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Thyroid

Ki-67 and CK-19 are predictors of locoregional recurrence in papillary thyroid carcinoma

Ki-67 e CK-19 sono fattori predittivi di ricaduta locoregionale nel carcinoma papillare della tiroide

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SUMMARY

Most patients with papillary thyroid carcinoma have good prognosis; however, recurrence rates and the need of salvage treatment remain a significant problem for 5-40% of patients. Although several risk classifications based on clinicopathological prognostic factors are used, it is not possible to predict which patients will have a higher risk of recurrence. The objective of the study is to analyse the impact of cytokeratin-19 and Ki-67 immunoexpression as predictive markers of the risk of recurrence in papillary thyroid carcinoma. This is a retrospective case-control study, including 42 patients with papillary thyroid carcinoma and 42 controls. The groups were matched by gender, age and pathological staging T and N. Slides were made by the microarray tissue system. Multivariate logistic regression was applied to identify an independent risk factor for recurrence. Of the 42 selected cases, 30 patients (71.4%) were female and 12 (28.6%) were male, ranging in age from 10 to 80 years (median of 39 years). Most patients (64.3%) had tumors at initial T staging (T1-T2). Half of the sample was classified as low risk according to the American Thyroid Association (ATA) risk stratification. Follow-up time ranged from 46 to 196 months, with time to recurrence from 2 to 106 months (median, 30 months). CK-19 and Ki-67 immunoexpression had a statistically significant association with the risk of recurrence (p = 0.029 and p = 0.007, respectively). In multivariate logistic regression analysis, immunoexpression for these markers was an independent risk factor for locoregional recurrence (OR-9,64; CI-1.14-81.01 and OR-3,21; CI-1.32-7.94, respectively). The immunohistochemical analysis of the Ki-67 and CK-19 markers is useful to predict tumour recurrence in patients with papillary thyroid carcinoma.

KEY WORDS: thyroid neoplasms, immunohistochemistry, recurrence, biomarkers, carcinoma papillary

RIASSUNTO

La maggior parte dei pazienti affetti da carcinoma papillare della tiroide godono di una prognosi favorevole tuttavia il 5-40% di essi possono essere colpiti da ricaduta di malattia e dover affrontare una chirurgia di salvataggio. Nonostante la presenza di diverse classificazioni di rischio e fattori prognostici clinicopatologici, non è possibile identificare con certezza i pazienti con più alto rischio di ricaduta. Lo scopo di questo studio è analizzare Ki-67 e CK-19 come fattori predittivi di ricaduta nel carcinoma papillare della tiroide. Abbiamo effettuato uno studio retrospettivo caso controllo che ha incluso 42 pazienti affetti da carcinoma papillare della tiroide e 42 controlli. I gruppi sono stati stratificati per genere, età, staging del T e N. Dei 42 pazienti, 30 erano di sesso femminile e 12 di sesso maschile, con un'età compresa fra i 10 e 80 anni (media 39 anni). Il 64,3% dei pazienti erano affetti da tumori T1-2. Metà del campione è stato classificato come a basso rischio secondo la classificazione della American Thyroid Association (ATA). Il tempo di follow-up è variato dai 46 a 196 mesi, con un periodo libero da malattia compreso fra i 2 e 106 mesi (media 30 mesi). L'immunoe-

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spressione di CK-19 e Ki-67 è associata in maniera statisticamente significativa con il rischio di ricaduta (p = 0,029 and p = 0,007, rispettivamente). Abbiamo effettuato un'analisi di regressione multivariata in cui si è evidenziato che l'immunoespressione di questi due marcatori è risultata un fattore di rischio indipendente per le recidive locoregionali (OR-9,64; CI-1,14-81,01 e OR-3,21; CI-1,32-7,94, rispettivamente). L'analisi immunoistochimica di Ki-67 e CK-19 è utile allo scopo di predire il rischio di ricaduta nei pazienti affetti da Carcinoma papillare della tiroide.

PAROLE CHIAVE: *neoplasie tiroidee, immunoistochimica, recidiva, biomarkers, carcinoma papillare*

Introduction

Papillary carcinoma is the most common well-differentiated thyroid carcinoma, corresponding to more than 80% of cases. It usually has a slow growth rate and can metastasise to cervical lymph nodes without affecting, however, overall survival rates [1-3]. In most series of patients with papillary thyroid carcinomas, the reported specific disease survival rate is up to 98% and 93% at 5 and 10 years, respectively. However, in long-term follow-up, the recurrence rate is about 28% [4-6].

