# Increased Expression of Transketolase-Like-1 in Papillary Thyroid Carcinomas Smaller Than 1.5 cm in Diameter Is Associated With Lymph-Node Metastases

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**BACKGROUND.** Patients with small papillary thyroid carcinoma (PTC) may have a high incidence of regional lymph-node (LN) metastases at presentation, and these are considered to be an independent risk factor for tumor recurrence. A mutated transketolase transcript (TKTL1) has been found up-regulated in different human malignancies, and strong TKTL1 protein expression has been associated with aggressiveness and poor patient survival in several epithelial cancers.

**METHODS.** TKTL1 protein expression was analyzed in 256 consecutive cases of PTCs $\leq$1.5 cm by immunohistochemistry with a specific anti-TKTL1 antibody. RNA analysis was performed by real-time polymerase chain reaction (PCR) in all cases for which frozen material was available, which resulted in 55 fragments of PTC.

**RESULTS.** Increased levels of TKTL1 transcript were detected in 50 of 55 analyzed tumors compared with their corresponding normal tissues. Significant differences in TKTL1 transcript levels were found between cases of PTC with and without LN metastases. In primary tumors, immunoreactivity for TKTL1 was detected in the majority of cases, ranging from 0% to 95.0% (mean, 50.11% $\pm$ 27.75%). A significant association was found between TKTL1 protein expression and the presence of multifocality, bilaterality, extrathyroidal extension, vascular invasion, sclerosis, and LN metastases. In cases with LN metastases, a positive correlation was found between the TKTL1 protein expression in primary tumors and the number of metastatic LNs as well as the diameter of the largest metastatic area in LNs.

**CONCLUSIONS.** These findings suggest that TKTL1 overexpression in PTC $\leq$1.5 cm may be considered a factor that facilitates tumor growth and progression. ***Cancer* 2008;113:936–44.** © *2008 American Cancer Society.*

**KEYWORDS: papillary thyroid carcinoma (PTC), tranketolase-like-1 (TKTL1), lymph-node metastases, pentose phosphate pathway (PPP), aerobic glycolysis.**

Papillary thyroid carcinoma (PTC) is the most frequent thyroid cancer, accounting for 85% to 90% of all thyroid malignancies. Improvement in diagnostic tools, such as ultrasonography, fine-needle aspiration biopsy, and pathologic procedures allows the identification of a huge number of small cancers, which, in the majority of cases, are PTCs associated with a good prognosis.[1-4]

Several studies have focused on thyroid cancers no larger than 1 cm in diameter (classified as microcarcinomas), but it is not entirely clear whether 1.0-cm tumor size should be considered a threshold for risk evaluation or whether carcinomas larger than 1.0 cm, but still small (no larger than 1.5 cm. in diameter), can also be expected to have a similar favorable clinical behavior to that of tumors no larger than 1.0 cm.[2,3,5] Because they are often found in


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thyroid glands resected for other lesions, small PTCs are often described as occult or incidental. Nevertheless, patients presenting with small PTCs may also have a high incidence of regional lymph-node (LN) metastases, which are the main route for the spread of PTC cancer cells.[6]

Several reports suggest that presence of LN metastases decreases cure rates and represents an independent risk factor for tumor recurrence.[7-10] However, the clinical management of LN metastasis, including the extent of initial surgery and the proper indication for radioiodine therapy, is still controversial.

During tumorigenesis, cells acquire genetic alterations that are responsible for modifications in their metabolism. Cancer cells characteristically present increased glucose uptake that is metabolized by anaerobic degradation, which leads to production of large amounts of lactate even in the presence of oxygen. This phenomenon is known as aerobic glycolysis or the "Warburg effect," and it has been described as the main metabolic alteration in malignant conversion.

Several biochemical and molecular studies have described possible mechanisms by which alterations in glucose metabolism may evolve during cancer development.[11-13] These mechanisms include mitochondrial defects and malfunction, adaptation to hypoxic tumor microenvironment, oncogenic signaling, and an abnormal expression of metabolic enzymes.[11,12]

