to discriminate benign nodules from their malignant counterparts, this is essential for the management of treatment [2]. A diagnosis of AUS/FLUS should not be made on more than 7–10% of all FNABs, and a repeat biopsy is indicated because the reported rate of malignancy is 5–15% [3,4]. As only small proportions of such patients undergo surgery, the real incidence of malignancy is controversial. In recent studies, the rate of malignancy in repeatedly biopsied AUS/FLUS patients ranged

between 6% and 48%, and no statistically significant differences in terms of age, gender, ultrasonographical or scintigraphical features, or number of FNAB interventions to explore benign and malignant nodules were evident [5-7]. The lack of specific and accurate diagnostic techniques has encouraged researchers to search for different biomarkers [8,9].

A relationship between inflammation and thyroid cancer has been established previously [10]. The neutrophil-to-lymphocyte ratio (NLR) is a simple indicator of systemic inflammation [11-13]. The NLR was higher in patients with PTC (Papillary Thyroid Cancer) and was associated with poor prognosis [14,15].

Tumor cells secrete various cytokines, which stimulate platelets [16]. An elevated mean platelet volume (MPV) indicates that the platelets are enzymatically and metabolically active [17]. A relationship between an elevated MPV and disease has been reported in various cancers, including PTC [18-20].

Our aim was to evaluate the MPV and NLR in patients diagnosed with AUS/FLUS by FNAB whose long-term pathology was cancer.

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Material and Method

This retrospective study included 101 patients who were diagnosed with AUS/FLUS by FNAB and who underwent surgery. The demographic characteristics, clinical and pathological features, history of thyroiditis and radiation exposure, thyroid function test results, and complete blood counts after FNAB were recorded prospectively. Thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), and thyroid autoantibodies were measured using a direct chemiluminescence method (Advia Centaur XP, Siemens, Dublin, Ireland). The normal ranges are as follows; FT3: 1.8 to 4.7 pg / ml, FT4: 0.8 to 2.6 pg / ml, and TSH: 0.4 to 6 μ IU / ml. Complete blood counts were obtained using an automated hematological analyzer (Cell-Dyn Sapphire; Abbott Diagnostic Division, Santa Clara, California, USA). The MPV and NLR were recorded. The NLR was calculated by dividing the number of neutrophils by that of lymphocytes.

Exclusion criteria; aged <18 years; history of radiotherapy of the neck; no surgery performed; acute or chronic inflammation; cardiac, renal, or liver failure; any malignancy except thyroid cancer; anticoagulant drug use; and/or surgery and/or revascularization in the past 6 months.

Thyroid sonographic imaging were performed at Endocrinology Clinic in our institution, using 11-MHz linear probe and LOGIQ 3. Ultrasound-guided FNABs were performed using 22-gauge needles and 10-ml syringes. Dominant nodule size was recorded for each biopsied nodule. All subjects provided informed consent.

All cases were reviewed by an expert cytopathologist using a six-tiered diagnostic system, the criteria of which were essentially identical to those of the BS: 1) nondiagnostic, 2) benign, 3), AUS/AUFL, 4) follicular neoplasm/suspicion of follicular neoplasm, 5) suspicion of malignancy, and, 6) malignant. AUS/FLUS was defined by the presence of atypical cells and microfollicular structures and little or no colloid [2,12].

Surgical resection was recommended for patients with a repeat diagnosis of AUS or a suspicious or definite malignancy on a repeat FNAB. However, depending on clinical assessments of parameters such as nodule size, growth, imaging characteristics, and patient preference, surgical resection was also recommended for some other patients. Histologically, the nodules were categorized as benign or malignant. This study was approved by our Local Clinical Study Medical Ethics Committee.

Statistical Analysis

Data were analyzed with the SPSS 18.0 statistical package program (SPSS Inc., Chicago, Illinois, USA). The distribution pattern of data was assessed by the Kolmogorov-Smirnov test. Categorical analysis was performed using a two-tailed χ^2 test. Student's t-test was used to evaluate the statistical significance of differences in the mean values of continuous variables. A receiver operator characteristic (ROC) curve was constructed for RDW to determine a cut-off value for the prediction of malignancy. A p-value of less than 0.05 was considered significant.