Several clinical and pathological features have been shown to predict the more aggressive behaviour of thyroid carcinoma, but the most useful prognostic factors in well-differentiated thyroid carcinoma are patient age, tumour size, tumour invasion, presence of distant metastasis and tumour dedifferentiation [7]. However, a significant number of cases without these characteristics can present locoregional recurrences. Thus, predicting outcomes in thyroid neoplasms is not reliable using clinicopathological information alone.

In order to search for new tools to improve the ability to predict which patients will have a better or worse outcomes, immunohistochemical (IHC) has been used to evaluate different markers in papillary thyroid carcinoma, both as a diagnostic tool and as a prognostic factor [8]. Cytokeratin-19 (CK-19) and Ki-67 are some of the markers used in prognostic evaluation in papillary thyroid carcinoma [7,9-12]. Currently, Ki-67 is regarded as one of the most promising markers for assessing cell proliferation activity. In clinical practice, it is used to estimate the prognostic factor in several different malignant tumours, such as mammary, thyroid and neurological tumors [11]. In thyroid neoplasms, there are studies that analyse both the diagnostic and prognostic value of this marker. Dwivedi et al. [13] analysed the expression of Ki-67 and observed greater expression of this marker in papillary thyroid carcinomas in relation to non-neoplastic lesions. Miyauchi et al. [14] found that Ki-67 was an independent prognostic factor for disease-free survival.

Ck-19 is present in the simple or glandular epithelium; however, enhanced immune expression is seen in some pathological conditions, as in tumours of epithelial origin. It is a sensitive marker for papillary carcinomas, usually with a strong diffuse plasma reactivity, while in benign thyroid lesions and normal thyroid tissue, a focal and light reactivity is observed [15,16]. Cheung et al. [15] demonstrated diffuse immune reactivity in 66% of papillary thyroid carcinomas. While the significance of CK-19 immunoexpression for differential diagnosis of thyroid lesions has been widely used and debated, its value as a prognostic factor in papillary carcinoma is still uncertain. In hepatocellular carcinoma and intrahepatic cholangiocarcinoma, it is well recognised that patients with immunoexpression for CK-19 have worse prognosis, with the level of immunoexpression significantly related to tumour aggressiveness and postoperative recurrence [17,18].

Most studies that investigate immunohistochemical markers for papillary thyroid carcinoma do so in order to differentiate between benign and malignant neoplasms, and few studies look for prognostic markers [7,9,11,12]. The aim of this study was to analyse the impact of the immunoexpression of CK-19 and Ki-67 on the risk of patients with papillary thyroid carcinoma.

Materials and methods

This is a retrospective case-control study including patients with thyroid papillary carcinoma who were treated surgically for curative purposes from January 1, 2000 to July 31, 2010. The case group was formed of all patients who presented locoregional tumour recurrence and underwent surgical salvage treatment. The control group was formed by patients who did not present locoregional recurrence. Data from the control group were matched with those from the case group according to age, gender and T and N staging. Exclusion criteria were: 1) patients submitted to initial treatment or salvage treatment at another institution; 2) unresectable locoregional relapse; 3) only distant recurrence; 4) biochemical relapse, with no identifiable site of recurrence; 5) records with incomplete information about the disease; 6) less than two years post-recurrence follow-up; 7) loss of follow-up was considered when the patient did not return on a scheduled appointment twice in the stipulated period.

All tumours were staged according to the American Joint Committee on Cancer tumor-node-metastasis (TNM) staging system (7th edition). The case group had analysed

samples of the initial tumour and the specimen from the salvage surgery. The control group had only the specimen of the primary surgery analysed. The study was evaluated and approved by the Research Ethics Committee of the A.C. Camargo Cancer Center. All demographic, clinical, pathological and therapeutic information were collected from the electronic medical records from the A.C. Camargo Cancer Center by a single investigator (AORV), using a standardised form.