Transketolase (TKT) is a thiamine diphosphate-dependent enzyme that controls the production of nucleic acid ribose synthesis in the nonoxidative part of the pentose phosphate pathway (PPP).[14] In cancer, inhibition of TKT enzyme activity suppresses tumor growth and metastases.[15,16] Three human *TKT* genes have been described, *TKT*, transketolase-like-1 (*TKTL1*), and transketolase-like-2 (*TKTL2*). During evolution, mutations in the *TKTL1* gene have led to the production of tissue-specific transcripts different in size, which encode an enzymatically active TKT protein with unusual enzymatic properties and different smaller protein isoforms. TKTL1 enzyme presents altered substrate specificity and reaction modus.[17] TKTL1 up-regulation in tumors induces an abnormal oxygen-independent glucose usage and a lactate-based matrix degradation.[15,18] TKTL1 mRNA expression levels have been found to be up-regulated in several tumors. Noteworthy is that high levels of TKTL1 have been reported in gastric, bladder, breast, lung, prostate, and pancreatic carcinomas.[19-23] Furthermore, TKTL1 overexpression is associated with the aggressiveness of colon, urothelial, ovarian cancers, and laryngeal squamous cell carcinomas, and its overexpression correlates with poor patient survival.[16,22,24] Interestingly, the inhibition of *TKTL1* induces a reduction in cell proliferation.[16,20,23,25]

In thyroid cancer, TKTL1 protein expression has previously been evaluated in follicular, papillary, and undifferentiated variants, whereas abundant TKTL1 expression has been identified within the cytoplasm.[16]

The aims of the present study were to investigate the expression of TKTL1 in a series of papillary thyroid carcinomas $\leq$1.5 cm and its relation to defined clinicopathologic features and LN metastases.

## MATERIALS AND METHODS

### Case Selection

In the present study, we included a group of 256 consecutive patients who had undergone total or near-total thyroidectomy between January 2001 and June 2006, with histologic diagnosis of PTC $\leq$1.5 cm in diameter. In 159 cases, clinical signs were present (clinical or nonincidental carcinomas), whereas in the remaining 97 cases, the tumor was an incidental finding (incidental carcinomas) after a lobectomy or near-total thyroidectomy performed for other diseases (multinodular goiter in 70 cases, toxic multinodular goiter in 10, toxic adenomas in 8, Hashimoto thyroiditis in 7, and Graves disease in 2). Patients with incidental carcinomas underwent total thyroidectomy and/or LN dissection within 6-12 months of diagnosis. Furthermore, laterocervical LNs were dissected when macroscopically involved. Sixty-seven patients presented regional LN metastases, 56 with nonincidental and 11 with incidental carcinomas. This retrospective study was performed in accordance with the rules of the Institutional Review Board of the Faculty of Medicine (University of Palermo).

### Histopathologic Evaluation and Tumor Staging

Tumor specimens were obtained from the archives of the Department of Human Pathology, University of Palermo. For each case, all histologic slides were reviewed by 2 pathologists (V. R. and A. M.), who were unaware of the clinical data, and histologic diagnoses were reassessed according to the World Health Organization histologic classification of thyroid tumors.

All primary tumors included in this study were classified as classical variants of PTC; tumors with morphological features of the follicular variant of PTC were not included. Tumors were staged according to the 6th edition of the Cancer Staging Manual,[26] where T (extent of the primary tumor) and N

(regional LN metastases) were determined on the basis of pathologic data, and M (evidence of distant metastasis) were based on findings at the first postoperative $^{131}$I-whole-body scan.

Five-μm-thick sections were hematoxylin and eosin-stained and reassessed with regard to intraglandular multifocality, bilaterality, extrathyroidal extension, vascular invasion, grade of sclerosis (according to the amount of fibrous stroma coexisting with malignant cells), degree of encapsulation (encapsulated or nonencapsulated), and maximum diameter of the primary lesion. Sections of LN metastases were reassessed with regard to compartment involvement, number of involved LNs, and diameter of the largest metastatic area.

### RNA Preparation From Ex Vivo Tissue Specimens

RNA analysis was performed in all cases for which frozen material was available, which corresponded to 55 PTC tissue fragments (14 cases were with LN metastases and 41 without LN metastasis) and their corresponding normal tissue specimens, derived from uninvolved thyroid glands. Tissue fragments were obtained from nonincidental tumors nodules >1 cm by means of careful identification by an experienced pathologist. These tissue fragments were immediately stored in RNA (Sigma, Milan, Italy) at −80°C until their subsequent use. Total RNA from normal and cancerous thyroid tissues was extracted by using the RNeasy Mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions.

### Real-Time Polymerase Chain Reaction (PCR) Analysis

RNA was reverse-transcribed by using the Quanti Tect reverse transcription kit (Qiagen) according to the manufacturer's instructions. Real-time PCR was performed with SYBR Green I dye and the Quanti Tect SYBR Green PCR kit (Qiagen) and was performed with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, Calif) and conducted by using the following parameters: 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, and 68°C for 1 minute. All amplification reactions were performed in triplicate, and the relative quantification of *TKTL1* gene expression was calculated by using the comparative Ct method (ΔΔCt). Levels of mRNA expression were expressed after normalization with endogenous-control β-actin. Data processing and statistical analysis were performed by using ABI PRISM SDS software v. 2.1 (Applied Biosystems).

