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Accuracy of World Health Organisation-grade parameters (necrosis and mitotic activity) and foci of vascular invasion in predicting prognosis of papillary thyroid carcinoma. A case–control validation study

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Accuracy of World Health Organisation-grade parameters (necrosis and mitotic activity) and foci of vascular invasion in predicting prognosis of papillary thyroid carcinoma. ?A case–control validation study

Aims: Tumour necrosis and/or increased mitoses define high-grade papillary thyroid carcinoma (PTC). It is unclear whether angioinvasion is prognostic for PTC. Cut-offs at five or more mitoses/2 mm^2 and four or more angioinvasive foci have been empirically defined based upon data from all forms of aggressive non-anaplastic thyroid carcinomas. Performance of tumour necrosis, mitoses and vascular invasion in predicting distant metastases when specifically applied to PTC is undefined.

Methods: We analysed 50 consecutive PTC cases with distant metastases (DM-PTC): 16 synchronous and 34 metachronous. A total of 108 non-metastatic PTC (N-DM-PTC, 15.0-year median follow-up) were used as controls. Invasive encapsulated follicular variant PTC was excluded. Necrosis, mitoses and

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Abbreviations: AUC, Areas Under the Curve; ColC-PTC, Columnar Cell Papillary Thyroid Carcinoma; C-PTC, Classic Papillary Thyroid Carcinoma; DHGTCs, Differentiated High-Grade Thyroid Carcinomas; DM, Distant Metastases; DM-PTC, Papillary Thyroid Carcinoma with Distant Metastasis; DOC, Death of other Causes; DOD, Death of Ddisease; DS-PTC, Diffuse Sclerosing Papillary Thyroid Carcinoma; EFVPTC, Encapsulated Follicular Variant of Papillary Thyroid Carcinoma; FU, Follow Up; IFV-PTC, Infiltrative Follicular Variant of Papillary Thyroid Carcinoma; N-DM-PTC, Papillary Thyroid Carcinoma without Distant Metastases; PTC, Papillary Thyroid Carcinoma; ROC, Receiver Operating Characteristics; ST-PTC, Solid Trabecular Papillary Thyroid Carcinoma; TC-PTC, Tall-Cell Papillary Thyroid Carcinoma; WHO, Word Health Organisation; WL-PTC, Warthin-Like Papillary Thyroid Carcinoma.

angioinvasion were quantified. Receiver operating characteristics (ROC) and area under the curve (AUC) analyses determined best sensitivity and specificity cut-offs predictive of distant metastases.
Results: Metastases correlated with necrosis (any extent = 43.8% all DM-PTC, 53.1% metachronous DM-PTC versus 5% N-DM-PTC; $P < 0.001$), mitoses ($P < 0.001$) and angioinvasion ($P < 0.001$). Mitoses at five or more per 2 mm^2 was the best cut-off correlating with distant metastases: sensitivity/specificity 42.9%/97.2% all DM-PTC (AUC = 0.78), 18.8%/97.2% synchronous DM-PTC (AUC = 0.63), 54.6%/97.2% metachronous DM-PTC (AUC = 0.85). Angioinvasive foci at five or more was the best cut-off correlating with distant metastases: sensitivity/specificity 36.2%/91.7% all DM-PTC (AUC = 0.75), 25%/91.7% synchronous DM-PTC (AUC = 0.79) and 41.9%/91.7% metachronous DM-PTC (AUC = 0.73). Positive/negative predictive values (PPV/NPV) were: necrosis 22.6%/98.2%; five or more mitoses 32.3%/98.2%; five or more angioinvasive foci 11.8%/97.9%. After multivariable analysis, only necrosis and mitotic activity remained associated with DM-PTC.
Conclusion: Our data strongly support PTC grading, statistically validating World Health Organisation (WHO) criteria to identify poor prognosis PTC. Angioinvasion is not an independent predictor of DM-PTC.