A TMA Block was constructed using 1.0 mm diameter representative samples of each tumour tissue taken from the recipient paraffin block, with papillary thyroid carcinoma samples in the case group at initial (n = 42) and relapse (n = 42) and in the control group (n = 42). All specimens were collected in duplicate. The slides were stained by IHC CK-19 (Clone RCK107, Dako), Ki-67 (Clone MIB-1, Dako). The reaction for labeling the samples was performed by dewaxing and hydrating the histological sections, with subsequent antigenic recovery in the pressure chamber in citrate solution (pH 6.0). Exposure to hydrogen peroxide 3% for 5 min; incubation of the slides with primary antibodies diluted in previously established titers in PBS buffer; incubation of the slides with Post Primary Block (Novolink Max Polymer-Leica Biosystems) for 30 minutes; Incubation of slides in substrate solution with chromogen diaminobenzidine (DAB, Sigma) 5 minutes; Fractionation with Harris haematoxylin for 30 seconds, followed by washing with distilled tap water; Assembly of the slides in Entellan neu (Merck, 1.07961.0100, Darmstadt, Germany). The negative control was obtained through the same tissue analysed, included in each series of immunohistochemical staining, with the omission of the primary antibody. The standardisation of pathological interpretation took into account the expected positivity for each marker: Cytokeratin 19 shows cytoplasmic positivity and Ki-67, nuclear positivity. The absence of staining was considered nonspecific and negative. Cytokeratin-19 was analysed by a score that classified the labeling in three categories: (0) no cell marking, (1) < 25% positive, (2) > 25% positive. Ki-67 was also classified into three categories: (0) no cell marking, (1) < 10% positive, (2) > 10% positive [19,20].

The information collected was stored in a computerised database made specifically for the study and statistical analysis was performed using SPSS (Statistical Package for Social Science, SPSS version 18.0 for IOS) statistical software. The categorical qualitative variables were presented through descriptive statistics (numbers and percentages), while continuous quantitative variables were presented by means of the median, mean and standard deviation. For analysis of the association of immunoexpression of biological markers (CK-19 and Ki-67) with the clinicopathological aspects of the patients, as well as the comparison between cases and controls, the χ^2 Test or the Fisher's exact test were used as appropriate. The McNemar test was used to ascertain the differences in the comparison between two paired qualitative samples (before and after). The Student's T test was used to compare the means between the continuous variables, whose normality distributions were confirmed through the Shapiro-Wilk Test.

Overall and disease-free survival were analysed using the Kaplan-Meier method. The follow-up time was considered from the date of surgery until the date of the last information and, in the cases of death, until the date of death. The period between the date of surgery and the occurrence of relapse was considered to calculate the probability of disease-free (or recurrence-free) survival.

Multivariate logistic regression was performed aiming to identify possible independent associations. All the significant associations observed in the univariate analyses were included in the regression model. Odds ratio (OR) values were determined to investigate the intensity of associations. In all tests statistical significance was considered for a value of $p \leq 0.05$.

Results

During the 10 year period, 2140 thyroidectomies were performed for thyroid carcinoma in the institution, in which, 1743 were histologically diagnosed as papillary thyroid carcinoma. Cases with incomplete clinical or pathological information (n = 178) or lost to follow-up (n = 305) were excluded. Of the remaining cases, 82 presented recurrence, but 40 cases had to be excluded because they did not meet the inclusion criteria. At the end of this process, 42 cases were obtained with all clinical information on treatment and follow-up present, as well as paraffin blocks suitable for the available immunohistochemical analysis.

The clinical characteristics of cases and control groups are described in Table I. The follow-up time of the 42 patients in the study group ranged from 46 to 194 months (median, 117 months). The time to recurrence ranged from 2 to 106 months (median, 30 months). The site of locoregional recurrence was the lateral chains of cervical lymph nodes [25 patients (59.5%)], followed by cervical lymph nodes of the central compartment [10 patients (23.8%)]. Three patients (7.1%) presented recurrence in the thyroid bed and three patients also presented local and lateral recurrences simultaneously. One patient presented recurrence in the thyroid bed and bilateral lateral lymph nodes.

All patients with recurrence were submitted to salvage surgical treatment according to the site of recurrence.

