Clinical Surgery

The significance of neutrophil/lymphocyte ratio as a possible marker of underlying papillary microcarcinomas in thyroidal goiters: a pilot study

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KEYWORDS:
Thyroid; Cancer; Goiter; Papillary thyroid microcarcinoma; Neutrophil-to-lymphocyte ratio; Biomarker

Abstract

BACKGROUND: Preoperatively elevated neutrophil/lymphocyte ratio (NLR) is a negative prognostic factor of survival in various types of cancers. A retrospective study was conducted to examine if preoperative elevation of NLR is associated with higher risk for incidental papillary thyroid microcarcinoma (PTMC).

METHODS: The study sample consisted of 26 patients with benign goiters, 31 patients with incidental PTMC, 26 patients preoperatively diagnosed with thyroid cancer, and 26 healthy controls. NLRs were compared regarding thyroidal pathology.

RESULTS: The mean preoperative NLR was significantly elevated in patients with PTMC and thyroid cancer. In addition, the third and fourth quartiles of NLR included only patients with either PTMC or thyroid cancer. No significant differences in NLR occurred between patients with multifocal and unifocal PTMC.

CONCLUSIONS: NLRs were significantly elevated in patients with incidental PTMC and thyroid cancer. The findings of this pilot study indicate that NLR should be considered an easily accessible biomarker for detecting incidental PTCM; nevertheless, further studies are required to confirm these preliminary results.

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Papillary thyroid microcarcinomas (PTMCs) are defined as thyroid carcinomas measuring ≤ 1 cm. In the vast majority of patients, the presence of PTMC is an incidental finding, detected during histopathologic examination of thyroid gland specimens that are surgically removed because of concomitant benign thyroid goiters. Despite the fact that PTMCs are generally considered to be rather benign entities with excellent prognosis, recent studies have found mortality of up to .5% in patients with PTMC, as a result of thyroid cancer progression, suggesting that a more aggressive approach should be adopted in confronting PTMCs. Thus, the need to assess biomarkers to enhance the capability of detecting PTMCs is evident, because early diagnosis of these nascent malignancies can guarantee excellent outcomes for these patients.

In general, the process of the development of malignancy has been firmly associated with impaired function of
the immune system. Neutrophil/lymphocyte ratio (NLR) has been proposed as a reliable indicator of the host’s inflammatory status; elevation of NLR has proved to be an independent predictor of poor prognosis in patients with cancer, associated with inefficient endogenous anticancer defense capability of the immune system. Until now, this index has not been evaluated as a prognostic factor in thyroid cancer. Moreover, to the best of our knowledge, NLR has also never been evaluated as a potential biomarker in the context of detecting underlying malignancy in theoretically benign neoplasia. The aim of this study was to assess the significance of NLR with respect to the presence of PTMC in benign goiters of the thyroid gland in patients with no preoperative evidence of concurrent thyroidal malignancy.

Methods

We performed a retrospective analysis, enrolling 109 patients who underwent surgery in our department between January 2010 and December 2011. More specifically, our study sample consisted of 57 patients who were submitted to total thyroidectomy because of the presence of goiters, without preoperative evidence of underlying PTMC; 26 patients preoperatively diagnosed with thyroid carcinoma, who were also submitted to total thyroidectomy; and 26 healthy subjects, who were referred to our department for hernia repair and served as a control group. The exclusion criteria were positive histologic findings of any kind of thyroiditis, as well as active infection or other known malignancy, chronic inflammatory or autoimmune diseases, presence of hematologic disorders, and current or long-term steroid treatment, because any of these factors could alter the NLR. The presence of thyroiditis in the removed specimens was evaluated by a highly experienced pathologist (consultant); in the presence of any inflammatory elements in the final histopathology report, the patient was excluded from the study sample. Regarding the presence of thyroiditis in the control group, the exclusion of relative pathology was made after thorough clinical examination, basic laboratory screening negative for active inflammation, and absence of a history of thyroidal disorders. Each patient’s medical record was reviewed independently by 2 physicians of our department to ensure that exclusion criteria were accurately met in every candidate for enrollment in our study. NLR was calculated as the ratio of the total count of neutrophils divided by the total count of lymphocytes in fasting morning blood samples obtained from our patients during the process of routine preoperative general blood testing. The blood samples of all patients were obtained in the morning before the scheduled operation (thyroidectomy with or without lymph node dissection, depending on the presence of preoperative findings suggestive of thyroid malignancy, and open hernia repair for the control group), between 7:30 and 9 AM, to standardize the known impact of circulating hormones (circadian rhythm) on the number and subtype distribution of the various white blood cell indices. Moreover, the blood samples obtained were fasting, in accordance with the department’s protocol for routine preoperative evaluation of patients scheduled for elective surgery, which is applied for the standardization of preoperative values of biochemical tests. Finally, the conditions of the proanalytic phase (from the moment blood was drawn until the final blood sample analysis) were standardized in all cases, in accordance with the relevant protocols for handling blood samples as set by the Department of Microbiology.