Expression differences between tumor and corresponding normal tissue were calculated and are expressed as fold induction in tumor samples relative to the corresponding normal sample.

The following primers were used for amplification: *TKTL1* (TAACACCATGACGCCTACTGC; CATCC TAACAAGCTTTCGCTG), *TKT* (TGTGTCCAGTGCATA GTGG; ACACTTCATATACCCGCCCTAG), *TKTL2* (AAA CTAGGCTTATTTCTAAAAAGTCAAG; GGCTTTGCTTTA AAAGAAACAG) by referring to Coy et al,[15] and *β-actin* (CCTAAAAGCCACCCCACTTCTC; ATGCTATCAC CTCCCCTGTGTG). Primers for *TKT*, *TKTL1*, *TKTL2*, and *β-actin* were designed by using PRIMER and Primer Express (PE Applied Biosystems) software.

### Immunohistochemical Study

Immunohistochemical analyses were performed on 5-μm-thick paraffin-embedded sections of thyroid tumors and LN metastases. Antigen unmasking was performed in 10 mM sodium citrate (pH 6.0) in a microwave oven for 1 minute at 450W followed by 5 minutes at 100 W. Endogenous peroxidase was inhibited with 3% $H_2O_2$ for 5 minutes. Endogenous avidin-biotin was blocked by a biotin blocking system (Dako, Glostrup, Denmark) for 10 minutes. Unspecific staining was blocked with 1% goat serum for 30 minutes. Sections were subsequently exposed to mouse anti-TKTL1 (clone JFC12T10; mouse IgG2b) antibody (15 μg/mL), or isotype-matched control (Dako) 1 hour at room temperature as previously described by Coy et al.[15] Slides were rinsed in Tris-buffered saline (TBS), incubated with biotinylated antimouse immunoglobulins for 30 minutes at room temperature, and treated with streptavidin-peroxidase (LSAB2 Kit, Dako). Staining was revealed by using 3-amino-9-ethylcarbazole (AEC) substrate (Dako) and counterstaining with aqueous hematoxylin.

The non-neoplastic tissue present on the same slides was considered to be a healthy control. Moreover, 20 random specimens, deriving from uninvolved contralateral thyroid gland in cases with nonbilateral tumors, were analyzed for TKTL1 expression.

### Immunohistochemical Evaluation

For the evaluation of TKTL1 immunoreactions, primary lesions and metastatic tissues were examined for evidence of staining. Specimens were analyzed under a light microscope (Dialux 20 with a plan 40× objective, aperture 0.65, 10× ocular; Leitz, Wetzlar, Germany) with an eyepiece graticule to facilitate cell counting. For each case, a minimum of $10^3$ cells was counted, and the percentage of tumor cells, stained with TKTL1 antibody, was regarded as the labeling index (LI). For multifocal tumors and multi-

ple LN metastases, evaluations were assessed on different areas of all lesions. Twenty random cases were evaluated separately by 2 different pathologists (A. M. and V. R.); because the variation was less than 5%, the first pathologist's data were used.

### Statistical Analyses

Continuous variables were analyzed as mean value ± standard deviation (SD). Rates and proportions were calculated for categorical data. For categorical variables, differences were analyzed by means of the $\chi^2$ test and Fisher exact test when appropriate. As continuous variables were without normal distribution, we used nonparametric tests, and differences were analyzed by the Mann-Whitney *U*-test. Differences between paired continuous variables were analyzed by means of the Wilcoxon test. Correlations among continuous variables were determined by the use of Spearman test. Group comparisons for quantitative variables were performed by using the Kruskal-Wallis nonparametric test. $P < .05$ was considered statistically significant. All analyses were performed with Statistical Package for Social Science (SPSS for Windows, v. 11.0; Chicago, Ill).

## RESULTS

The clinicopathologic characteristics of the 256 patients who underwent surgery for the classic variant of small PTCs (≤1.5 cm) are shown in Table 1. Patient age ranged from 17 to 76 years (mean, 42.34 ± 14.48 years), and there was a strong predominance of female patients (82.4%). At presentation, tumor size was no larger than 1.0 cm in 169 cases and was between 1.1 and 1.5 cm in 87 cases.