Table I - Clinicopathological characteristics of selected cases and controls

Variable	Categories	Cases N (%)	Control N (%)	p
Age	Variation Median	10-80 39	14-77 39	0.659
Gender	Female Male	30 (71.4) 12 (28.6)	30 (71.4) 12 (28.6)	1.000
Size of node	Variation Median	2-50 mm 12 mm	2-80 mm 10 mm	0.669
Multifocality	Yes No	18 (42.9) 24 (57.1)	13 (31) 29 (69)	0.366
Extrathyroid extension	Yes No	16 (38.1) 26 (61.9)	11 (26.2) 31 (73.8)	0.350
Blood Vascular Invasion (BVI)	Yes No	2 (4.8) 40 (95.2)	0 (0) 42 (100)	0.494
Lymphatic Vascular Invasion (LVI)	Yes No	4 (9.5) 38 (90.5)	2 (7.1) 39 (92.9)	1.000
Perineural Invasion (PNI)	Yes No	3 (7.1) 39 (92.9)	0 (0) 42 (100)	0.241
Pathological T staging	pT1-T2 pT3-T4	27 (64.3) 15 (35.7)	27(64.3) 15 (35.7)	1.000
Pathological N staging	pN0 pN1a pN1b	21 (50) 18 (42.9) 3 (7.1)	25 (59.5) 15 (35.7) 2 (4.8)	0.476
ATA risk classification	Low risk Intermediary risk High risk	21 (50) 17 (40.5) 4 (9.5)	30 (71.4) 9 (21.4) 3 (7.1)	0.129
Type of surgery	Total thyroidectomy Partial thyroidectomy	42 (100) 0 (0)	40 (95.2) 2 (4.8)	0.494
Iodine (I131)	Yes No	35 (83.3) 7 (16.7)	33 (78.6) 9 (21.4)	0.782
Recurrence	Local Level VI Levels II-V Local + level VI + levels II-V Level VI +levels II-V Local + distant Without recurrence	3 (7.1) 11 (26.2) 24 (57.1) 1 (2.4) 2 (4.8) 1 (2.4) 0 (0)	(0) (0) (0) (0) (0) (0) 42 (100)	0.000

P-value obtained by the Chi-Square Frequency Test or Fisher's Exact.

Of the three patients with recurrence in the thyroid bed, two required the use preoperative radioactive technetium injection and a probe for identification of local recurrence, which was confirmed in pathological examination, with no evidence of lymph node involvement. One patient had local recurrence with invasion of the cricoid cartilage and underwent right vertical hemilaryngectomy. There were also no lymph nodes involved in the specimen. There was no association of locoregional recurrence with distant metastasis.

As for the pathological analysis of the recurrences, all confirmed the diagnosis of papillary carcinoma. The number of lymph nodes dissected in lateral neck dissections (levels II to V) ranged from 17-97 (mean of 49 lymph nodes). Positive lymph nodes found in the specimen ranged from 1-10 (mean of 3.3 lymph nodes). The number of lymph nodes dissected in the central compartment dissections (level VI) ranged from 3-10 (mean of 5.3 lymph nodes), with positive lymph nodes found in the specimen ranging from 1-3 (mean of 1.8 lymph nodes).

Twenty-eight patients (66.7%) underwent adjuvant radioiodine therapy with doses varying from 108 to 389 mCi (mean of 239.6 mCi). Only the patient that underwent partial laryngectomy was submitted to adjuvant teleradiotherapy. This patient had no previous iodine uptake prior to salvage surgery (diagnosed through increased thyroglobulin and local uptake seen in PET-CT). No patient in the study group underwent chemotherapy.

Regarding the status at the end of the follow-up, 39 patients (92.9%) were alive and without evidence of disease, 2 patients (4.8%) died due to other causes (both with second primary tumors – lung and breast – and died from complications of these neoplasms). One female patient had recurrent disease in the lateral cervical levels (new relapse), but she was pregnant and decided to undergo salvage surgery after puerperium. The patient continues regular prenatal and follow-up examinations and no lymph node uptake has been observed so far.

Overall survival at 10 years for the case group was 95.2% (Fig. 1). In the control group, follow-up ranged from 51 to 205 months, with a median time of 112 months. No patient presented locoregional recurrence. Two patients (4.8%) died due to causes not related to thyroid cancer (1 breast cancer and 1 CNS cancer). Overall survival for the control group at 10 years was also 95.2%. Disease-free survival in the group that presented recurrent disease was 21.4% at 5 years.

