World Cancer Research Journal WCRJ 2021; 8: e1915

THE ROLE OF PREOPERATIVE LEVELS OF SERUM IL-6, IL- 8 AND TNF-α AND CONVENTIONAL INFLAMMATORY PARAMETERS IN THE DETECTION OF METASTATIC FORMS OF PAPILLARY THYROID CANCER

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Abstract – *Objective:* *Inflammation is an important part of cancer pathophysiology. Preoperative detection of inflammatory parameters like cytokines and conventional inflammatory parameters (CIP), as well as their correlation, could be helpful in early detection of M-PTC. We aimed to find the preoperative serum levels of IL-6, IL-8, and TNF-αand conventional inflammatory parameters (NLR, PLR, platelets, and hs-CRP), and their correlation with demographic and clinical and pathological characteristics of patients and their disease in patients with papillary thyroid cancer (PTC), metastatic papillary thyroid cancer (M-PTC), and in a group of healthy subjects (HS). We also aimed to determine the best possible pair of cytokines and/or conventional inflammatory parameters that may help in the early detection of M-PTC.*

Patients and Methods: *A cross-sectional study which involved 123 subjects, divided into three groups: 43 subjects with M-PTC, 48 subjects with PTC and 34 HS. Data were taken using a survey and from a medical record and blood samples were taken preoperatively. Standard laboratory tests were performed immediately, and sera were frozen, as the cytokine analysis was performed by ELISA after a sufficient number of patients had been collected.*

Results: *NLR, PLR, Platelets, hs-CRP, and IL-6 differentiated subjects with metastases from those without specific cut-off values. In the multivariate analysis, hs-CRP had a greater ability to predict metastases than platelets. None of the parameters tested were able to differentiate patients with PTC from healthy subjects. The greatest number of parameters (IL-6, NLR, PLR, platelets, and hs-CRP) was in correlation with tumor size and fewer with lymphovascular invasion (hs-CRP and NLR).*

Conclusions: *None of the parameters tested distinguished benign from malignant disease. NLR, PLR, platelets, IL-6 and especially hs-CRP are useful markers of M-PTC.*

KEYWORDS: *Thyroid cancer, Papillary, Cytokines, C-reactive protein, Blood platelets, Neutrophils, Lymphocytes.*

INTRODUCTION

Papillary thyroid cancer (PTC) shows a high potential for regional spread and metastasis, and regional lymphatic metastases were found in 15-90% of all PTC cases [1-4].

Most clinical guidelines recommend near-total or total thyroidectomy as an optimal treatment for all preoperatively diagnosed PTCs[5]. The issue of prophylactic central compartment neck dissection is still the subject of debate, but the general recommendations suggest prophylactic dissection

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of the clinically negative neck only in high-risk groups of patients [6,7]. The primary form of treatment for M-PTC is total thyroidectomy with neck dissection and postoperative radioiodine therapy. There is a consensus that the presence of metastases increases the risk of recurrence to lymph nodes and mortality in general[8]. Reoperations of these patients increase the risk of operative complications, such as recurrent nerve and parathyroid gland injuries, which can be life-threatening. This is the reason while controlling regional recurrences poses the greatest challenge for the surgeon[9].

Inflammation is a component of a non-specific immune response, and the inflammatory process is one of the ways in which malignant disease occurs and progresses. It is involved in tumor initiation and promotion, angiogenesis, and metastasis[10]. Only a small number of cancers are the result of genetic mutations, and the largest number of them (up to 90%) are caused by somatic mutations and environmental factors. Up to 20% of environmental factors are the result of some form of chronic inflammation, i.e. chronic inflammation and infectious diseases account for 25% of all cancer causes[11]. Key markers of inflammation are cytokines and conventional inflammatory parameters (CIP). In thyroid cancer, cytokine dysregulation is found in both benign and malignant diseases. They are produced mainly by tumor-infiltrating, but also by the follicular cells themselves, and in both diseases, they can be easily detected in blood samples[12]. The prognostic role of the systemic inflammatory response (SIS) has been studied in a number of cancers[13]. SIS is the effect of tumor hypoxia or necrosis or a result of local tissue damage[14]. CIP are markers of SIS, they include Neutrophil/Lymphocyte Ratio (NLR), Platelet/Lymphocyte (PLR), Platelets, and hs-CRP. They are cheap and affordable, they reflect the immune and inflammatory response, and numerous studies have linked them to a poorer tumor prognosis[15,16].

