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Original article

Napsin a expression across subtypes of thyroid carcinoma: An immunohistochemical diagnostic encounter with prognostic correlates

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Abstract. *Background and aim:* Novel aspartic proteinase of pepsin family A (Napsin A) is a diagnostic marker for pulmonary adenocarcinoma. Recently, it was detected in carcinomas of various organs including thyroid carcinomas (TCs), raising a diagnostic challenge especially when combined with positive thyroid transcription factor-1 (TTF-1). *Methods:* This retrospective study investigates the frequency of Napsin A immunohistochemical (IHC) expression across subtypes of TC focusing on its association with the prognostic parameters. Sixty-three TC patients who underwent thyroidectomy were enrolled. After collecting the clinicopathological, laboratory, surgical, therapeutic and survival data, IHC was applied to TC tissue microarray-prepared sections using anti-Napsin A. IHC scoring divided TCs as: Napsin A positive & negative. Statistical and survival analyses were performed using SPSS version 26. *Results:* Napsin A was expressed in 17.5% of TCs with 100% expression in anaplastic TC and 19.5% expression in papillary TC. Other TC subtypes were negative. Statistically significant associations were noticed between Napsin A and some less favorable TC prognostic variables as the involvement of both lobes, anaplastic histopathology, larger tumor size, higher pathological stage, and a shorter mean OS and DFS of patients (all P ≤0.05). *Conclusions*: Napsin A is predominately expressed in anaplastic and papillary TC subtypes. In patients with a possible metastatic lung carcinoma or malignancy of unknown origin co-expressing Napsin A and TTF-1, the diagnosis of TC should be considered and supported with a panel of other TC markers. Considering its less favorable prognostic associations, Napsin A may be added as a molecular marker for TC risk stratification, and treatment targeting. However, the other subtypes must be evaluated in a larger series to support these conclusions. (www.actabiomedica.it)

Key words: thyroid carcinoma, Napsin A, frequency, therapy, prognosis

Introduction

Novel aspartic proteinase of the pepsin family A (Napsin A, TAO1/TAO2) belongs to the endopeptidase A1 family, such as Cathepsin E, renin, and pepsin and is encoded by the NAPSA gene located at chromosome 19q13.3 (1). Napsin A complementary DNA encodes a 420-amino acid polypeptide

consisting of 4 regions: a 24-residue signal peptide, a 40-amino acid pro-part, the mature enzyme of 336 amino acids, and a C-terminal extension of 18 residues. The mature protein contains 3 predicted disulfide bonds and 3 potential N-linked oligosaccharide attachment sites. It also contains an RGD motif, a recognition motif for integrin binding, in the C terminus. Human Napsin A shares 72.6% amino acid identity with the mouse homolog. Northern blot analysis detected Napsin A expression predominantly in the lung, and at lower levels in the kidneys, pancreatic acini and ducts, spleen, and leukocytes. Immunohistochemical studies revealed high expression of Napsin A in the kidney and lung but low expression in the spleen (2).

On a functional basis, Napsin A is involved in the maturation of prosurfactant protein B in type II pneumocytes, in phagocytosis by alveolar macrophages and the lysosomal protein catabolism in renal proximal convoluted tubules where it might be involved in cell differentiation (3,4).

In 1999, Chuman et al. (5) cloned Napsin A from lung adenocarcinoma. They noted that since Napsin A is expressed in greater than 90% of primary lung adenocarcinomas but not in other malignancies, it is a potential marker for this carcinoma. Further studies demonstrated the utility of Napsin A for stratifying lung adenocarcinoma into different prognostic subgroups (6-9), and its therapeutic pertinency in drug-resistant lung cancer has been explicated (10).

Recently, Napsin A expression has been confirmed in different tumor types including clear cell adenocarcinomas of the ovary; endometrium; and uterine cervix (11,12), papillary; clear cell; and chromophobe renal cell carcinomas (13), endometrial serous carcinoma (14), gastrointestinal mucinous carcinomas (15), and thyroid carcinomas (16,17). Yet, the frequency of Napsin A expression in different carcinomas and the impact of this expression on the diagnostic effectiveness of Napsin A remain contentious owing to the limited number of studies in this field.

Thyroid transcription factor 1 (TTF-1) is one of the most commonly utilized immunohistochemical markers in the diagnosis of lung cancers (18), and dual-marker staining of TTF-1 and Napsin A is widely accepted among pathologists to confirm the diagnosis of a primary pulmonary adenocarcinoma (19). Hence, the possible combined expression of Napsin A and TTF-1 in thyroid carcinoma raises a concern due to the chances of misdiagnosis of a metastatic thyroid carcinoma as primary lung adenocarcinoma (17); especially when taking into consideration that 84% of single organ distant metastasis from thyroid carcinomas occur in the lung, and that 94% of multiorgan metastases from thyroid carcinomas involves the lung with one or more other metastatic sites (20). Further complexity is added by the existence of genetic and pre-analytical factors that compromise the integrity of the thyroglobulin protein in papillary thyroid carcinomas (PTCs), and by the existence of calcitonin-negative medullary thyroid carcinomas (MTCs) (21). Although quite rare, adenocarcinoma of lung origin might also metastasize to the thyroid gland many years after the initial diagnosis (22).

For the aforementioned reasons, this work aimed to determine the frequency of Napsin A immunohistochemical expression across various subtypes of thyroid carcinomas focusing on its possible association with the clinicopathological parameters, as well as therapeutic and survival outcomes. This may spotlight its therapeutic applicability in a subset of thyroid carcinoma patients as previously addressed in lung adenocarcinoma.