**TABLE 1**
**Clinical and Histopathologic Features of 256 Small PTCs**

| Parameters | No. (%) |
|---|---|
| Clinical signs | |
| Nonincidental | 159 (62) |
| Incidental | 97 (38) |
| Age, y | |
| <45 | 136 (53) |
| ≥45 | 120 (47) |
| Sex | |
| Women | 211 (82.4) |
| Men | 45 (17.6) |
| Diameter of primary lesion | |
| ≤1 cm | 169 (66) |
| 1.1-1.5 cm | 87 (34) |
| Multifocality | |
| Present | 81 (31.6) |
| Absent | 175 (68.4) |
| Bilaterality | |
| Present | 47 (18.4) |
| Absent | 209 (81.6) |
| Extrathyroidal extension | |
| Present | 45 (17.6) |
| Absent | 211 (82.4) |
| Lymph node metastases | |
| Present | 67 (26.8) |
| Absent | 189 (73.2) |
| Vascular invasion | |
| Present | 14 (5.5) |
| Absent | 242 (94.5) |
| Sclerosis | |
| Present | 10 (3.9) |
| Absent | 246 (96.1) |
| Encapsulation | |
| Present | 103 (40.2) |
| Absent | 153 (59.8) |

PTC indicates papillary thyroid carcinoma.

### TKTL1 mRNA Levels in Thyroid Carcinoma

TKTL1 mRNA levels were detected by reverse-transcriptase (RT) real-time polymerase chain reaction (PCR) in 55 PTC fragments and in their corresponding normal tissues and expressed as fold induction of TKTL1 expression in tumor samples compared with corresponding normal specimens. TKTL1 mRNA expression ranged from 0.7 to 134 relative levels. Of the 55 PTC samples analyzed, 5 samples without LN metastases showed TKTL1 transcript levels consistently low and with similar levels to their corresponding normal tissues. On the basis of the presence of LN metastases, we divided specimens in 2 subgroups, 14 cases with LN metastases and 41 without LN metastasis. In the group with LN metastases, TKTL1 mRNA levels ranged from 48.3 to 134 (mean 86.76 ± 25.01). In the group without LN metastases, TKTL1 mRNA levels ranged from 0.7 to 97 (mean, 39.66 ± 27.30); these differences proved to be significant ($P < .001$) (Fig. 1).

Moreover, we investigated TKT and TKTL2 mRNA expression levels in the same samples. Relevant differences were not found between normal tissues and tumor samples in cases with and without LN metastases (data not shown).

### Immunohistochemistry of TKTL1

Immunohistochemical staining was performed to identify the presence of the TKTL1 protein. Considering primary tumors of the classical variants of PTC, we have found a predominantly cytoplasmatic staining with strong positive tumor cells for TKTL1, whereas the adjacent nonneoplastic tissue, as well as control samples of uninvolved contralateral gland, the stromal and follicular cells did not show immunoreactivity for TKTL1 (Fig. 2A).

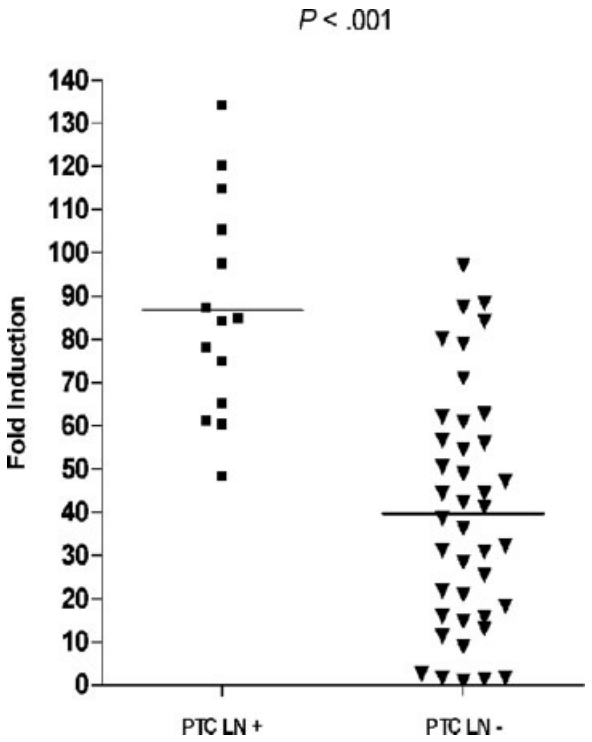


**FIGURE 1.** Transketolase-like-1 TKTL1 mRNA expression levels in papillary thyroid carcinoma (PTC) tissue fragments from nonincidental nodules >1 cm (14 with lymph-node [LN] metastases and 41 without LN metastasis). For quantification of TKTL1 transcripts, expression differences between tumor and corresponding normal tissue were calculated and are shown as fold induction in tumor samples relative to the corresponding normal samples. Levels of mRNA expression were expressed after normalization with endogenous control β-actin. Results are mean values of 3 independent experiments. Differences were analyzed by the Mann-Whitney *U* test; statistical results were considered significant for $P < .05$.