Preoperative detection of these parameters, as well as their correlation, could be helpful in early detection of M-PTC, which would allow optimization of the therapeutic approach to these patients.

The aim of this study was to determine the preoperative levels of serum IL-6, IL-8 and TNF-α and CIP: NLR, PLR, Platelets and hs-CRP and their correlation with demographic and clinical and pathological characteristics of patients and their disease (sex, age, BMI, TSH, tumor size, extrathyroid extension, lymphovascular invasion, site of metastasis, tumor stage according to AJCC) in three groups of patients: those with PTC, M-PTC, and in a control group (CG). Also, the goal was to determine the best possible pair of cytokines/CIP in the early detection of M-PTC.

PATIENTS AND METHODS

The research was cross-sectional and was conducted at the Tertiary Care Clinic for Ear, Nose, and Throat Diseases and at the Department of Laboratory Diagnostics. The study included 123 patients, divided into three groups: 48 patients with PTC, 43 patients with M-PTC and 34 controls. The minimum number of patients was defined statistically and the distribution into groups was defined by the inclusion and exclusion criteria. Included were patients with pathohystologically confirmed PTC, as well as patients with cervical lymph node status suggestive for PTC. Included were also patients who were otherwise healthy, did not have previous neck operations, acute or chronic inflammatory diseases, and were older than 12 years.

The control group consisted of patients admitted to the hospital for nasal septum and/or nasal pyramid surgery, patients with vocal cords polyps, and patients with congenital neck cysts, i.e. patients with diseases not related to inflammation. All of these patients did not have thyroid diseases, other primary tumors, they denied allergic reactions to food and/or medication and were older than twelve years.

Blood was taken preoperatively, and patients underwent surgery depending on the main diagnosis. Total thyroidectomy with or without neck dissection was performed, and the control group was also operated. The findings of the differential blood count and the highly sensitive C-reactive protein were completed the same day, and the required values of inflammatory parameters were calculated. Analyzes of patients' sera for IL-6, IL-8, and TNF-α were performed using human TNF-α total Platinum ELISA Kit BMS2034 (96 tests) (Affymetrix / eBioscience), human IL6 Elisa Kit BMS213 / 2 (96 tests) (Invitrogen, Carlsbad, CA, USA), and human IL8 / NAP-1 Elisa Kit BMS 204-3 (96 tests) (Invitrogen, Carlsbad, CA, USA) for quantitative detection.

Statistical analysis

Statistical analysis was performed using the SPSS program (Version 19, SPSS Inc., Chicago, IL, USA). To test the difference, Kruskal-Wallis and Mann-Whitney tests were used, and to test a connection, Spearman's correlation test was used.

Univariate and multivariate binary logistic regression examined the influence of independent predictors (NLR, PLR, platelets, hs-CRP, IL-6, IL-8, TNF-α, TSH) on the outcome of the dependent variable "metastasis". The ROC curve showed whether these tested parameters could be mark-

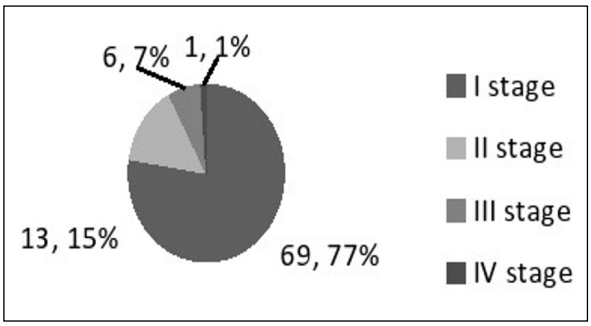

Fig. 1. Tumor stage.

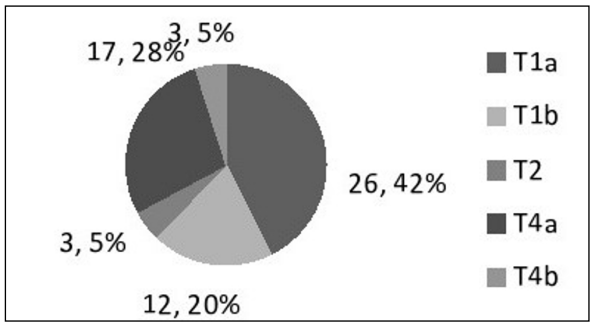

Fig. 2. Tumor size.

ers of M-PTC. Based on the Youden index, we determined the optimal cut-off for each variable, followed by sensitivity and specificity. Accepted statistical significance was at the level of $p < 0.05$.