Patients and methods

Patient selection and data collection

This cross-sectional retrospective study was conducted upon ethical approval of the research proposal (code R.23.02.2055) from Institutional Research Board (IRB) at the Faculty of Medicine, Mansoura University, Egypt. Sixty-three surgically-managed thyroid carcinoma patients were included in the study based on electronic database search at Oncology Center at Faculty of Medicine, Mansoura University (OCMU) during the period between September 2013 to January 2022. These comprised 41 papillary thyroid carcinomas (PTCs), 6 poorly-differentiated thyroid carcinomas (PDTCs), 3 anaplastic thyroid carcinomas (ATCs), 3 Hürthle cell carcinoma, 6 medullary thyroid carcinomas (MTCs), and 4 follicular thyroid carcinomas (FTCs); diagnosed on the basis of histopathological and immunohistochemical examination.

Inclusion criteria were the availability of relevant demographic, clinical, laboratory, surgical, therapeutic (type and response) and follow-up data including local, nodal or distant relapses to calculate the disease-free survival (DFS), and mortality data to calculate the overall survival (OS). Accessibility to the gross pathology reporting data, hematoxylin and eosin (H&E)-stained excision specimen sections, previous immunohistochemistry slides (TTF-1, thyroglobulin, Cytokeratin19, Calcitonin and CD56 are routinely used as applicable per case), and the formalin-fixed, paraffin-embedded tissue blocks from the tumor was ascertained with the aims of: histopathological review, confirmation of diagnosis, evaluation of microscopic prognostic parameters, pathological staging (by two pathologists H.S. and A.A.), and preparation of tissue-microarray (TMA) blocks to apply further Napsin A immunohistochemistry (IHC). The collected data are represented in tables 1 and 2. Patients lost for follow-up, inaccessibility of histopathology archived data/material or pre-operative therapy were considered as exclusion criteria.

Preparation of TMA and IHC

Two TMA paraffin blocks were prepared using the semiautomated technique with Manual Tissue Arrayer (MTA-1, cat.no.MP06, 0.6mm punch-size, Estigen Tissue Science, Estonia), including three representative tissue cores from each tumor. Cores of normal and pathological tissues were inserted in each block to serve as navigation tools and act as positive (kidney and lung) and negative controls (skeletal muscle and liver) for Napsin A. About 4µm-cut TMA paraffin sections were immunostained manually by applying the standard avidin-biotin peroxidase method using anti-Napsin A primary antibody (at a 1:200 dilution, mouse monoclonal antibody; Dako, Denmark). As per datasheet instructions, heat-mediated antigen retrieval was performed in 10mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature.

IHC scoring

The immunostained slides of Napsin A were scored semi-quantitatively by two pathologist co-authors (H.S. and A.A.) using an ordinary light microscope; independently. The scoring was adopted from Wu et al. (17) where coarse granular cytoplasmic staining of tumor cells was only considered. Staining extent was classified as follows: < 1% = negative; score 1 = 1 to 25% of tumor cells positive; score 2 = > 25 to 50%; score 3 = > 50%. Staining intensity was characterized as faint =0, weak=1, moderate=2, or strong=3. The intensity and percentage scores were then added to obtain a total immunostaining score, and a score ≥ 2 was considered positive.

Statistical analysis

Data were analyzed using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 26). The normality of data was first tested with a one-sample Kolmogorov-Smirnov test. Qualitative data were described using numbers and percentages. Association between categorical variables was tested using the Chi-square test while the Fischer exact test. The Monte Carlo test was used when the expected cell count was less than 5. Continuous variables were presented as mean ± standard deviation (SD) for normally distributed data and median (Min-Max) for non-parametric data. Mann Whitney test was used to compare two groups (non-parametric). The Kaplan-Meier test was used for survival analysis and the Log-rank test determined the statistical significance of differences among curves. For all above mentioned statistical tests done, the threshold of significance is fixed at 5% level (P-value). The results were considered significant when the P ≤0.05. The smaller the P-value obtained, the more significant the results.

Ethical standards

The study was conducted upon local IRB ethical approval (code R.23.02.2055). H&E slides and paraffin tissue blocks of the studied thyroid carcinomas were reverted to archives following the completion of the study procedure. No additional surgical or non-surgical interventions were applied to the patients as a part of the study whose anonymity and confidentiality were secured throughout and after the study by using code numbers instead of names. All procedures

were done following the current revision of the Helsinki Declaration of medical research involving human subjects (23).

Results

Surgical and post-operative therapy and follow-up data

Patients ranged in age from 15 to 86 years (mean 46.26±16.26) and included 13 (20.6 %) males and 50 (79.4%) females. Concerning the surgical procedures, 56 patients (88.9%) underwent total thyroidectomy 56 (88.9%), 1 patient (1.6%) underwent debulking & tracheostomy, and 1 patient (1.6%) underwent a hemithyroidectomy, however, for the remaining 5 cases (7.9%) the type of surgery was not documented. Forty-eight (76.2%) patients received adjuvant therapy. Therapeutic regimens included the use of radioactive iodine (RAI) in 39 (61.9%), radiotherapy in 4 (6.3%), chemotherapy in 1 (1.6%), RAI plus external-beam radiotherapy (EBRT) in 2 (3.2%) and RAI plus radiotherapy to the bone in 2 (3.2%) cases. Relapses occurred in 14 (22.2%) of patients including 11 cases (17.5%) of local relapse and 3 (4.7%) cases of both local and distant relapses. During the follow-up period, 11 (17.5%) of patients died including 9 (14.3%) patients who died due to disease-related factors and 2 (3.2%) patients who died due to disease-unrelated factors.