Results

Of the 101 patients, 87 were female (86.1%) and 14 were male

(13.9 %). The mean age was 49.01 ± 11.59 (20-80) years; the mean TSH level 2.04 ± 2.09 μ IU / ml (0.01–11.87); the mean FT4 level 0.98 ± 0.43 pg / ml (0.54–3.96); and the mean FT3 level 3.71 ± 0.80 pg / ml (2.15–7.46). Of all patients, 26 (25.7%) had solitary nodules and 75 (74.3%) had multinodular goiter. The malignancy rate in patients with cytological atypia was 33.7% (34 patients). We divided patients in two groups according to postoperative histopathological results: benign and malignant. There were no differences between the two groups in terms of age, gender, incidence of solitary or multinodular goiters, or TSH or FT4 levels (Table 1). No chronic lymphocytic thyroiditis was found among study population. Median preoperative Red Cell Distribution Width (RDW) level was 13.90 in patients with benign thyroid, while it was 14.40 in patients with malignant thyroid. RDW level was found to be an independent variable correlated with malignancy ($p=0.034$, Table 1). 14.0 was set as the cutoff value for RDW levels, as a result of ROC curve analysis. ROC analysis revealed an area under the curve of 0.59 (Figure 4). Values below 14.0 were regarded as negative; whereas 14.1 and higher values were regarded as positive RDW result. However, both the MPV and NLR were significantly higher in malignant nodules than in histopathologically benign thyroid nodules ($p=0.029$ and $p=0.027$, respectively) (Figure 1, 2, 3).

Table 1. Demographic and laboratory differences between benign and malignant postoperative histopathology

	Benign (n=67)	Malignant (n=34)	P value
Mean Age (years)	49.93 ± 10.62 (26-80)	47.21 ± 13.27 (20-80)	0.231
Gender (Female/Male)	56/11	31/3	0.373
Nodule (Solitary/MNG)	21/46	5/29	0.093
TSH (mIU/m)	2.27 ± 2.33 (0.01-11.87)	1.56 ± 1.39 (0.01-5.5)	0.175
FT4 (ng/dl)	0.97 ± 0.48 (0.54-3.96)	1.01 ± 0.31 (0.56-2.12)	0.242
FT3 (pg/ml)	3.55 ± 0.60 (2.15 -4.82)	4.04 ± 1.03 (2.63-7.46)	0.024
MPV (fL)	8.78 ± 0.96 (7-11)	9.20 ± 1.10 (6.9-11.4)	0.029
NLR	1.95 ± 0.84	2.39 ± 0.96	0.027
RDW	13.90	14.40	0.034

MNG: Multinodular goiter, TSH: Thyroid stimulating hormone, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio, RDW: Red cell distribution width