RESULTS

The study included 123 subjects, divided into three groups: 41 M-PTC patients (33.3%), 48 (39.0%) PTC patients, and 34 (27.6%) controls. There were more female patients (67.5%), while almost one third patient was male (32.5%).

The average age was 46.47 ± 14.23 years; the youngest patient was 12 and the oldest was 75 years old. Subjects with PTC were the oldest (49.65 ± 13.31 years), followed by controls (44.91 ± 12.15 years), and M-PTC patients (44.05 ± 16.37 years). There was no statistically significant difference between the groups p (ANOVA) = 0.137. The frequency of tumor stages and different tumor sizes are shown on Figure 1 and Figure 2, respectively.

There were 41 M-PTC patients, 21 (51.2%) of them had paratracheal and lateral metastases, 15 (36.6%) only paratracheal and 5 (12.2%) only lateral metastases. The preoperative values of tested parameters and their difference are shown on Table 1.

The tested difference in IL-6, NLR, PLR, platelets and hs-CRP between PTC and M-PTC and M-PTC and controls was statistically significant ($p<0.05$), while the difference between PTC and controls was not statistically significant ($p>0.05$). Mann-Whitney test showed that the differences in IL-8 and TNF-α values were statistically significant only between M-PTC and controls, $p= 0.016$ and 0.010, respectively (Table 1).

IL-6 values were in moderately strong and positive correlation with CRP, and in weak and positive with platelets, i.e. higher IL-6 values were found in patients with higher CRP and higher platelet counts (Table 2).

IL-8 and TNF-α values were not in correlation with CRP, NLR, PLR, and platelets $p> 0.05$. Among all tested characteristics, IL-6 values were in strong and positive correlation only with BMI, in moderately strong and positive with tumor multifocality, and in mild and positive correlation with tumor size. Higher IL-6 values were found in patients with higher BMI, larger tumor size and tumor multifocality (Table 3).

IL-8 and TNF-α values were not in correlation with demographic and clinical and pathological characteristics of patients and their disease ($p> 0.05$).

NLR values were in mild and positive correlation with tumor size and LVI, i.e. higher NLR values were found in patients with a larger tumor

TABLE 1. The values of tested differences in different groups of patients.

Patient group	*IL-6*	*IL-8*	*TNF-α*	*NLR*	*PLR*	*Platelets*	*Hs-CRP*
PTC	$^{\#}$4.7(4.2-5.2)	$^{\#}$18.1(15-26)	$^{\#}$22 (22-29)	$^{\#}$1.5(1.2-1.9)	$^{\#}$113.0(94.3-146.1	$^{\#}$244(199-282)	$^{\#}$0.9(0.5-2.7)
M-PTC	*6(5.1-10.2)	*21.5(16.5-34)	*24 (21-30.5)	*2.1(1.6-2.9	*171.2(41-198.1)	*290(247-334)	5.7(2.1-8.7)
HS	$^{\$}$4.5(3.6-5.1)	$^{\$}$16.8(11.7-23.5)	$^{\$}$21(16.5-23.7)	$^{\$}$1.6(1.3-2.1)	$^{\$}$123.5(101.3-153.2	$^{\$}$243(200-292)	0.5(03-0.7)
***p*-value**							
PTC. M-PTC	$p^{*\#}$=0.001	$p^{*\#}$>0.05	$p^{*\#}$>0.05	$p^{*\#}$=0.001	$p^{*\#}$=0.001	$p^{*\#}$<0.05	$p^{*\#}$<0.05
M-PTC-healthy subjects	$p^{*\$}$=0.001	$p^{*\$}$=0.016	$p^{*\$}$=0.010	$p^{*\$}$=0.001	$p^{*\$}$=0.001	$p^{*\$}$<0.05	$p^{*\$}$<0.05
PTC-healthy subjects	$p^{\#\$}$=0.405	$p^{\#\$}$>0.05	$p^{\#\$}$>0.05	$p^{\#\$}$=0.405	$p^{\#\$}$=0.501	$p^{\#\$}$=0.854	$p^{\#\$}$=0.854

TABLE 2. Correlation of IL-6 with CRP, NLR, PLR and Platelets.

	Spearman's rho	CRP	NLR	PLR	Platelets	BMI	Tumor size	Tumor multifocality
IL-6	Correlation Coefficient	0.346(**)	0.185	0.139	0.218(*)	.288	.233	.321
p	*p*	0.001	0.071	0.177	0.033	.004	.042	.005
	N	96	96	96	96	96	76	76

TABLE 3. Correlation of IL-6 with clinical and pathohistological characteristics of patients.