Frequency of Napsin A expression in thyroid carcinoma and across subtypes

Out of 63 thyroid carcinomas, Napsin A was expressed in 11 (17.5%) of carcinomas distributed as: 8/41 PTCs (19.5%) and 3/3 ATCs (100%). All other thyroid carcinoma subtypes including: PDTC, Hürthle cell carcinoma, MTC, and FTC didn't display any Napsin A expression (Figures 1 and 2).

Associations between Napsin A and the demographic, clinical and laboratory data

Napsin A expression was significantly associated (P=0.007) with the involvement of both thyroid lobes (42.9% of cases) by carcinoma as compared to carcinomas involving either of the right or left lobes or the isthmus (0, 20 and 0% of cases respectively). All other demographic, clinical and laboratory parameters demonstrated in Table 1 revealed no association with Napsin A expression.

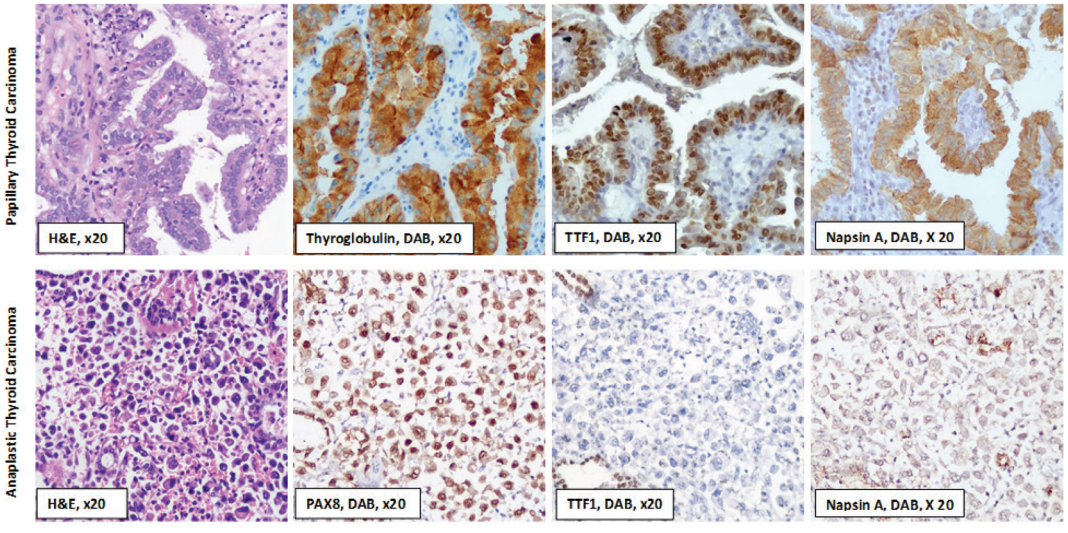

Figure 1. Napsin A positive immunostaining in thyroid carcinomas. Napsin A-positive papillary thyroid carcinoma with positive staining for thyroglobulin, thyroid transcription factor (TTF1) (upper row). Napsin A-positive anaplastic thyroid carcinoma with positive immunostaining for PAX8 and negative immunostaining for TTF1 (lower row). H&E; hematoxylin and eosin, DAB; diaminobenzidine.