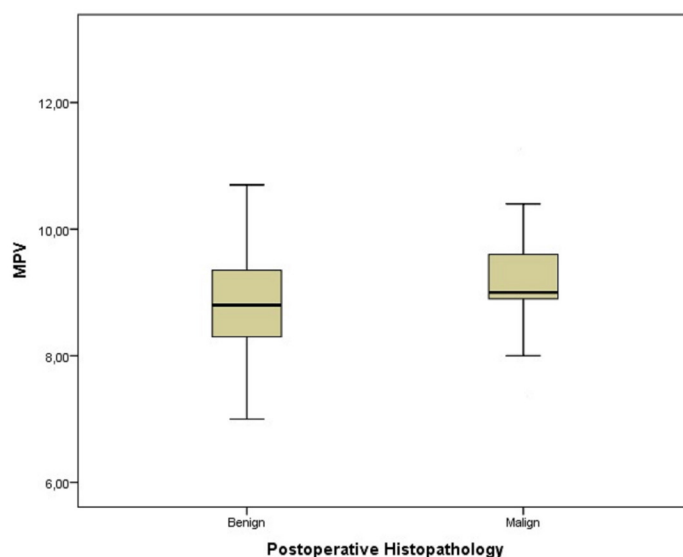


Figure 1. Mean platelet volume levels in benign and malignant thyroid nodules

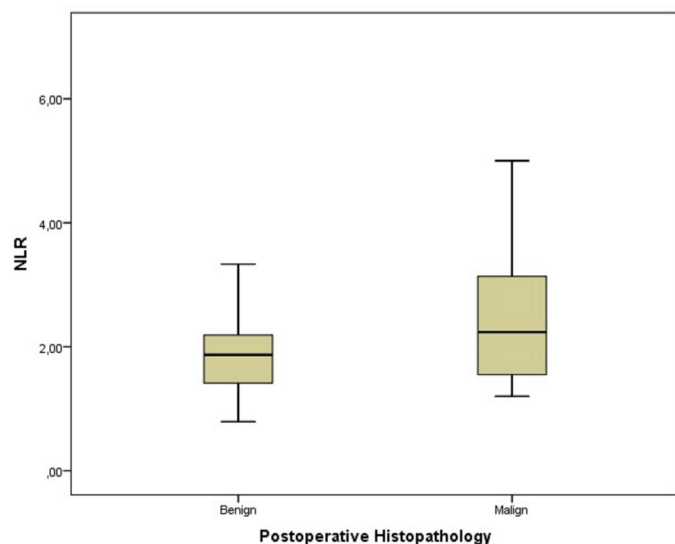


Figure 2. Neutrophil-lymphocyte ratio in benign and malign thyroid nodules

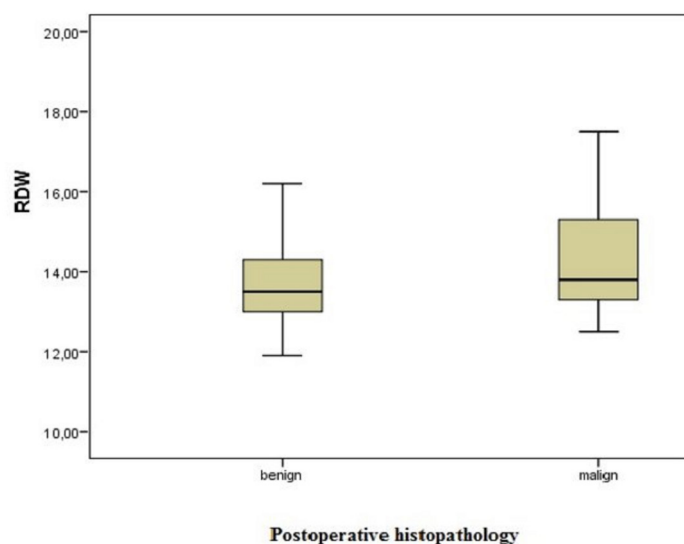


Figure 3. Red cell distribution width in benign and malign thyroid nodules

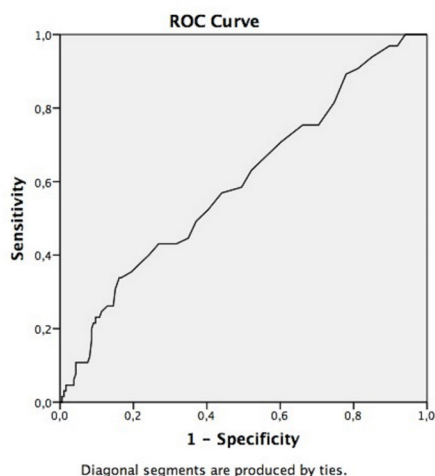


Figure 4. ROC curve for red cell distribution width

Discussion

To manage AUS/FLUS patients, BS recommends repeating FNAB

at appropriate intervals, as the anticipated risk of malignancy is low, at 5–15% [2-4]. However, most recently published data exhibit a higher malignancy risk than that anticipated by BS (6–48%)[3,5,7,21]. As the risk is even higher than that of Bethesda 4, the BS guidelines should be reconsidered [2]. Some studies have even suggested that the AUS category be changed to the AUS/follicular neoplasia category [22]. Similar to other studies, we found a high rate of malignancy (33.7%) in this research. No statistically significant difference was evident between benign and malignant nodules in terms of clinical or ultrasonographical features upon either single or repetitive FNAB [5]. In the same study, some patients whose cytological results suggested AUS/FLUS underwent surgery without repeat FNAB, and the malignancy rate was 77.6% [5]. Paul et al. [21] found no statistically significant difference in the malignancy rate among patients who underwent surgery immediately after a single AUS diagnosis and those with two successive AUS FNAB diagnoses (both 41–43%). Cuhaci et al. [7] reported that the malignancy rate was 22.8%. In that study, there were no differences between patients with benign and malign histopathological grades in terms of age, gender, ultrasonographical or scintigraphical features, or nodule size. These authors also did not find any difference between patients who underwent single or repeated FNAB. Likewise, we found no differences between patients with benign and malignant histopathological results in terms of age, gender, thyroid function test results, or the rate of solitary or multiple nodules.