In primary tumors, immunoreactivity for TKTL1 was detected in the majority of cases, ranging from 0% to 95.0% (mean 50.11 ± 27.75%). According to the results obtained from real-time PCR analysis, all 5 samples that showed TKTL1 transcript levels consistently low and similar to their corresponding normal tissues also showed very low TKTL1 LI.

Expression of TKTL1 protein was also detected in LNs metastatic tumor tissues, ranging from 21.9% to 97.2% (mean, 83.21 ± 13.18%) (Fig. 2B). On examining the distribution of tumors without LN metastases (Group I) and with LN metastases (Group II), significant differences were not found for sex, age, diameter of primary lesion, and encapsulation; however, statistical analysis demonstrated a significant association, apart from clinical signs, between nodal status and intraglandular multifocality, bilaterality, extrathyroidal extension, vascular invasion, sclerosis, and TKTL1 protein expression, as shown in Table 2.

Taking into consideration only cases with LN metastases (Group II), immunoreactivity for TKTL1 in primary tumors ranged from 19.6% to 95.0% (mean, 80.48 ± 16.76%); a difference was not found in TKTL1 protein expression in relation to primary tumors and their paired LN metastases (80.48 ± 16.76% vs 83.21 ± 13.18%; $P = .063$) or to compartment involved (N1a or N1b [78.4 ± 9.47% vs 87.54 ± 7.65%; $P = .071$]), whereas a positive correlation was found between the TKTL1 protein expression in primary tumors and the number of metastatic LNs ($\rho = 0.651$; $P < .001$), as well as between the TKTL1 protein expression and the diameter of the largest metastatic area in LNs ($\rho = 0.555$; $P < .001$).

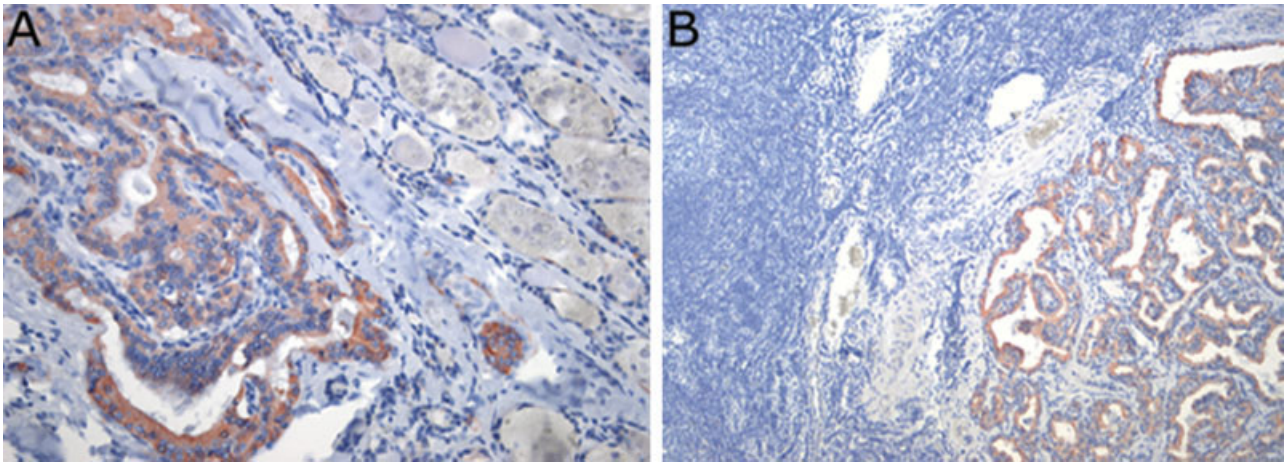


**FIGURE 2.** Cytoplasmatic immunostaining for transketolase-like-1 (TKTL1). (A) TKTL1 expression detected in tumor cells but not in the surrounding stromal cells and in the adjacent nonneoplastic tissue (original magnification ×200). (B) TKTL1 staining in lymph nodal metastatic tissue: positive staining in 79% of tumor metastatic cells (original magnification ×100).