The patients included in the study were initially categorized in 4 groups. Group 1 consisted of patients with incidental PTMC, who underwent surgery for goiter; group 2 included patients who underwent surgery for goiters, who were negative for PTMC and the presence of histologic examination of the removed gland; group 3 included patients submitted to thyroidection for preoperatively diagnosed thyroid cancer; and group 4 consisted of the control patients. In these 4 groups, we assessed the possibility of existing differences regarding the mean NLR. Sequentially, we calculated the quartile distribution of NLR in the sample of our study and accordingly divided our patients into 4 quartiles, with respect to NLR of each patient. We then performed statistical assessment of any possible correlation of the recorded demographic data and histologic findings among the groups corresponding to the quartile distribution of NLR.

Our statistical analyses were performed using SPSS version 16.0 (SPSS, Inc, Chicago, IL). All continuous and categorical variables were assessed for normal distribution. Comparison of NLR between the initial groups was performed using 1-way analysis of variance, while statistical correlations after quartile splitting of our sample were carried out using Kruskal-Wallis tests for testing NLR quartiles and continuous variables and $\chi^2$ tests when testing NLR quartiles and categorical variables. $P$ values <.05 were considered statistically significant. Patients enrolled in the control group were sex and age matched with the patients who underwent thyroidectomy (no statistical significant differences in age and sex among groups 1–4).

Results

Briefly, the entire cohort’s demographic characteristics were as follows: the mean age was 54.1 years (range, 28–72 years); 51 were men and 58 were women; and the mean NLR among all patients was 2.5 (range, 0.7–6.9) (Table 1). The statistical analysis demonstrated that older patients had significantly higher NLRs ($P < .001$). Among patients who were submitted to total thyroidectomy without preoperative diagnosis of underlying malignancy ($n = 57$), in 26 (46%) of the histopathologic examinations of the removed specimens revealed the presence of PTMCs. Thus, the initial categorization of our study sample was transformed as follows: 26 patients with PTMC (group 1), 31 patients with
benign goiters (group 2), 26 patients preoperatively diagnosed with thyroid cancer (group 3), and 26 controls (group 4). The mean NLRs in each of these groups were 3, 1.9, 3.4, and 1.8 respectively, with the differences being statistically significant between groups 1 and 2 (P < .001), groups 1 and 4 (P < .001), groups 2 and 3 (P < .001), and groups 3 and 4 (P < .001), indicating a clear elevation of NLR in thyroid malignancy compared with benign thyroid disorders and controls (Fig. 1). No statistical significance was attained when comparing mean NLRs among the groups with incidental PTMC and preoperatively diagnosed thyroid cancer. Regarding the group with incidental PTMC, local lymphatic spread in the harvested local minor lymph nodes was detected in none of the patients (0%) (1 or 2 in all cases, situated around the isthmus). No significant correlation was found between elevated NLR and the size of the incidental PTMC (mean PTMC size, 6 ± 2.9 mm).

Sequentially, we separated our patients into quartiles with respect to NLR and assessed the existence of statistically significant differences regarding the distribution of groups 1 to 4, according to the categorization of our patients into quartiles NLR. The first quartile (group A) included patients with NLRs ranging from .7 to 1.65, the second quartile (group B) included patients with NLRs >1.65 to 2.5, the third quartile (group C) included patients with NLRs >2.5 to 3, and the fourth quartile (group D, with the highest values of the ratio) included patients with NLRs >3 to 6.9. After this categorization, group A numbered 50 patients, while groups B, C, and D numbered 45, 11, and 3 patients, respectively. Groups C and D (14 patients) included only patients with incidental PTMC and thyroid cancer, meaning that only patients with thyroid malignancy had preoperative NLRs >2.5. Statistical significance (P < .001) was reached when assessing the existence of potential differences in the distribution of groups 1 to 4 according to NLR quartiles (groups A–D); in other words, elevated NLR was significantly associated with the presence of thyroid malignancy, either cancer or PTMC (Table 2). Moreover, no statistical significance was obtained when assessing age in NLR quartiles, as well as when comparing NLRs between patients with multifocal and unifocal PTMC.