	Spearman's rho	BMI	Tumor size	Tumor multifocality
IL-6	Correlation coefficient	.288	.233	.321
p	*p*	.004	.042	.005
	N	96	76	76

size and patients with LVI. PLR values were in mild and positive correlation with TSH, tumor size, ENE, LVI, and LI (p <0.05), i.e. higher PLR values were found in patients with higher values of these characteristics. Platelet values were in mild and positive correlation with tumor size and LVI (p <0.05) i.e. higher platelet counts were found in patients with larger tumors and present LVI. Platelet values were also in mild and negative correlation with tumor stage (p <0.05), i.e. subjects with a lower tumor stage had a higher platelet counts. Hs-CRP values were in mild and positive correlation with BMI, tumor size and LVI, and in moderately strong and positive correlation with tumor multifocality (p <0.05), i.e. higher CRP values were found in patients with higher BMI, larger tumor, with LVI present, and tumor multifocality (Table 4).

NLR, PLR, platelets and hs-CRP values were not in correlation with other demographic and clinical and pathological characteristics of patients, p> 0.05.

Univariant binary logistic regression showed that from all tested parameters, IL-6, NLR, PLR, platelets, and hs-CRP were statistically significant predictor of metastases (OR=1,199, OR = 1.808, OR = 1.189, OR = 1.147 and OR = 1,048 respectively; p<0.05) (Table 5).

For increase of IL6 and NLR by 1, the chance that the patient gets a metastasis increases by 20% in our sample (it ranges between 1% and 42% in population), and by 1.8 times in our sample in

TABLE 4. Statistically significant correlations of NLR, PLR, Platelets, and Hs-CRP with clinical and pathohistological characteristics of patients.

Rho values	Tumor size	LVI*	Tumor stage	ENE*	LI*	Multifocality	BMI	TSH	
NLR	0.255	0.244	–	–	–	–	–	.	
PLR	0.378	0.258	–	0.266	0.267	–	–	0.245	*p*-value-
Platelets	0.221	0	-0.364	–	–	–	–	–	<0.05
Hs-CRP	0.327	0.259	–	–	–	0.457	0.220	–	

TABLE 5. Predictive ability of independent variables on the dependent variable: metastases (yes / no).

							95.0% C.I.for EXP(B)	
	B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
NLR	.592	.244	5.873	1	**.015**	**1.808**	1.120	2.920
PLR1	.173	.051	11.752	1	**.001**	**1.189**	1.077	1.313
TR1	.137	.043	10.297	1	**.001**	**1.147**	1.055	1.247
CRP1	.047	.011	17.438	1	**.000**	**1.048**	1.025	1.071
TNF	.004	.005	.451	1	**.502**	**1.004**	.993	1.014
IL8	.014	.014	1.131	1	**.287**	**1.015**	.988	1.042
IL6	.181	.089	4.164	1	**.041**	**1.199**	1.007	1.427
TSH	.085	.090	.883	1	**.347**	**1.088**	.912	1.299

TABLE 6. Multivariant predictive significance of independent variables on the dependent variable: metastases (yes / no).

							95.0% C.I. for EXP(B)	
Model 1		*B*	*S.E.*	*Wald*	*df*	*Sig.*	*Exp(B)*	*Lower*	*Upper*
NStep 1(a)	NLR	-.058	.299	.037	1	.847	.944	.525	1.696
	PLR1	.123	.077	2.517	1	.113	1.130	.972	1.315
	TR1	.129	.063	4.114	1	.043	1.137	1.004	1.288
	CRP1	.056	.017	10.184	1	.001	1.057	1.022	1.094
	IL6	.130	.084	2.425	1	.119	1.139	.967	1.342
	Constant	-7.334	2.078	12.454	1	.000	.001		
Step 2(a)	PLR1	.117	.070	2.752	1	.097	1.124	.979	1.289
	TR1	.131	.063	4.369	1	.037	1.140	1.008	1.289
	CRP1	.055	.017	10.316	1	.001	1.057	1.022	1.093
	IL6	.127	.082	2.424	1	.119	1.136	.968	1.334
	Constant	-7.390	2.062	12.839	1	.000	.001		
Step 3(a)	PLR1	.123	.069	3.117	1	.077	1.130	.987	1.295
	TR1	.135	.061	4.941	1	.026	1.144	1.016	1.288
	CRP1	.051	.016	10.003	1	.002	1.053	1.020	1.087
	Constant	-6.664	1.916	12.097	1	.001	.001		

NLR case (it ranges from 1 to 2.9 times in the population). If the PLR and platelets increase by 10, the chance that the patient gets a metastasis increases by 19% in our sample (8% to 31% in population) and 15% in our sample in platelets case (between 5% and 25% in the population).