**TABLE 2**
**Features of 256 Small PTCs in Relation to Lymph Nodes Metastases**

| | Group I Node− n=189 Mean [SD] | Group II Node+ n=67 Mean [SD] | *P** |
|---|---|---|---|
| Age | 40.07 [14.7] | 43.25 [13.7] | NS |
| Diameter of primary lesions | 10.37 [3.4] | 11.04 [3.3] | NS |
| TKTL1 LI | 39.34 [22.4] | 80.48 [16.7] | <.001 |
| | **No. (%)** | **No. (%)** | ***P*†** |
| Presence of multifocality | 30 (15.9) | 51 (76.1) | <.001 |
| Presence of bilaterality | 5 (2.6) | 42 (62.7) | <.001 |
| Extrathyroidal extension | 19 (10.0) | 26 (38.0) | <.001 |
| Encapsulation | 71 (37.6) | 32 (47.7) | NS |
| Vascular invasion | 7 (3.7) | 7 (10.4) | .037 |
| Presence of sclerosis | 1 (0.5) | 9 (13.4) | <.001 |
| Incidental | 86 (45.5) | 11 (16.4) | <.001 |

PTC indicates papillary thyroid carcinoma; NS, not significant; LI, labeling index.
The distribution of tumors with absence of lymph-node metastases (Group I) and with presence (Group II), shows a significant association, apart from clinical signs, between node status and TKTL1 protein expression, intraglandular multifocality, bilaterality, extrathyroidal extension, vascular invasion, and sclerosis. For continuous variables, nonparametric tests were used, and differences were analyzed by the Mann-Whitney *U*-test. For categorical variables, differences were analyzed by means of the chi-square test and Fisher exact test, when appropriate. Statistical results were considered significant for *P* values <.05.
*Mann-Whitney test; significant values when *P*<.05.
†Chi-square test; significant values when *P*< .05.

**TABLE 3**
**Evaluation of TKTL1 Expression in Relation to PTC Characteristics**

| | | TKTL1 % | | |
|---|---|---|---|---|
| **Parameters** | | **No.** | **Mean (SD)** | ***P**** |
| Multifocality | Absent | 175 | 42.68 (25.9) | |
| | Present | 81 | 66.16 (24.8) | <.001 |
| Bilaterality | Absent | 209 | 43.42 (25.9) | |
| | Present | 47 | 79.86 (11.6) | <.001 |
| Extrathyroidal extension | Absent | 211 | 46.22 (26.6) | |
| | Present | 45 | 68.37 (25.6) | <.001 |
| Vascular invasion | Absent | 242 | 48.55 (27.2) | |
| | Present | 14 | 77.14 (23.8) | <.001 |
| Sclerosis | Absent | 246 | 48.82 (27.5) | |
| | Present | 10 | 81.83 (7.1) | <.001 |

PTC indicates papillary thyroid carcinoma.
The TKTL1 protein expression in primary tumors was significantly associated with multifocality, bilaterality, extrathyroidal extension, vascular invasion, and sclerosis. The differences were analyzed by the Mann-Whitney *U*-test. Statistical results were considered significant for *P* values <.05.
*Mann-Whitney test; significant values when *P*<.05.

Examination of the TKTL1 protein expression in primary tumors did not show significant differences for sex, age, tumor diameter, encapsulation, or clinical signs. Statistical analysis, however, demonstrated a significant association between TKTL1 protein expression and multifocality, bilaterality, extrathyroidal extension, vascular invasion, and sclerosis (Table 3).

By applying an arbitrary score analysis based on intraglandular multifocality, bilaterality, tumor extrathyroidal extension, vascular invasion, and grade of sclerosis (range from score 0 = none of the selected evaluation criteria present, to score 5 = all 5 selected criteria present) and then subdividing the 256 patients, we found that no patients were included in score 5 and that cases with LN metastases were found in 8 of 8 (100%) patients with score 4, in 21 of 26 (80.8%) patients with score 3, in 16 of 21 (76.2%) patients with score 2, in 8 of 45 (17.8%) patients with score 1, and in 14 of 156 (9%) patients with score 0 (Fig. 3). By analyzing TKTL1 protein expression, we found that the progressive increase of TKTL1 LI was significantly associated with the worst score: the mean value of TKTL1 protein expression was 39.41 ± 25.43% in score 0, 47.94 ± 26.85% in score 1, 69.19 ± 17.57% in score 2, 77.73 ± 12.79% in score 3,