There was a significant association between the presence of higher Ki-67 expression in the group that presented relapse, compared to the control group, where the majority did not present expression for this marker or, when present, was classified as weak ($p = 0.007$). Similarly, CK-19 was immunoexpressed in both, but in the case group, a greater number of patients with higher grades of immunoexpression were shown ($p = 0.029$) (Tab. II).

There was no significant association among clinical and histopathological variables (gender, age, staging, multifocality, extrathyroidal extension, blood vascular invasion, perineural invasion and ATA risk classification). There was a significant association between CK-19 immunoexpression and lower lymphatic vascular invasion (LVI) rate ($p = 0.046$).

Multivariate analysis by logistic regression of

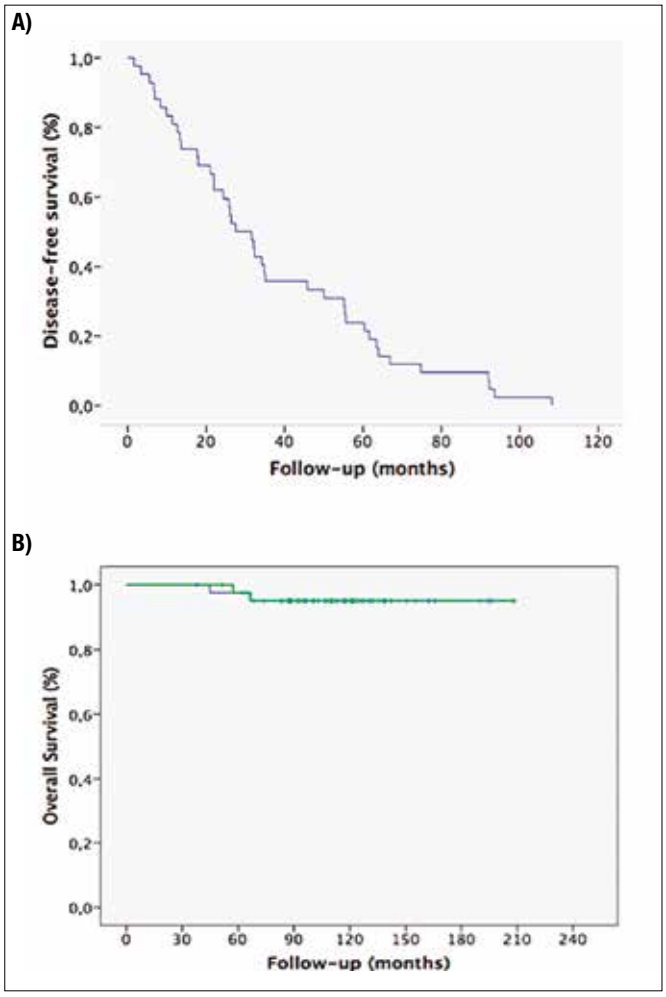

Figure 1. A) Recurrence-free survival of 42 patients with carcinoma papillary thyroid cells included in the group of cases; **B)** Global survival between the studied case and control groups. (Method of Kaplan-Meier).

Table II. Immunoexpression of CK-19 and Ki-67 in the case and control groups.

Markers IHQ	Cases (%)	Control (%)	p
CK-19:			
negative	1 (2.4)	8 (19.0)	0.029
< 25%	5 (11.9)	3 (7.1)	
> 25%	36 (85.7)	31 (73.8)	
Ki-67:			
negative	14 (33.3)	26 (61.9)	0.007
< 10%	25 (59.5)	16 (38.1)	
> 10%	3 (7.1)	0 (0)	

P-value obtained by Fisher's Exact Test.

immunoexpression of the cytokeratin-19 and Ki-67 markers showed that the presence of immunoexpression of CK-19 and Ki-67 were significant independent predictors of the risk of recurrence, with a confidence interval of 1.14-81.01 for CK-19 and 1.32-7.94 for Ki-67 (Tab. III). There was no significant association between ATA classification and CK-19 or Ki-67 immunoexpression (Tab. IV).

We also performed an analysis combining risk stratification according to ATA and Ki-67 immunoexpression. The cases were reassembled as follows: a) low risk: patients classified as low risk ATA/Ki-67-; b) intermediate risk: patients with low risk ATA/Ki-67+, intermediate risk ATA/Ki-67- or + and high risk ATA/Ki-67-; c) high risk: patients high risk ATA/Ki-67+. We observed that in the group of cases, 76% of the patients classified as low ATA risk were reclassified as intermediate after the new stratification with the inclusion of Ki-67. In the control group, 3 patients classified as high risk were reclassified as intermediate risk. When we analysed the locoregional recurrence rate after the new stratification, we observed that 100% of patients reclassified as high risk presented tumour recurrence (Tab. V).