For increase of CRP by 0.1, the chance that the subject gets a metastasis increases by 5% in our sample, and it ranges between 3% and 7% in the population.

TNF-α, IL-8 and TSH univariantely in our sample are not significant predictors of metastases (p> 0.05).

Multivariant binary logistic regression examined the influence of independent predictors, which univariately showed a significant prediction for metastases in subjects with thyroid cancer (NLR, PLR, platelets, hs-CRP and IL-6) (Table 6).

We obtained Model 1 in the third step where the values of Cox & Snell R2 = 0.438 and Nagelkerke R2 = 0.585, showed that the set of variables could explain between 43.8%- 58.5% of the variance. Furthermore, the results of the Hosmer and Lemeshow test supported the claim that the model was good, ie $\chi^2 = 7.5$; $p = 0.784$.

Platelets and CRP showed a statistically significant (p <0.05) and independent effect by the "Backward Stepwise" method in three steps. CRP had a higher prediction (Wald = 10.3) compared to platelets (Wald = 4.9) in the identification of thyroid cancer.

IL-6 values of 5.25 have the highest Youden index= 0.528, sensitivity (75.6%) and specificity (77.1%) are at this cross section the highest; i.e. IL-6 values > 5.25 may differentiate M-PTC and PTC patients.

IL-8 and TNF-α values could not distinguish patients with M-PTC compared to the PTC patients (p = 0.283 and p = 0.362 respectively) (Table 7).

NLR values of 1.81 have the highest Youden index=0.433; sensitivity (68.3%) and specificity (75.0%) are at this cross section the highest; i.e. NLR values> 1.81 can distinguish M-PTC and PTC patients. PLR values of 113.23 have the highest Youden index = 0.423, i.e. PLR at this cross section has the highest sensitivity (90.2%) and the highest specificity (52.1%). PLR values> 113.23 may differentiate M-PTC and PTC patients. Platelet values of 262.5 have the highest Youden

TABLE 7. AUC for tested parameters for distinguishing M-PTC and PTC patients.

	IL-6	*IL-8*	*TNF-α*	*NLR*	*PLR*	*Platelets*	*Hs-CRP*
AUC	0.764	0.572	0.561	0.736	0.756	0.721	0.812
Statistical error	0.057	0.066	0.067	0.054	0.051	0.055	0.046
p	0.0001	0.283	0.362	0.0001	0.0001	0.0001	0.0001
95% CI	0.653-0.875	0.442-0.701	0.430-0.692	0.630-0.841	0.656-0.856	0.613-0.829	0.721-0.903

index = 0.416, i.e. platelets at this cross section have the highest sensitivity of 70.7% and specificity of 71.8%. Platelet counts > 262.5 may differentiate M-PTC and PTC. CRP values of 5.65 have the highest Youden index = 0.491, i.e. CRP at this cross section has the highest sensitivity of 51.20% and the highest specificity of 91.80%. CRP values> 5.65 may differentiate M-PTC and PTC patients.

DISCUSSION

There is a cytokine dysregulation present in all thyroid diseases, and significantly higher levels of IL-6, IL-7, IL-10 and IL-13, as well as significantly lower levels of IL-8 exist in patients with benign and malignant thyroid disease[17]. IL-6 is a multifunctional cytokine; it has both pro- and anti-inflammatory properties, and has a central role in the regulation of the inflammatory and immune response[18]. In our study, patients with M-PTC have the highest values of IL-6 6 (5.1-10.2) and IL-6 cannot differentiate between PTC and control group. The study of Martins et al[19] found that the serum levels of IL-6, but also IL-2 and IL-2R, IL-6R, IL-8, IL-10 and IL-12 could differentiate benign from malignant thyroid disease; sensitivity of IL-6R was 56%, specificity 63%, PPV 60% and NPV 59% (p <0.0001). The same findings were showed by Kobawala et al[12] (AUC = 0.708, p <0.001) and Provatopoulou et al[20]. In our study, IL-6 distinguishes M-PTC from healthy subjects, and IL-6 values greater than 5.25 differentiate M-PTC from PTC patients.