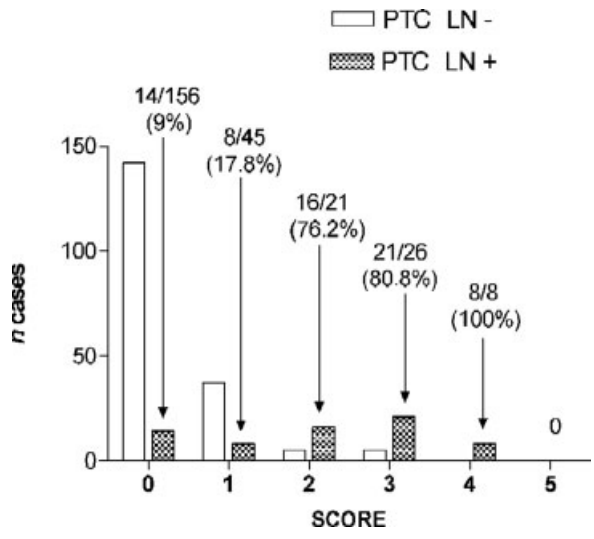


**FIGURE 3.** Subdivision of the 256 patients according to our scoring system, based on intraglandular multifocality, bilaterality, tumor extrathyroidal extension, vascular invasion, and grade of sclerosis [(ranging from a score of 0 (none of the selected evaluation criteria present) to a score of 5 (all 5 selected criteria present)].

and 92.55 ± 5.96% in score 4, with significant differences between scores 0 and 2, scores 0 and 3, and scores 0 and 4. Moreover, all patients in scores 4 and 3 and 19 of 21 (90.5%) in score 2 showed values of TKTL1 protein expression >50% (Fig. 4).

To identify a TKTL1 LI value of the primary PTC tumors and to determine whether the probability of the presence of LN metastases was more likely, we

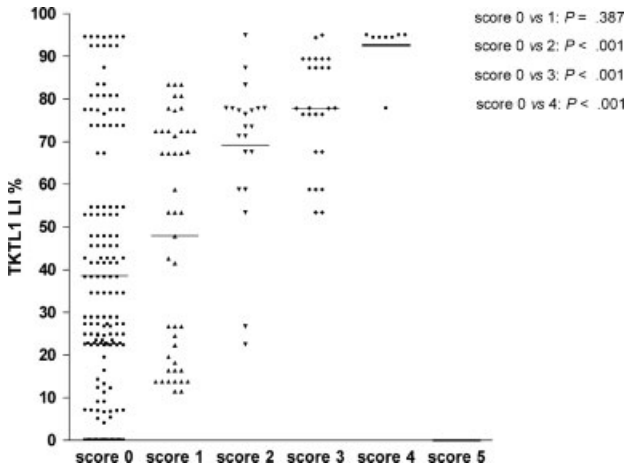


**FIGURE 4.** Transketolase-like-1 (TKTL1) protein expression in 256 cases of small papillary thyroid carcinomas (PTCs) subdivided according to our scoring system. The progressive increase of TKTL1 LI was significantly associated with the worst score. The mean value of TKTL1 protein expression was 39.41 ± 25.43% in score 0, 47.94 ± 26.85% in score 1, 69.19 ± 17.57% in score 2, 77.73 ± 12.79% in score 3, and 92.55 ± 5.96% in score 4, with significant differences between scores 0 and 2, scores 0 and 3, and scores 0 and 4 (differences were analyzed by Kruskal-Wallis test; statistical results were considered significant at $P<.05$). All patients in scores 4 and 3 and 19 of 21 (90.5%) in score 2 showed values of TKTL1 protein expression >50%.

considered an arbitrary value corresponding to the 50th percentile of the TKTL1 LI in Group 0; this value was 34.5%. Finally, 63 (94%) of the 67 cases with LN metastases showed a TKTL1 LI >34.5%, and only 4 (6%) showed a TKTL1 LI ≤34.5%. The association between the presence of LN metastases and TKTL1 LI >34.5% proved to be highly significant (odds ratio, 17.32; 95% confidence interval [CI], 6.06-49.51; $P < .001$).

## DISCUSSION

In this study, we evaluated the clinicopathogic characteristics and the TKTL1 expression of a large series of small PTCs ≤1.5 cm in diameter. All of our patients were treated with total or near-total thyroidectomy, and this allowed us to identify multifocal or bilateral cancer foci with high accuracy and to maximize sensitivity of the postoperative $^{131}$I-whole-body scan. We found that approximately 18% of small PTCs had bilateral foci and/or extrathyroidal extension, and approximately 27% presented regional LN metastases. Many studies have highlighted the importance of the presence of initial LN metastases in papillary cancer. Hay et al[27] analyzed 535 microcarcinomas and found that local relapse was related to the presence of metastatic LNs and to the extension of initial surgery. Baudin et al[28] observed that the most effective predictor of local relapse is the presence of multiple foci, correlated with the presence of metastatic LNs at presentation. Our work extends the correlation of the presence of metastatic LNs at presentation to intraglandular bilaterality, sclerosis, extrathyroidal extension, and vascular invasion.