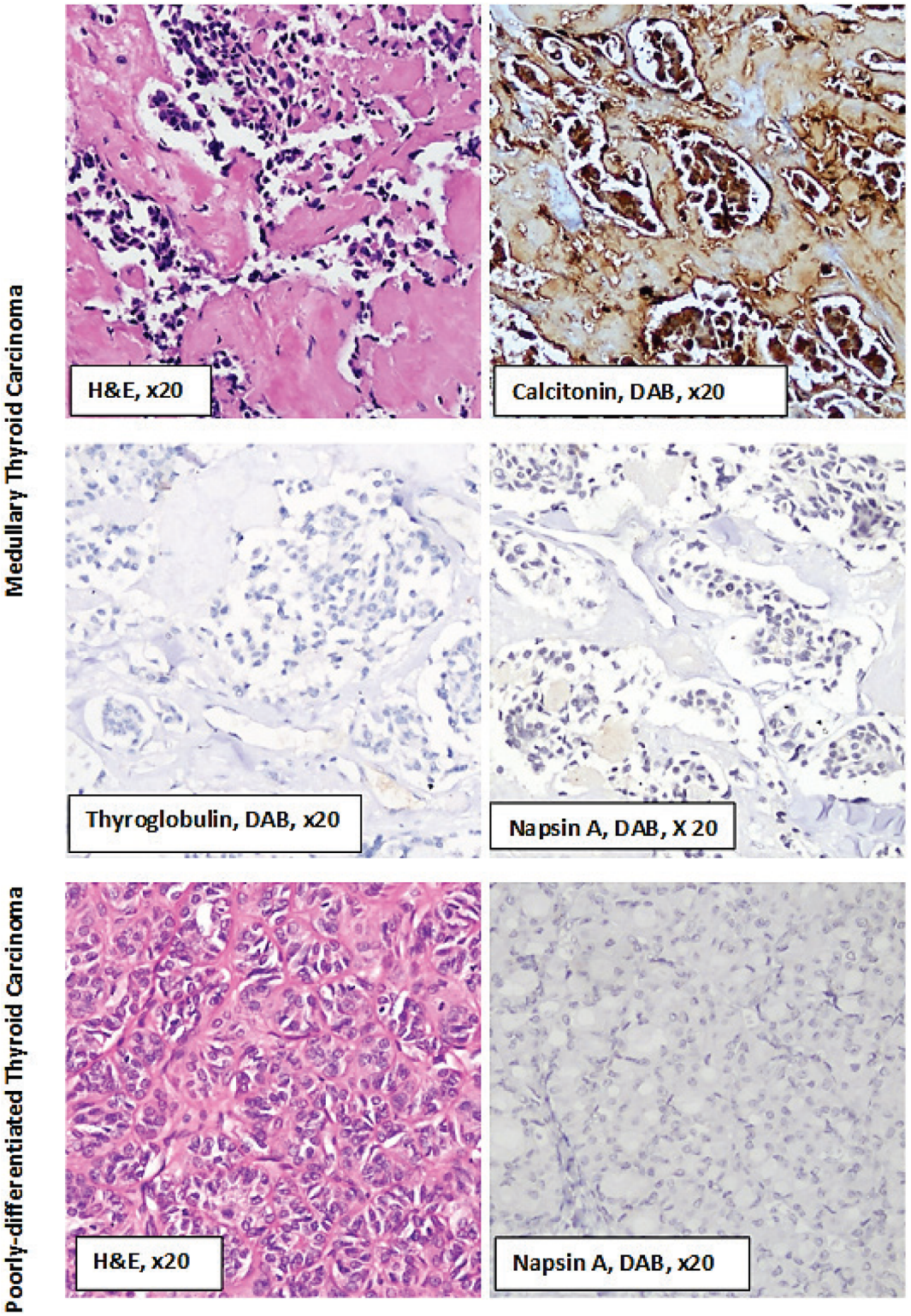

Figure 2. Napsin A negative immunostaining in thyroid carcinomas. Napsin A-negative medullary thyroid carcinoma with positive immuno-staining for calcitonin and negative staining for thyroglobulin (upper 2 rows). Napsin A-negative poorly-differentiated thyroid carcinoma (lower row). H&E; hematoxylin and eosin, DAB; diaminobenzidine.

Table 1. Associations between Napsin A expression and the demographic, clinical and laboratory data

Variables	Total (%)	Napsin A Immunohistochemical Expression				χ^2 (P value)
		Positive (n=11)		Negative (n=52)		
		No/median	%/min-max	No/median	%/min-max	
Age						
≤50y	40 (63.5%)	7	17.5	33	82.5	χ^2 =0.001
>50y	23 (36.5%)	4	17.4	19	82.6	P=0.991
Family history						
Yes	1 (1.6%)	0	0	1	100	FET
No	62 (98.4%)	11	17.7	51	82.3	P=1.00
Smoking						
Yes	7 (11.1%)	1	14.3	6	85.7	χ^2 =0.055
No	56 (88.9%)	10	17.9	46	82.1	P=0.814
BMI						
<30	29 (46.0%)	6	20.7	23	79.3	χ^2 =0.389
>30	34(54.0%)	5	14.7	29	85.3	P=0.533
Performance Status (ECOG) score						
0	7 (11.1%)	0	0	7	100	MC
1	40(63.5%)	5	20	32	80	P=0.63
2	15(23.8%)	3	20	12	80	
3	1(1.6%)	0	0	1	100	
Neck swelling						
Yes	61 (96.8%)	11	18	50	82	FET
No	2 (3.2%)	0	0	2	100	P=1.00
Cervical lymph nodes						
Yes	25 (39.7%)	6	24	19	76	χ^2 =1.23
No	38 (60.3%)	5	13.2	33	86.8	P=0.267
Cervical lymph nodes						
No	40 (63.5%)	5	12.5	35	87.5	MC
Unilateral	17 (27.0%)	3	17.6	14	82.4	P=0.095
Bilateral	6 (9.5%)	3	50	3	50	
Hoarseness of voice						
Yes	14 (22.2)	4	28.6	10	71.4	χ^2 =1.542
No	49 (77.8%)	7	14.3	42	85.7	P=0.214
Retrosternal extension						
Yes	11 (17.5%)	4	36.4	7	63.6	χ^2 =3.30
No	52 (82.5%)	7	13.5	45	86.5	P=0.069
Distant Metastasis						
Yes	12 (19.0%)	3	25	9	75	χ2 =0.585
No	51 (81.0%)	8	15.7	43	84.3	P=0.444
Site of primary lesion						
LT lobe	23 (36.5%)	0	0	23	100	MC
RT lobe	25 (39.7%)	5	20	20	80	**P=0.007***
Both	14 (22.2%)	6	42.9	8	57.1	
Isthmus	1 (1.6%)	0	0	1	100	

Variables	Total (%)	Napsin A Immunohistochemical Expression				χ^2 (P value)
		Positive (n=11)		Negative (n=52)		
		No/median	%/min-max	No/median	%/min-max	
Clinical staging						
I	23 (36.5%)	3	13	20	87	MC
II	20 (31.7%)	3	15	17	85	P=0.734
III	6 (9.5%)	2	33.3	4	66.7	
IVB	2 (3.2%)	0	0	1	100	
IVC	12 (19.0%)	3	25	9	75	
TSH Median (Min-Max)	1.75 (0.01- 102.00)	2.05 (0.03- 19.00)		1.70 (0.01- 102.00)		Z =0.691 P=0.490
Serum thyroglobulin Median (Min-Max)	4.25 (0.04- 2133.00)	3.00 (1.30- 309.00)		4.75 (0.04- 2133)		Z=0.021
Serum antithyroglobulin						
Positive	30 (47.6%)	3	10	27	90	MC
Negative	4 (6.3%)	2	50	2	50	P=0.093
NA	29 (46.0%)	6	20.7	23	79.3	
Total	63 (100%)	11	17.5	52	82.5	

Abbreviations: χ^2, Chi square test; FET, Fisher exact test; MC, Monte carlo test; ECOG, Eastern Cooperative Oncology Group, CEA; carcinoembryonic antigen, TSH; thyroid stimulating hormone, *Significant P≤0.05.