IL-6 values are in correlation with CRP, platelets, and BMI, which was also found in the study of Warakomski et al[21]. By logistic regression, waist circumference ≥ 88 in women and ≥102 cm in men, upper tertiary IL-6 and leptin were associated with a higher clinical stage of the disease. IL-6 in our study was also in correlation with tumor size and multifocality and Kobawala et al[20] also found a significant positive correlation with larger tumor size, as well as with extrathyroid extension, the presence of distant metastases and poor overall survival.

IL-8 has a significant role in acute inflammatory response where it has a chemotactic function for neutrophils, and in chronic inflammatory conditions such as cancer, where it has mitogenic and angiogenic function[22]. Its values are highest in the group of M-PTC patients 21.5 (16.5-34) and, therefore, IL-8 can differentiate M-PTC from healthy subjects only and cannot distinguish benign from malign disease. On the other hand, Martins et al[19] have demonstrated that IL-8, among other cytokines such as IL-2, IL-2R, IL-6, IL-6R, IL-8, IL-10 and IL-12, differentiates benign from malignant thyroid disease with a sensitivity of 50%, specificity 76%, PPV 68% and NPV of 60% (p = 0.0025)[19]. Kobawala et al[23] have demonstrated significantly higher levels of IL-8 and interferon-alpha (IFN-α) in all patients with thyroid disease (including thyroid cancer patients- AUC-0.767) compared to healthy individuals. It also had the ability to discriminate between early and late stages of thyroid cancer patients (AUC-0.749). In our study, IL-8 was not in correlation with other parameters nor with the characteristics of patients, nor a predictor of tumor metastases.

The role of TNF-α in cancer is quite complex; in some tumors it induces cell death, i.e. it has an antitumor effect, and in others it stimulates the proliferation, survival, migration and angiogenesis of tumor cells[24]. Subjects with M-PTC have the highest values of TNF-α 24 (21-30.5), and TNF-α, just like IL-8, can only differentiate M-PTCs from healthy subjects, and cannot differentiate benign from malign disease, nor it can predict metastases.

TNF-α increases the metastatic potential of thyroid tumors increasing the membrane expression of the chemokine receptor CCR6, selectively bounded by CCL20, which is involved in the metastasis of thyroid cancer[25].

The TNF-α value in our study did not correlate with other tested parameters, nor with the demographic and clinical and pathological characteristics of patients. In a study by Ari et al[26] TNF-α has a positive and significant association with PLR and leukocytes. In a study by Zhang et al[27], it correlates with IL-17, and is proved to be a useful indicator of a poorer prognosis of patients with PTC.

The role of NLR in thyroid cancer is still the subject of debate[15]. This is, among others, the result of heterogeneity of the statistical methodology and large range of sample sizes of previous studies; studies range from 41 to 3364 cases per sample[28]. Galdiero et al[29] found a correlation between tumor-infiltrating neutrophils and the size of thyroid cancer and it is also known that the lymphocytic infiltration i.e. autoimmune diseases occur more frequently in the thyroid gland than in other organs. In our study, NLR was the largest in the M-PTC group 2.07 (1.6-2.9), it could differentiate between patients with M-PTCs and PTC and healthy controls, which confirms the thesis about the correlation with more advanced tumors. In the study by Cho et al[30], NLR thresholds of 3.8 discriminate between papillary on the one hand, and poorly differentiated and anaplastic thyroid cancer on the other (p = 0.035, p = 0.002, and p = 0.025). The study by Manatakis et al[28] found

higher NLR values in metastatic tumors (3.12 ± 1.07) compared to those without metastases (2.41 ± 1.02; $p = 0.03$). In our study, NLR values greater than 1.81 may distinguish subjects with metastasis from those without. In a study by Wen et al[31] the ability of NLR to predict metastases is small, with an AUC of 0.603 ($p = 0.109$), and in the study by Lang et al[32], NLR did not correlate with the risk of central occult neck metastases. In our study, NLR is in correlation with tumor size, and LVI. Liu et al[15] found that cancer patients in the larger NLR tercile had a significantly larger tumor size ($p = 0.004$), as well as a study by Ozmen et al[33].

Increased PLR is a result of increased number of megakaryocytes, i.e. total number of platelets in the peripheral blood, as a consequence of the compensatory response of the immune system and a result of a decrease in the total number of lymphocytes due to the suppression of the immune response. In our study, PLR is not able to discriminate benign from malignant disease, while study by Ari et al[26] found significantly higher PLR values in patients with thyroiditis (139.1 ± 52.0; $p < 0.001$) and PTC patients (136.7 ± 57.0; $p = 0.003$) than in healthy controls. PLR values in our study were the highest in the M-PTC group 171.2 (123.4-198.1) and the PLR values greater than 113.23 can distinguish M-PTC patients.