Considering these results, it is clearly demonstrated that patients with elevated NLRs had greater risk for concurrent thyroid malignancy. Nevertheless, although it would be of great interest to propose an NLR cutoff value above which patients with thyroidal goiters may be at higher risk for concurrent PTMC, such an attempt would be ill advised because of the relatively small sample size of our pilot study. Nevertheless, as demonstrated above, only patients with thyroidal malignancy, either preoperatively diagnosed cancer or incidental PTMC, had NLRs >2.5.

**Comments**

Impaired function of the immune system represents a turning point concerning the endogenous capability of intercepting carcinogenesis, with inflammation and cancer pathogenesis being strongly associated. Moreover, apart from the negative impact of chronic inflammation in cancer progression, various studies have demonstrated that carcinogenesis itself enhances chronic inflammation, which has led to the evaluation of inflammation markers as possible predictors of survival and cancer-related complications. The 2 most frequently used markers of active inflammatory status in patients with cancer are C-reactive protein and NLR, because they are reliable and widely available in daily clinical practice, and their sensitivity in predicting survival rates has been supported by a large number of studies in various types of cancer. With respect to the pathophysiologic interpretation of preoperatively elevated NLR, it appears that there are many possible explanations regarding its wide application in predicting survival in patients with cancer. More specifically, low lymphocyte counts have been associated with generalized suppression of the immune systems of patients with cancer. Moreover, coexisting neutrophilia is believed to occur because of either paraneoplastic activity of the primary tumor or the production of granulocyte colony–stimulating factor.

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (men/women)</th>
<th>Age (y), mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTMC</td>
<td>26 (12/14)</td>
<td>53.6 ± 14.2</td>
</tr>
<tr>
<td>Benign goiters</td>
<td>31 (12/19)</td>
<td>51.8 ± 13.6</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>26 (13/13)</td>
<td>55.4 ± 12.6</td>
</tr>
<tr>
<td>Controls</td>
<td>26 (14/12)</td>
<td>55.4 ± 12.0</td>
</tr>
<tr>
<td>Total</td>
<td>109 (51/58)</td>
<td>53.9 ± 13.0</td>
</tr>
</tbody>
</table>

PTMC = papillary thyroid microcarcinoma.
through an interaction between malignant cells and bone marrow granulocytic cells.\textsuperscript{11–14} As a result, elevation of NLR seems justified in patients with cancer with limited immune anticancer capability. Nevertheless, the underlying mechanisms of neutrophilia and lymphopenia in patients with cancer remain a matter of intensive scientific research.

These findings have led to further examination of the significance of preoperative NLR in terms of the prediction of overall survival, disease-free survival, and response to chemotherapy in various types of cancers.\textsuperscript{15–20} As far as thyroid malignancy is concerned, NLR until now has not been examined in any study as a potential prognostic factor of survival. In addition, no studies have assessed NLR elevation as a possible indicator of underlying malignancy in benign neoplastic tumors.

The aim of our pilot study was to investigate the possible application of preoperative NLR elevation as an indirect marker of incidental PTMC in patients submitted to thyroidectomy because of the presence of thyroidal goiters. The findings of our study clearly support that the evaluation of preoperative NLR may be a useful means of assessing risk for the presence of PTMC in thyroidal goiters. Although further studies with larger samples are required to examine this association in patients with thyroid neoplasia, our preliminary results suggest that elevated NLR could be used as a potential biomarker of the necessity of close monitoring in patients with presumably benign goiters. From a more clinical point of view, elevation of NLR could stratify the group of patients with goiters who could possibly be candidates for thyroidectomy or for a more intensive protocol of monitoring the progression of the thyroidal pathology. Moreover, NLR could possibly serve as a marker of closer follow-up after thyroidectomy, to increase the possibility of early detection of recurrence and lymphatic or even distant metastases, because this ratio has been associated with higher risk for recurrence and metastatic spread in previous studies.\textsuperscript{21} The significance of our findings increases considering that, to the present time, confrontation of PTMC remains a matter of scientific debate,\textsuperscript{22,23} while the incidence of PTMC is explosively increasing\textsuperscript{24,25} and NLR stands as an emerging biomarker of cancer activity in an increasing number of types of solid malignancies (Table 3).\textsuperscript{26–28,30–33}