Associations between Napsin A and the surgical, pathological, therapy and follow-up data

Amidst the surgical, pathological, therapeutic and follow-up parameters demonstrated in Table 2, the histopathological subtype; the median gross tumor size; and the pathological stage were significantly associated with Napsin A expression (P=0.005, 0.05 and 0.014 respectively) as immunopositivity occurred more frequently in ATC and PTC (100% and 19.5% respectively), larger size tumors (median 4cm. versus 3cm. in negative tumors) and in higher stage carcinomas (50% of stage IV carcinomas).

Kaplan-Meier survival analysis

Table 3 demonstrates the associations between the tested variables and both Overall Survival (OS) and Disease-Free Survival (DFS) in 63 patients diagnosed with thyroid carcinomas of different subtypes using the Log-rank test. OS was significantly associated with patients above 50 years old at diagnosis (P≤0.001), presence of hoarseness of voice (P=0.002), retrosternal extension (P=0.007), distant metastasis (P=0.001), ATC histopathological subtype (P≤0.001), pathological stage IV (P≤0.001), lymph node dissection (P=0.012), lymphovascular emboli (P≤0.001), extrathyroidal extension (P≤0.001), immunohistochemical positivity for Napsin A (P=0.047), and the patients eligible to adjuvant therapy (P=0.003) as significantly shorter mean OS was verified in these groups. DFS was significantly associated with the presence of retrosternal extension (P=0.01), pathological stage IV (P=0.011), lymphovascular invasion (P≤0.001), and immunohistochemical positivity for Napsin A (P=≤0.001) as significantly shorter mean DFS was verified in these groups (Figure 3).

Discussion

When Napsin A was first defined about 25 years ago, it was believed to be a lung-specific marker that is used to differentiate subtypes of non-small cell lung

Table 2. Associations between Napsin A and the surgical, pathological, therapy and follow-up data.

Variables	Total (%)	Napsin A Immunohistochemical Expression				χ^2 (P value)
		Positive (n=11)		Negative (n=52)		
		No/median	%/min-max	No/median	%/min-max	
LN dissection						
Yes	36 (57.1%)	4	11.1	32	88.9	χ^2 =2.35
No	27 (42.9%)	7	25.9	20	74.1	P=0.125
Extent of LN dissection						
NA	28 (44.4%)	7	25	21	75	MC
Central	14 (22.2%)	1	7.1	13	92.9	0.524
Lateral	5 (7.9%)	1	20	4	80	
Both	16 (25.4%)	2	12.5	14	87.5	
Extrathyroidal extension						
Yes	17 (27.0%)	5	29.4	12	70.6	MC
No	44 (69.8%)	6	13.6	38	86.4	0.286
NA	2 (3.2%)	0	0	2	100	
Histopathological subtype						
PTC	41 (65.1%)	8	19.5	33	80.5	MC
MTC	6 (9.5%)	0	0	6	100	**P=0.005***
Hurthle cell carcinoma	3 (4.8%)	0	0	3	100	
ATC	3 (4.8%)	3	100	0	0	
PDTC	6 (9.5%)	0	0	6	100	
FC	4 (6.3%)	0	0	4	100	
Pathology Max. T. size (cm)						χ2 =1.96
Median (Min-Max)	3.0 (0.5- 13.0)	4.0 (2.5- 13.0)		3.0 (0.5- 10.0)		**P=0.05***
Number of positive LNs						Z=1.184
Median (Min-Max)	3 (1- 30)	2 (1- 3)		3 (1- 30)		P=0.236
Side of involved LNs						
NA	41 (65.1%)	7	17.1	34	82.9	MC
Right	7 (11.1%)	2	28.6	5	71.4	0.764
Left	11 (17.5%)	1	9.1	10	90.9	
Both	4 (6.3%)	1	25	3	75	
Lymph-vascular invasion						
Yes	20 (31.7%)	3	15	17	85	MC
No	38 (60.3%)	6	15.8	32	84.2	0.451
NA	5 (7.9%)	2	40	3	60	
Capsular invasion						
Yes	28 (44.4%)	6	21.4	22	78.6	χ2 =0.551
No	35 (55.6%)	5	14.3	30	85.7	P=0.458
Pathological stage						
I	20 (31.7%)	0	0	20	100	MC
II	21 (33.3%)	4	19	17	81	**P=0.014***
III	14 (22.2%)	3	21.4	11	78.6	
IV	8 (12.7%)	4	50	4	50	
TTF1 IHC expression						
Negative	2 (3.2%)	1	50	1	50	MC
Positive	18 (28.6%)	3	16.7	15	83.3	0.505
NA	43 (68.3%)	7	16.3	36	83.7	
Adjuvant therapy						
Yes	48 (76.2%)	7	14.6	41	85.4	χ2 =1.15
No	15 (23.8%)	4	26.7	11	73.3	P=0.282

Variables	Total (%)	Napsin A Immunohistochemical Expression				χ^2 (P value)
		Positive (n=11)		Negative (n=52)		
		No/median	%/min-max	No/median	%/min-max	
Relapse status						
Yes	14 (22.2%)	4	28.6	10	71.4	FET
No	49 (77.8%)	7	14.3	42	85.7	P=0.589
Death						
Yes	11(17.5%)	3	27.3	8	72.7	χ2 =0.89
No	52(82.5%)	8	15.4	44	84.6	P=0.345
Total	63 (100%)	11	17.5	52	82.5	

Abbreviations: χ^2, Chi square test; FET, Fisher exact test; MC, Monte carlo test; IHC; immunohistochemistry, LN; lymph node, *Significant P≤0.05.

carcinoma; being frequently expressed in pulmonary adenocarcinoma but not in squamous cell carcinoma. Soon after, it was shown that Napsin A is also expressed in a wide range of human tumors and normal tissues (1). Because the lung is a frequent site for metastasis of various malignancies, it is of paramount importance to determine the frequency of Napsin A expression in other tumor types of potential metastasis to the lung.