Patients with higher preoperative PLR values have a higher incidence of Lateral Nodal Metastases (LNM) ($p = 0.018$), which confirms its relation to advanced forms of cancer[16]. Patients with larger tumor size, extranodal extension, LVI, LI, and higher TSH values also have higher PLR values, while there is no correlation with other characteristics. In the study by Kim et al[16] PLR was also significantly higher in patients with larger tumor size (> 1 cm, $p = 0.021$), as well as in the study by Ozmen et al[33]. In this study, higher PLR was associated with higher thyroglobulin levels, making it a marker of poorer survival (the AUC value for PLR is 0.796; $p = 0.000$)[33].

Platelets play a significant role in cancer progression and metastasis by stimulating neoangiogenesis, growth, and dissemination of tumor cells. Tumor cells activate platelets through various mechanisms, from the release of platelet-activating mediators, i.e. ADP, thromboxane A, direct cell-to-cell contact, to cytokine production[34]. Our results showed that platelets do not have the ability to differentiate benign from malignant tumors, and other authors have the same results[35,36]. Patients with metastatic carcinomas have the highest platelet count, i.e. 290 x10^9/L (247-334 x10^9/L) and platelet values greater than 262.5 can differentiate patients with metastases from those without. There is a positive correlation of platelets with tumor size and LVI, and negative with higher tumor stage. A positive association with T3 tumor size and extrathyroid extension has been found in other studies[35]. No correlations were found with other clinical and pathological characteristics of the patient, including hormonal status (TSH), which was confirmed in other studies[37].

Elevated CRP levels have been found in many cancers. CRP is either initially elevated due to a series of inflammatory processes that cause mutations in tumor suppressor genes and genes involved in DNA repair, thereby promoting carcinogenesis, or is released by inflammatory cytokines, such as IL-6 and IL-1β[38].

CRP values in our study were the highest in the group of M-PTC patients 5.7 mg/dl (2.1-8.7) and CRP does not have the ability to distinguish between PTC and healthy subjects. A small ability of CRP to detect differentiated thyroid cancer (AUC value 0.58; $p = 0.28$) was also found by Ozmen et al[33]. Dossus et al[39] found that CRP, along with leptin, IL-6, and TNF-α was also not associated with a risk of developing thyroid cancer in either sex, which confirmed also a Sweden with the Apolipoprotein-related mortality risk study (AMORISS Apolipoprotein-related MOrtality RISk)[40].

CRP values greater than 5.65 can differentiate subjects with metastases from those without. In our study, CRP is in correlation with tumor size, multifocality, LVI, and BMI. Ozmen et al[33] also found a positive correlation with a poorer tumor profile, predominantly with tumor size and patient age. Elevated serum IL-4, IL-10 and hs-CRP levels were found in patients with persistent and/or recurrent disease compared to those without recurrence ($p < 0.001$), and positive correlations were found with thyroglobulin (Tg) (r between 0.48 and 0.56; $p < 0.005$) and antithyroglobulin antibodies (TgAb) (r between 0.63 and 0.80; $p < 0.002$). They also found a positive association with thyroglobulin levels measured six months postoperatively, making CRP also an indicator of poorer survival[33]. Shorter survival in patients with higher CRP was also found in a study by Shimura et al[41]. Preoperative CRP values ≥ 0.155 mg/dL are an independent prognostic factor for relapse-free survival in patients with differentiated thyroid cancer (HR = 6.334; 95% CI 1.023-39.234; $p = 0.037$). The study also included metastatic cancers, which is a great advantage, but it had a deficiency in a small sample (45 patients) [41].

As it is obviously, none of these examined parameters can differentiate benign from malignant thyroid disease, so they cannot be a predictor of malignancy. The reason for this may be the predominantly indolent behavior of PTCs. M-PTCs, on the

other hand, has a more aggressive clinical behavior, which is accompanied by a more advanced degree of inflammation, and the inflammatory markers in these tumor forms are, therefore, higher.

Limitations of this work include primarily the fact that the data were obtained from one institution. This limits the sample size, but generally fits the average of previous studies. By including a larger number of institutions, we would gain in diversity, in the number of patients, but we would also get a clearer perception of the role of these parameters. Cross-sectional character of the study was also a limiting factor. Including a follow-up period would significantly contribute to a better understanding of the problem.