Taking into account the relatively small sample of patients enrolled in our study, it would be ill advised to propose a cutoff NLR that would be more safely associated with underlying malignancy. Because there are no previous data on the evaluation of NLR concerning both thyroid malignancies and lesions that share characteristics similar to the microcarcinomas (extent, progression, and biologic behavior), we decided to include an analysis based on the quartile distribution of NLR, following the excellent study by Azab et al,\textsuperscript{17} who were the first group to assess NLR in breast cancer and thus had no previous relevant data on

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Statistical significance ($P &lt; .001$) was obtained when assessing the existence of potential differences in the distribution of groups 1 to 4 according to NLR quartile (groups A–D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PTMC</td>
</tr>
<tr>
<td>Group A (first quartile)</td>
<td>6</td>
</tr>
<tr>
<td>NLR $&gt; 1.65$</td>
<td>23.1%</td>
</tr>
<tr>
<td>Group B (second quartile)</td>
<td>15</td>
</tr>
<tr>
<td>NLR $&gt; 1.65–2.5$</td>
<td>57.7%</td>
</tr>
<tr>
<td>Group C (third quartile)</td>
<td>5</td>
</tr>
<tr>
<td>NLR $&gt; 2.5–3$</td>
<td>19.2%</td>
</tr>
<tr>
<td>Group D (fourth quartile)</td>
<td>0</td>
</tr>
<tr>
<td>NLR $&gt; 3–6.9$</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>26 (100.0%)</td>
</tr>
</tbody>
</table>

NLR = neutrophil/lymphocyte ratio; PTMC = papillary thyroid microcarcinoma.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Presentation of the increasing rate of application of NLR as a prognostic factor of survival in various types of cancer (selected publications in leading journals in 2012 alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Type of malignancy</td>
</tr>
<tr>
<td>Gondo et al\textsuperscript{26}</td>
<td>Bladder cancer</td>
</tr>
<tr>
<td>Dutta et al\textsuperscript{27}</td>
<td>Gastric cancer</td>
</tr>
<tr>
<td>Kwon et al\textsuperscript{28}</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>He et al\textsuperscript{29}</td>
<td>Nasopharyngeal carcinoma</td>
</tr>
<tr>
<td>Pinato et al\textsuperscript{30}</td>
<td>Malignant pleural mesothelioma</td>
</tr>
<tr>
<td>Wang et al\textsuperscript{32}</td>
<td>Gastric cancer</td>
</tr>
<tr>
<td>Ohno et al\textsuperscript{33}</td>
<td>Renal cell carcinoma</td>
</tr>
</tbody>
</table>

NLR = neutrophil/lymphocyte ratio.
which to base their findings. We adopted their approach because, in any other case, on the basis of the conventional cutoff values associated with poor survival, we would in fact compare the interplay between thyroid malignancy and the immune system to that between the immune system and highly immune-regulating types of cancer, such as pancreatic, gastric, and colorectal cancer. Nevertheless, according to our results, only patients with preoperatively diagnosed thyroid cancer and incidental PTMC had NLRs >2.5; after grouping our patients according to NLR quartiles, we found that the third and fourth quartiles, corresponding to higher NLR values, included only patients with thyroidal malignancy, either cancer or incidental microcarcinomas. Moreover, approximately 20% of all patients with incidental PTMC and 35% of patients with thyroid cancer belonged in the top 2 quartiles, with this particular finding being a primitive indicator of the pool of patients who could benefit from coevaluation of NLR in the context of monitoring thyroid pathology. Finally, with respect to the fact that our study sample did not reflect the 3:1 female preponderance in thyroid operations, the ultimately equal numbers of male and female patients are a consequence of the nature of the hospital, which is a tertiary military hospital; thus, an above-average percentage of patients are inevitably men.

Although promising in terms of sensitivity, it is obvious that NLR, at least excluding the highest values, cannot be regarded as a specific detector of malignancy. As the exclusion criteria of our study highlight, NLR can be influenced in many common pathologic conditions. Nevertheless, it should be taken into account that NLR is always available in routine blood tests and does not increase the cost of the diagnostic strategy; this particular point represents a major advantage regarding its easy and cost-effective application. Also, as mentioned above, clinical suspicion of underlying malignancy can be increased with relative safety when a remarkable elevation of NLR is found.

In summary, our pilot study suggests that NLR elevation may be useful as a serum biomarker in terms of stratifying patients with thyroidal goiters regarding the possibility of having concurrent PTMC. Although of doubtful specificity, NLR appears to be a cost-effective and rather sensitive indicator of progressing thyroid malignancy in patients with goiters. Further studies are required to confirm our preliminary results and shed light on the possible association of thyroidal malignancy with NLR elevation.

References