Discussion

Papillary thyroid carcinoma is the most common histological subtype among malignant thyroid neoplasms, and usually has an indolent clinical course in most cases. In our study, we had a 6.5% rate of tumour recurrence, consistent with the literature reporting rates of 5-30% [21,22]. The follow-up period in this series was almost 10 years (118 months), which is necessary for proper evaluation because of the indolent tumour behaviour [23]. Possibly our low rate of relapse, close to the lower margin described in the literature, can be due to the fact that the majority of operated cases were pT1 (61.9%), also consistent with the significant change in the profile of cases of papillary carcinoma in the last decades, with a significant decrease in the number of advanced tumours treated [24,25]. Despite this, we had a significant number of early recurrences (median of 30 months), although all patients were staged during initial evaluation with preoperative cervical ultrasonography to identify probable lymph node metastases, and most were classified as low risk (50%) or intermediate risk (40.5%) by ATA risk stratification.

Although uncommon, relapses and deaths have been reported with some cases of papillary thyroid carcinoma [24,25]. Several clinical or histopathological prognostic factors are predictors of survival (TNM, ATA, MACIS, AGES, GAMES, EORTC, among others) and are not predictors of recurrence in patients with papillary carcinoma [26]. With this in mind, several authors have sought to identify molecular markers that could, in addition to the classic clinical and histopathological parameters, distinguish low- and high-risk patients for recurrences aiming to minimise the risk of overtreatment for low-risk patients [10-12,27-29].

Table III. Logistic regression analysis of CK-19 and Ki-67 immunoexpression between the case and control groups and the risk of recurrence.

Markers IHQ	Cases (%)	Control (%)	Odds Ratio	95% IC	p
CK-19:					
negative	1(2.4)	8 (19.0)	1	1.14-81.01	0.037
imunoexpress	41(97.6)	34 (81)	9.64		
Ki-67:					
negative	14 (33.3)	26 (61.9)	1	1.32-7.94	0.010
imunoexpress	28 (59.5)	16 (38.1)	3.25		

P-value obtained by Fisher's Exact Test.

Table IV. Relationship of expression of markers with ATA risk classification.

Markers IHQ	ATA risk classification (%)			p
	Low	Intermediary	High	
CK-19:				
negative	1 (2.4)	0 (0)	0 (0)	0.655
< 25%	3 (7.1)	1 (2.4)	1 (2.4)	
> 25%	17 (40.5)	16 (38.1)	3 (7.1)	
Ki-67:				
negative	5 (11.9)	0 (0)	0 (0)	0.380
< 10%	15 (35.7)	7 (16.7)	3 (7.1)	
> 10%	1 (2.4)	2(4.8)	0 (0)	

Table V. Locoregional recurrence rate among all patients studied (cases and controls) after a new risk stratification (ATA + Ki-67).

ATA + Ki-67 risk classification		Locoregional recurrence (%)
Low risk	Low ATA/Ki-67-	5/23 (21.7)
Intermediary risk	Low ATA/Ki-67+ Intermediary ATA/Ki-67+ or - High ATA/Ki-67-	34/58 (58.6)
High risk	High ATA/Ki-67+	3/3 (100.0)

In 2009, ATA published a risk stratification for patients with well differentiated thyroid carcinoma undergoing thyroidectomy, in order to select those patients with a higher risk of recurrence and/or disease persistence, with update of this guideline in 2015 [30]. There were three categories: low risk, intermediate risk and high risk of recurrence. Low-risk patients were defined as having intrathyroidal DTC with no evidence of extrathyroidal extension, vascular invasion, or metastases. Intermediate-risk patients demonstrated either microscopic extrathyroidal extension, cervical lymph node metastases, RAI-avid disease in the neck outside the thyroid bed, vascular invasion, or aggressive tumour histology. High-risk patients had gross extrathyroidal extension, incomplete tumour resection, distant metastases, or inappropriate postoperative serum Tg values. In our series, we were unable to identify patients at greater risk for tumour recurrence, since 90.5% of the cases belonged to the low an /or intermediate risk groups.