Thus, future research may certainly include the possible use of IL-6, IL-8 and TNF-α as possible targets in cancer therapy, because their role in advanced forms of tumors is also clearly confirmed by this study. Antibodies directed against IL-6 (Tocilizumab and Sarilumab) are already used in patients with rheumatoid arthritis (RA and JIA). Siltuximab is a monoclonal antibody of the IgG1κ class, indicated for the treatment of Castleman's disease in adult patients who are negative for immunodeficiency virus (HIV) and human herpes virus-8 (HHV-8)[42]. There are several monoclonal antibodies directed against TNF-α. They include infliximab (IFX) and adalimumab (ADA), indicated for the treatment of rheumatoid arthritis, inflammatory bowel disease, psoriasis and psoriatic arthritis; Golimumab (GOL), indicated for arthritis, spondylitis, psoriasis, and soluble TNF-α receptor etanercept (ETN), indicated for rheumatoid arthritis. Contraindications for their use include TB, as they can activate it, other severe infections such as sepsis, abscesses, and opportunistic infections, and moderate or severe heart failure (NYHA grade III / IV)[42,43]. IL-8, on the other hand, has been tested therapeutically in thyroid cancer. In this, *in vitro* study, it was used Reparixin, which by inhibiting CXCR1 and CXCR2 IL-8 receptors, significantly inhibits thyroid cell tumorigenicity in immunodeficient mice. Thus, it represents a new, potential strategy, for aggressive forms of thyroid cancer[44]. Bilusic et al[45] examined the effect of HuMax-IL-8 (BMS-986253) a new monoclonal antibody that inhibits IL-8. Fifteen subjects with metastatic or unresectable locally advanced solid tumors (a patient with papillary thyroid carcinoma, ovarian tumor, chondrosarcoma, esophageal tumor, and five patients with chordomas, four colorectal tumors, and two prostate tumors) were included in this study. 11 subjects (73%) had stable disease with a median treatment of 24 weeks (range 4-54 weeks), and serum IL-8 was significantly reduced during day 3 of HuMax-IL8 treatment compared to control (p = 0.0004), with a reduction in IL-8 at all dosing levels. Thus, although HuMax-IL-8 is safe and well tolerated, further studies are needed[45].

There are also a few studies done in order to research the nutraceutics in the treatment of the papillary thyroid cancer. Quagliariello et al[46] studied the effects of Quercetin, as a well-known bioactive molecule and potent anticancer, antioxidant and antiinflammatory agent.

They concluded that the pretreatment of patients with medullary and papillary human thyroid cancer with Quercetin or with Hialuronic Acid- Hydrogel of Quercetin at the indicate concentrations decrease significantly the level of IL8 and TNF alpha respectively in both cell lines. Also, HA-hydrogel of Quercetin has better antiinflammatory effects compared to its unformulated form.

Perna et al[47] treated the TPC-1 cells with the three different curcumin extracts and examined the levels of expression of different markers (proliferative, inflammatory, antioxidant, apoptotic). Curcumin showed anti-inflammatory, antioxidant properties, it influences the cell cycle with different effects among different extracts and it also influences cell metabolic activity vitality. Its therapeutic safe has needed to be verified *in vivo*. Nutraceutics are really powerful against acute and chronic diseases, as well as cancer itself, although their implementation in future has to be verified.

CONCLUSIONS

None of these examined parameters cannot differentiate benign from malignant thyroid disease, and thus are not predictors of malignancy. IL6, NLR, PLR, platelets and CRP have an ability to differentiate M-PTC and PTC patients with different cut-off values (5.25, 1.81, 113.23, 262.5 and 5.65, respectively). By multivariant binary logistic regression, platelets and CRP have a statistically significant and independent effect, with CRP having a higher ability of prediction.

TNF-α, IL8 and TSH univariantely in our sample are not significant predictors of metastases in PTC patients, they only differentiate M-PTC to healthy subjects, i.e. they discriminate only advanced forms of disease.

There is only correlation between IL-6 and CRP, and also IL-6 and platelets present. Most of parameters (IL-6, NLR, PLR, platelets and CRP) are in correlation with tumor size. IL-8 and TNF-α do not correlate with the pathohistological parameters of the disease. Therefore, IL-6, NLR, PLR, Platelets and CRP are more useful than IL-8 and TNF-α in detecting M-PTCs.

Ethical Committee:
The study was conducted according to Helsinki declaration and University Hospital Centre Zagreb, Croatia Policy.

Conflict of interest:
The Authors declare that there is no conflict of interest.

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