In an earlier study conducted in our country, the malignancy rate of AUS/FLUS was reported to be 35%, and it was found that the red cell distribution width was significantly higher in malign nodules [23].

Tumor cells release proinflammatory cytokines, chemokines, and growth factors; all of these stimulate platelets. Platelets exert proteolytic, angiogenic, and metastatic activities in inflammation, and play an important role in the formation and progression of various cancers [16,18-20].

Mean platelet volume (MPV), which is associated with functional changes in platelets, is easily calculated during routine blood counts [24,25]. It reflects platelet size, and MPV increases upon stimulation and production of platelets [17]. Large platelets are more metabolically and enzymatically active [17]. Platelets play an important role in tumor angiogenesis, and MPV has thus been suggested as a useful marker of angiogenesis [18-20]. Baldane et al. [19] reported that the MPV was higher in patients with PTC and was reduced significantly in the postoperative period. In another study, the MPV was elevated in patients with malignant thyroid disease patients [20]. Consistent with previous studies, we found that the MPV was higher in malignant than in benign nodules.

The NLR is important in determining the systemic inflammatory response, and other important processes in the development of malignancy are disorders of the immune system [10,11,13-25]. A low NLR reflects the absence of an adequate response to a tumor [26]. A low lymphocyte count reflects immune system suppression by tumor cells, and elevated neutrophil levels reflect paraneoplastic activity or secretion of cytokines resulting in tissue destruction [27,28]. Neutrophilia also reflects an excessive (but not effective) response to tumor destruction. Experimental studies have shown that neutrophils directly or indirectly induce tumor growth [15].

The NLR is easy and inexpensive to calculate and is applicable in many situations. Seretis et al. [14] have shown that an elevated NLR was associated with papillary microcarcinomas. The NLR also has been used to detect recurrences and distant metastases in one previous study [29]. In another study, the NLR was associated with increased tumor size, a poorly differentiated histological type, and a high risk of recurrence [15]. As we observed that the NLR was higher in malignant than in benign nodules, we suggest that both the MPV and NLR may serve as new biomarkers predicting malignancy in patients with AUF/FLUS.

Due to developing interest specifically in RDW levels in inflammatory and malignant diseases, we decided to investigate also RDW levels for statistical analysis. Among the histopathological outcome groups, significant difference was found between median RDW levels ($p < 0.05$). In literature, there are also studies reported high RDW levels as a predictor of malignancy. In a study evaluating obstructive jaundice, the RDW value was higher in the malignant group [30].

There are different studies in the literature reporting that MPV and NLR values correlate positively or negatively with thyroid function disorders [31-34]. Güneş et al. found that no significant relationship between apparently hypothyroidism, subclinical hypothyroidism, and NLR values of healthy volunteers [32]. A recent study by Ren et al. in a large group of patients found no statistical significance between the MPV levels of patients with hypothyroidism, hyperthyroidism and euthyroidism [33]. In our study, despite being mostly euthyroid in the benign and malignant group, it was present in patients with subclinical hypothyroidism and hyperthyroidism. Despite the variable association between NLR and MPV levels and thyroid function in current studies, in our study there was no statistically significant difference between malign and benign group according to the TSH levels.

The limitations of the current study included the small number of patients. Additionally, we did not consider the sonographic features or subtypes of AUS/FLUS. We also did not evaluate MPV and NLR reduction postoperatively.

Conclusion

Sonographic imaging is one of the primary tools for the evaluation of thyroid nodules. Predicting malignancy in undetermined thyroid nodules is a critical situation. We believe that the risk of malignancy for AUS/FLUS patients studied by thyroid FNABs is higher than that anticipated by the BS. MPV and RDW levels as well as may become another promising tool to predict malignancy in this group of patients.

Competing interests

The authors declare that they have no competing interest

Financial Disclosure

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