We evaluated the immunoexpression of two biological markers (Ki-67 and CK-19) in 42 patients with papillary thyroid carcinoma who presented with locoregional recurrences during the follow-up period. Cytokeratin-19 is one of the most widely used immunohistochemical markers for thyroid neoplasms, and is not usually produced by healthy thyroid cells. Its presence is therefore related to neoplastic transformation. There is strong evidence in the literature reporting weak positivity for CK-19 in benign lesions, but levels of variable expression in malignant thyroid lesions [7]. Although CK-19 immunoexpression has been used in the differential diagnosis of neoplastic thyroid lesions, its use as a prognostic factor is not well known. However, CK-19 has a well-defined prognostic value in liver tumours, where patients with strong immunoexpression for CK-19 have a significantly worse prognosis, characterising greater tumour aggressiveness in those with higher expression of this marker [17,18]. In the case of thyroid neoplasms, there is little information about the carcinogenic role of CK-19 or its value as a prognostic factor [7]. In our study, the immunoexpression of CK-19 was strongly expressed and significantly more evident in patients who presented recurrence of the disease compared to the control group. Only one case in the sample did not have immunoexpression for CK-19. Paradoxically, most patients in the case group with strong immunoexpression for CK-19 did not present lymphatic vascular invasion ($p < 0.05$). We did not find in the literature any correlation between lymphatic vascular invasion and CK-19 immunoexpression in papillary thyroid carcinoma.

Some studies have reported a significant correlation of the level of CK-19 immunoexpression in thyroid papillary carcinoma with extrathyroidal extension and pTNM staging [7]. These studies demonstrate that the high expression of CK-19 in tumour thyroid cells is associated with worse prognosis, which was also seen in our study; however, we did not find correlation of these levels with staging and extrathyroidal extension.

Ki-67 is a DNA-binding protein that is found primarily in the nucleus and is related to cell proliferation, being an important tumour marker. Its high immunoexpression is associated with worse prognosis in breast cancer and prostate cancer [31,32]. In thyroid, few studies have analysed the use of this marker as a prognostic factor. Ito et al. [10] demonstrated that Ki-67 was an independent prognostic factor for disease-free survival in patients with papillary thyroid carcinoma. In the present study, there was a significant association between the presence of Ki-67 expression in the group that presented recurrence,

compared to the control group, where the majority did not present expression for this marker or, when present, presented weak immunoexpression. No association was found for this marker with gender, age, T and N staging, multifocality, extrathyroidal extension, lymphatic vascular invasion, or ATA risk classification.

Tang et al. [12] showed higher immunoexpression of Ki-67 in papillary thyroid carcinomas > 1.0 cm and in the presence of thyroiditis, but without relation with multifocality, extrathyroidal extension and lymph node metastasis. Zhou et al. [11], on the other hand, observed a significant correlation between increased Ki-67 immunoexpression and extrathyroidal extension and lymph node metastasis. Pan et al. [20], in a meta-analysis on 51 studies in thyroid cancer, showed that patients with higher Ki-67 immunoexpression had worse survival. It was also associated with tumour size, lymph node metastasis and extrathyroidal extension.

In multivariate analysis, we observed that Cytokeratin-19 and Ki-67 were independent risk factors for locoregional recurrence in papillary thyroid carcinoma. It is, therefore, suggested that cell proliferative activity in the primary lesion is an important factor that reflects the probability of carcinoma recurrence. Therefore, these markers may be useful in clinical practice in order to predict tumour recurrence in patients with papillary thyroid carcinoma.

The proposed risk classification combining the expression of Ki-67 and ATA classification seems to have the potential to increase the ability to predict recurrences. In the group of cases, only 5 patients remained at low risk and 37 patients (88.1%) were classified as intermediate or high risk for recurrence. All the patients reclassified as high risk presented locoregional recurrences. Therefore, the use of this marker associated with the ATA classification must be tested in a larger independent sample to confirm its predictive value on the risk of recurrence.

Conclusions

Ki-67 and cytokeratin 19 immunoexpression is related to locoregional recurrence in papillary thyroid carcinoma, and these two markers are independent risk factors for tumour recurrence. The combination of Ki-67 immunoexpression may contribute to improve the predictive value of ATA recurrence risk classification, although these findings need to be confirmed in a larger independent patient sample.

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