TKTL1 protein has been found overexpressed in most human carcinomas and has been involved in tumor progression and invasion.[16] In our study, which compared tumors with their corresponding normal tissues, we found that TKTL1 transcript levels were significantly higher in the majority of cases that we analyzed. This finding demonstrates that high levels of TKTL1 transcript are a relevant phenomenon in small PTCs. Moreover, TKTL1 mRNA levels notably increased in primary PTCs with LN metastases compared with the group without LN metastases.

In primary tumors of the classic variant of PTCs ≤1.5 cm, we found that immunoreactivity for TKTL1 ranged from 0% to 95.0% and that many tumors present TKTL1 protein overexpression in accordance with results obtained from transcript analysis and with other studies that have involved different types of epithelial tumor entities. In our own series, the diameter of primary lesions was not associated with the presence of metastatic LNs at presentation and with the TKTL1 protein expression, whereas a positive correlation was found between the diameter of the largest metastatic area in LNs as well as the number of metastatic LNs and the TKTL1 protein expression in primary tumors. These data suggest that LN metastases at presentation in small PTCs probably depend on tumor biology and histologic characteristics more than on the diameter of the primary tumor.

Moreover, in our own series, the overexpression of TKTL1 protein was strongly associated with LN metastases. We also found a significant association between the TKTL1 expression and multifocality, bilaterality, extrathyroidal extension, vascular invasion, and sclerosis.

Overexpression of TKTL1 protein may cause an increase in the nonoxidative part of PPP activity, leading to the conversion of glucose to ribose required for nucleic acid synthesis. Moreover, the nonoxidative part of PPP is responsible for anaerobic glucose degradation.[14] Cancer cells characteristically acquire the capability of metabolizing high amounts of glucose by anaerobic degradation, which leads to production of large amounts of lactate even in the presence of oxygen.[29] This phenomenon is known as

the Warburg effect, and it has been described as the main metabolic adaptation in malignant alterations.[13] Glycolytic phenotype with increased glucose flux has been almost universally observed in clinical cancers by the use of positron emission tomography (PET) along with a glucose analog tracer [fluorodeoxyglucose (FdG)].[29,30]

Gatenby et al[11,31] proposed an acid-mediated tumor invasion model that provides a simple mechanism for malignant tumor growth. This mechanism includes promotion of angiogenesis, proteolytic cleavage of matrix proteins, and inhibition of immune response. Nonoxidative glucose degradation in tumor cells causes the excretion of protons from the tumor into surrounding normal tissues, thereby subjecting nontransformed cells adjacent to the tumor to an extracellular pH significantly lower than normal. The acid environment leads to the death of neighboring healthy cells via p53-dependent apoptosis pathways, as well as by degradation of the interstitial matrix, loss of intercellular gap junctions, enhanced angiogenesis, and inhibition of the host-immune response to tumor antigens. This enables tumor cells to remain proliferative and to migrate into surrounding normal tissues, thus producing invasive phenotypes.[32]

In our arbitrary scoring system, the progressive overexpression of TKTL1 was significantly associated with the worst score. Moreover, in our own series of the cases with LN metastases, 94% showed a TKTL1 LI >34.5%. Our data suggest that the arbitrary value of TKTL1 LI >34.5% may be considered a relative measure of risk for developing LN metastases.

Several studies usually distinguish malignant tumors from postulated benign tumor counterparts through the expression of specific molecular markers. It is widely accepted that a range of benign papillary lesions mimic papillary carcinoma, but there is no consensus that these are actually tumors' benign counterparts.[33] Studies of papillary carcinoma are, thus, deprived of baseline assessment of molecular-markers expression in the benign counterpart of malignant tumors. As it is important to provide diagnostic tests for distinguishing between clinically aggressive and clinically indolent forms of tumors, our findings suggest that the expression level of TKTL1 may indicate which small PTCs ≤1.5 cm in diameter may show aggressive behavior.

In conclusion, to our knowledge, the present study is the first analysis of TKTL1 protein expression in primary tumors of the classic variant of PTCs ≤1.5 cm; thus TKTL1 overexpression in PTC ≤1.5 cm may be considered a factor facilitating tumor growth and progression. Finally, it may also be extremely interesting to investigate an alternative effective way of inhibiting tumor progression by blocking the generation of energy and ribose mediated by TKTL1.

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