Thyroid cancer is a relatively common cancer ranking the 11th cancer worldwide with 586,202 newly-diagnosed cases; comprising about 3% of new cancer cases in 2020 (24). It encompasses a heterogenous group of pathological entities including rare and diagnostically challenging tumor subtypes. Thus, the diagnostic approach to thyroid tumors should take into consideration their morphological, immunohistochemical and molecular features (21).

In this work, Napsin A immunohistochemical expression was investigated in 63 thyroid carcinomas of different subtypes to reveal that 17.5% of thyroid carcinomas express this marker, and that it is expressed particularly in all anaplastic thyroid carcinomas (ATCs) and in a percentage of 19.5% of papillary thyroid carcinomas (PTCs), whilst poorly-differentiated thyroid carcinoma (PDTC), Hürthle cell carcinoma, medullary thyroid carcinoma (MTC), and follicular thyroid carcinoma (FTC) don't express this marker.

In comparison, Wu et al. (17) verified Napsin A expression in 23.8% thyroid tumors but in a different disruption across subtypes with positive expression in 30.3% of PTCs, 12.5% of PDTC, and 11.1% of ATC and negative expression in Hürthle cell carcinoma, MTC, FTC. Meanwhile Chernock et al. (16) demonstrated Napsin A expression in 15% of ATCs, 13% of PDCs and 100% of micropapillary pattern thyroid carcinoma. Regarding canine thyroid carcinomas, Napsin A was found to be the most sensitive in differentiating MTCs compared to PTC and FTC (25). Such partially conflicting results may be due to the limited number of studies, the use of different antibodies, the application of different staining protocols as well as different criteria to determine positivity in these studies. For many other tumor types, Napsin A expression has never been analyzed (1). Therefore, further studies are warranted in this field.

The contribution of Napsin A in the carcinogenesis and prognosis of lung cancer has been reported. Its immunopositivity was correlated with better prognostic variables such as small-size tumors, well-differentiated lung adenocarcinomas, low mitotic count, and a longer OS, while its absence was considered as an independent prognostic factor for reduced survival time (7-9). The favorable biological effects of Napsin A include (1) a synergistic inhibitory effect on cellular proliferation and a promotive effect on cell apoptosis of gefitinib-resistant A549s lung epithelial cell lines, (2) blocking the downregulation of E-cadherin and the upregulation of Vimentin in gefitinib-resistant cells, thus averting epithelial-mesenchymal transition (EMT), (3) re-sensitization of the resistant A549 cells to gefitinib by reversing EMT and inhibiting the activation of the integrin signalling pathway via focal adhesion kinase (FAK) (10). Also, Napsin A was found to suppress tumor growth in kidney cell lines

Table 3. Associations between overall survival (OS) and disease-free survival (DFS) and the studied prognostic variables

	Overall Survival (OS)/months					Disease-Free Survival (DFS)/months				
Variables	**Mean**	**Std. Error**	**95% CI**	**Log-rank test**	**P-value**	**Mean**	**Std. Error**	**95% CI**	**Log-rank test**	**P-value**
Age/years										
<50 y	57.583	1.397	54.8-60.3	13.56	**≤0.001***	40.270	2.729	34.9-45.6	0.477	0.490
≥50 y	44.844	7.345	30.4-59.2			45.861	4.990	36.1-55.6		
Gender										
Male	58.734	8.162	42.7-74.7	0.241	0.624	40.053	7.081	26.1-53.9	0.259	0.611
Female	60.230	3.648	53.1-67.3			42.878	2.725	37.5-48.2		
Cervical LN										
Yes	36.347	3.46	29.5-43.1	1.44	0.23	34.539	3.584	27.5-41.5	1.24	0.265
No	62.422	3.66	55.23-69.6			44.790	3.307	38.3-51.2		
Hoarseness of voice	41.286	9.581	22.5-60.1	9.59	**0.002***	33.338	8.114	17.4-49.2	2.75	0.097
Yes	64.770	2.717	59.4-70.1			44.978	3.189	38.7-51.2		
No										
Retrosternal extension										
Yes	30.000	6.390	17.4-42.5	7.29	**0.007***	24.675	7.277	10.4-38.9	6.69	**0.01***
No	62.906	3.032	56.9-68.8			45.218	2.732	39.86-50.5		
Distant Metastasis	35.048	8.162	19.1-51.04	11.61	**0.001***	42.333	8.607	25.4-59.2	0.011	0.916
Yes	64.083	2.858	58.5-69.7			43.335	3.081	37.2-49.3		
No										
Histopathological Type										
PTC	66.327	2.548	61.3-71.3	15.69	**≤0.001***	44.260	3.022	38.3-50.1	5.26	0.072
MTC	21.200	4.828	11.7-30.6			38.150	4.945	28.4-47.8		
PDTC	10.333	3.549	3.3-17.2			12.500	3.419	5.7-19.2		
Others	36.887	5.738	25.6-48.1							
Pathological Tumor stage										
I	40.738	2.822	35.2-46.2	19.48	**≤0.001***	41.406	2.243	37.0-45.8	11.04	**0.011***
II	69.500	3.500	68.5-70.4			48.520	3.377	41.9-55.1		
III	39.250	4.367	30.6-47.8			29.500	5.657	18.4-40.5		
IV	16.000	4.969	6.2-25.7			8.000	1.058	5.9-10.1		
Capsular invasion										
Yes	39.536	2.987	33.6-45.3	0.086	0.769	35.354	3.735	28.0-42.6	1.04	0.308
No	60.430	4.066	52.4-68.4			45.213	3.065	39.2-51.2		
LN dissection										
Yes	55.952	2.085	51.8-60.04	6.35	**0.012***	39.510	2.891	33.8-45.1	1.23	0.267
No	50.913	6.363	38.4-63.3			47.005	4.403	38.3-55.6		
Lympho-vascular invasion										
Yes	39.454	3.467	32.6-46.2	46.194	**≤0.001***	8.095	1.419	5.3-10.8	15.31	**≤0.001***
No	65.848	2.916	60.13-71.5			45.571	2.642	40.3-50.7		

Variables	Overall Survival (OS)/months					Disease-Free Survival (DFS)/months				
	Mean	Std. Error	95% CI	Log-rank test	P-value	Mean	Std. Error	95% CI	Log-rank test	P-value
Extrathyroidal extension										
Yes	34.655	4.510	25.8-43.4	27.91	**≤0.001***					
No	65.006	2.813	59.4-70.5							
Napsin A										
Positive	11.477	1.681	8.1-14.7	3.93	**0.047***	8.095	1.419	5.3-10.8	15.31	**≤0.001***
Negative	61.889	3.140	55.7-68.0			45.571	2.642	40.3-50.7		
Adjuvant therapy										
Yes	63.926	2.936	58.1-69.6	8.55	**0.003***	42.293	3.004	36.4-48.1	0.344	0.557
No	27.690	5.166	17.5-37.8			38.300	3.510	31.4-45.1		
Survival	59.648	3.194	53.38-65.90			42.842	2.788	37.3-48.3		

Abbreviations: LN; lymph node, Log-rank (Mantel-Cox); CI: confidence interval, *Significant P≤0.05.

independent of its catalytic activity (3). Concerning renal cell carcinoma, there was also a positive correlation between Napsin A expression and the low tumor grade and stage, and the late recurrence and long tumor-specific survival (1). Accordingly, it was plausible to assess the association between Napsin A expression and the prognostic clinicopathological parameters and survival outcomes in patients with thyroid carcinoma included in this study.

We demonstrated statistically significant associations between Napsin A expression and a considerable number of the less favorable prognostic variables in thyroid carcinoma notably involvement of both thyroid lobes, anaplastic carcinoma histopathology, larger-size-tumors, pathological stage IV, and a shorter mean OS and DFS of patients. Despite the statistically significant associations between the OS and DFS and several prognostic variables as: age above 50 years at diagnosis, hoarseness of voice, retrosternal extension, distant metastasis, surgical lymph node dissection, presence of lymphovascular emboli, extrathyroidal extension, and the eligibility to adjuvant therapy for the former; and the presence of retrosternal extension and lymphovascular invasion for the later; these factors were not found to be associated with Napsin A expression in the univariate analysis.

Likewise, Wu et al. (17) documented a significant correlation between Napsin A and lymph node metastasis after pathologic investigation and owed this finding to the expression of Napsin A in the more aggressive PTC variants mainly in the classic, diffuse sclerosis, tall cell and solid variants of PTC. Also, Napsin A expression was found to be associated with the more aggressive subtypes of thyroid carcinoma (16). Yet, there is only a small number of previous studies that analyzed the prognostic value of Napsin A expression in different cancers. In clear cell carcinomas of the endometrium, Fadare et al (26) did not find associations between Napsin A expression and survival or clinicopathological features. Such prognostic associations are contradictory to the data obtained from lung adenocarcinoma and renal cell carcinoma-based studies. This contradiction may indicate an organ-specific carcinogenic role of Napsin A; with a cancer-suppressing effect in lung and renal carcinoma on one side and a cancer-promoting effect in thyroid and endometrial carcinomas on the other side. A possible explanation is that Napsin A is not detected in the normal thyroid or endometrial tissue but is detected in normal lung and renal tissue and that this different behavior is related to the aberrant Napsin A expression in thyroid and endometrial carcinomas.

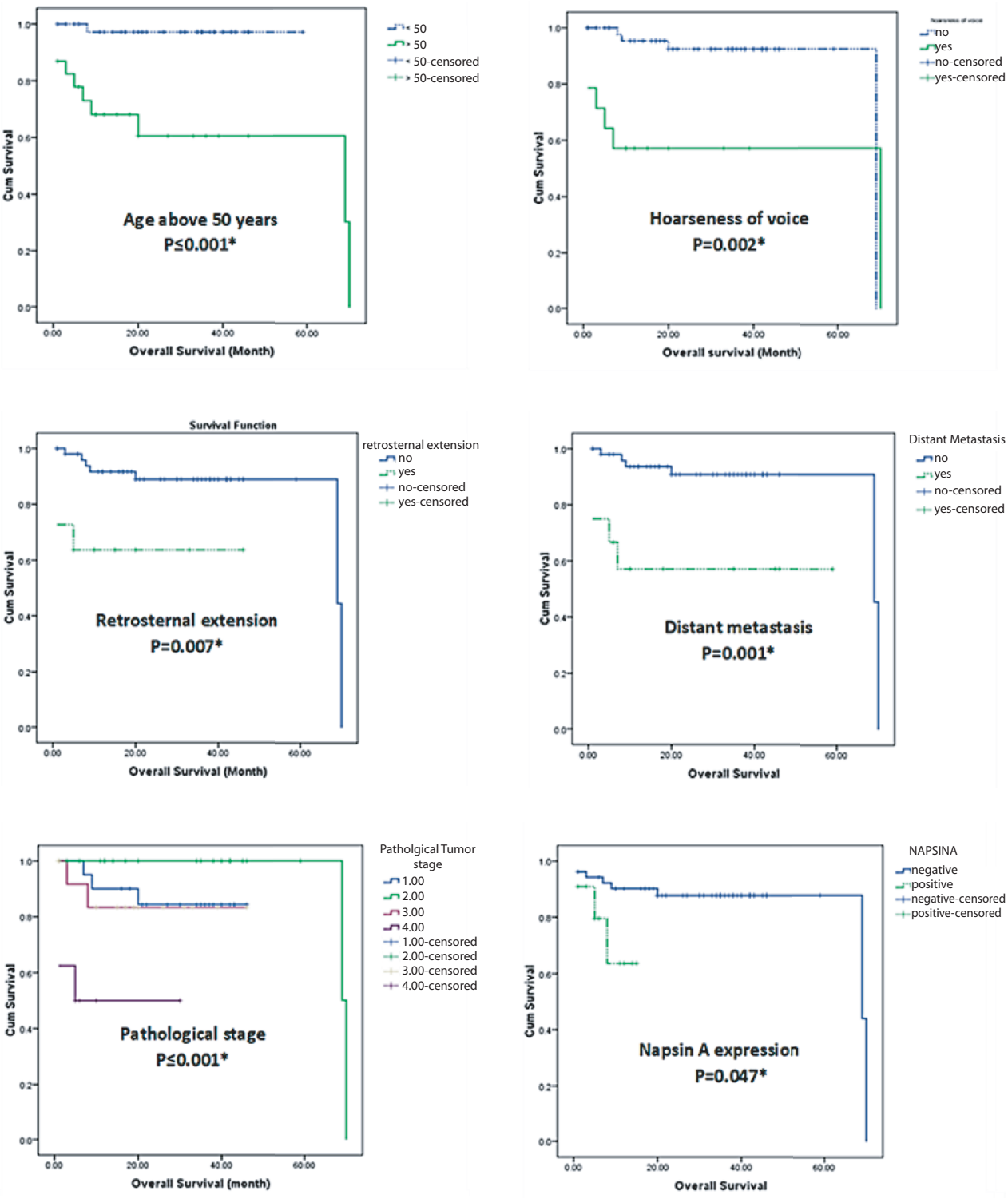

Figure 3. Kaplan-Meier Survival curves using Log-rank test demonstrate significant associations between Overall Survival (OS) and Diseases-Free Survival (DFS) with different variables in thyroid carcinoma patients. *Significant P≤0.05.

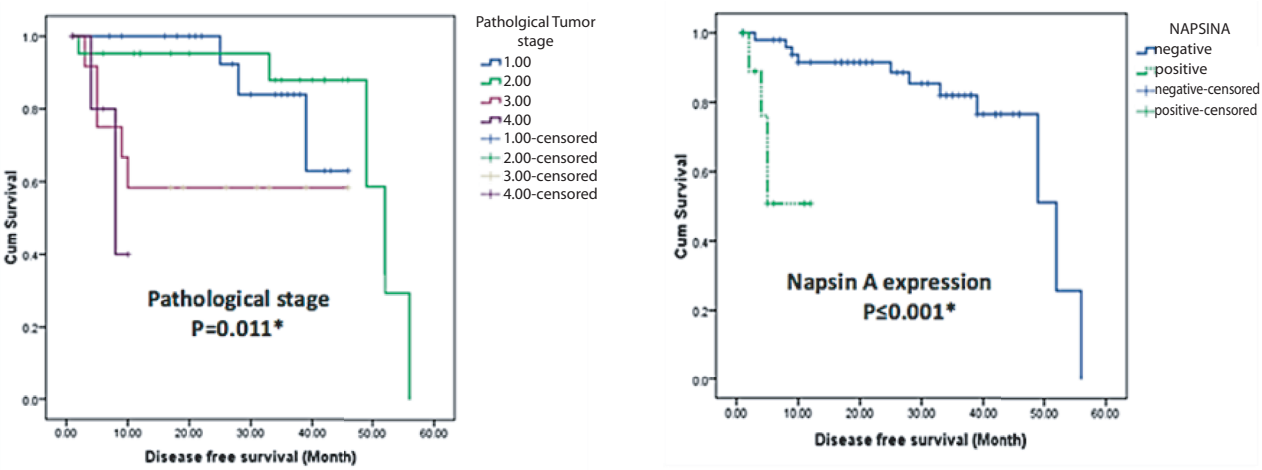

Figure 3. (*Continued*)

In conclusion, this work demonstrated Napsin A immunohistochemical expression particularly in ATC and in a considerable number of PTC. In patients with a possible metastatic lung carcinoma or malignancy of unknown origin with co-expression of Napsin A and TTF-1, the diagnosis of thyroid carcinoma should be taken into consideration and should be supported with a panel of other thyroid carcinoma-specific markers. Considering its less favorable prognostic associations, Napsin A may be added as a molecular marker for risk stratification, and treatment targeting in thyroid carcinoma to improve patient's survival. However, except for papillary thyroid carcinoma, the other subtypes must be evaluated in a larger series to support our findings across all types of TC.

Abbreviations: Napsin A: Novel aspartic proteinase of pepsin family A; TCs: thyroid carcinomas; TTF-1: thyroid transcription factor-1; IHC: immunohistochemical; DFS: disease-free survival; OS: overall survival; H&E: hematoxylin and eosin; TMA: tissue-microarray.

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Conflict of Interest: All authors participated equally based on specialty in study conception and design, data acquisition, supply of study materials, analysis, and interpretation, drafting and writing the final version of the manuscript, critical revision of the manuscript. H.S. and A.A. revised histopathology and interpreted immunohistochemistry. All authors have read and approved the final version of the manuscript.

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