Keywords: angioinvasion, differentiated high-grade thyroid carcinoma (DHGTC), distant metastasis, metachronous metastasis, mitoses, necrosis, papillary thyroid carcinoma

Introduction

Papillary thyroid carcinoma (PTC) is the most common malignancy originating from thyroid follicular cells. It is typically associated with long-term survival, but some cases have adverse outcomes due to distant metastases.[1] The current World Health Organisation (WHO) classification recognises differentiated high-grade thyroid carcinomas (DHGTCs) as invasive high-grade follicular cell-derived carcinomas that still retain the distinctive architectural and/or cytological properties of well-differentiated histotypes (papillary, follicular and oncocytic thyroid carcinoma), but share the same outcome of poorly differentiated carcinoma.[2] DHGTCs are defined by the presence of five or more mitoses per 2 mm^2 and/or tumour necrosis in an otherwise well-differentiated carcinoma. The vast majority of DHGTCs—approximately 90% in a large series[3]—are high-grade PTC, with a high rate of lymph node and distant metastases, both at diagnosis and during follow-up (FU).[3,4]

The extent to which the finding of tumour necrosis is of prognostic value is unclear when specifically applied to PTC. The cut-off for mitosis used to define DHGTC has been empirically defined, without statistical verification of whether five or more mitoses per 2 mm^2 represents the best value.[5] Angioinvasion is an established prognostic factor, and according to current WHO recommendations foci of vascular (blood vessel) invasion should be counted and reported in all encapsulated angioinvasive follicular[6] and oncocytic[7] carcinomas of the thyroid gland. Four angioinvasive foci represents the cut-off used to distinguish tumours with limited vascular invasion (fewer than four foci) having a better prognosis compared with those with extensive vascular invasion (four or more foci of invasion). The cut-off for angioinvasive foci has also been empirically defined,[8–10] without statistical verification of whether four foci indeed represent the best value. It is unclear if, and to what extent, vascular invasion may be useful for PTC risk stratification.[11–15]

The aim of this study is to investigate, in a consecutive monocentric series of PTC with distant metastasis (DM-PTC), the role of necrosis, mitoses and angioinvasion in predicting distant metastases, defining optimal cut-off values and their statistical performance.

Materials and Methods

STUDY DESIGN AND POPULATION

In this case–control study, PTC with distant metastases (DM-PTC) diagnosed between 1979 and 2013 were retrieved from the electronic files of the Pathology Unit of the AUSL-IRCCS of Reggio Emilia, Italy. All specimens were formalin-fixed and paraffin-embedded surgical resections. Inclusion criteria comprised histological PTC diagnosis with haematoxylin and eosin (H&E)-stained slides available for review, and evidence of distant metastasis at diagnosis or during FU. Carcinomas smaller than 1 cm in diameter, encapsulated follicular variant of PTC (EFVPTC)

and cases with anaplastic carcinoma component were excluded. Distant metastases were defined as synchronous when present at the time of initial diagnosis, metachronous when they developed during FU. Control PTC without distant metastases at diagnosis and without distant metastases for a FU period of at least 5 years (N-DM-PTC) were selected from the same files, using the same inclusion and exclusion criteria. Controls were matched for the study period (1979–2013). Frequency matching for nodal status was attempted. As it was not perfect, adjustment for the residual difference in pN distribution was applied (see Statistical analysis below). All cases were followed-up through mortality and cancer registries, pathology and outpatients' databases. FU was calculated from the date of histological diagnosis to that of last FU or to the date of patient death of disease (DOD) or of other causes (DOC). The study followed Institutional Review Board-approved protocols (AUSLRE 2017/0066257).

CLINICAL AND PATHOLOGICAL DATA

Demographic and clinical data were collected from patient charts, including age, sex, tumour site, date of last FU and state at last FU. Three pathologists (M.R., S.P., G.T.), blinded to clinical data and patient group (cases versus controls), reviewed all histology slides retrospectively. The following parameters were re-assessed and recorded: histological type, tumour–necrosis–metastasis (TNM) stage, presence of tumour necrosis (absent/focal/extensive), mitotic activity (mitoses/2 mm^2) and angioinvasion (total number of foci). All tumours were reclassified according to the 2022 WHO classification criteria for thyroid neoplasms[16] and restaged according to the latest American Joint Committee on Cancer protocols (AJCC, VIII edition, 2017).[17] Figure 1 represents histological features as they were evaluated for the study.

Tumour necrosis was defined by the presence of devitalised apoptotic cellular debris with karyorrhexis

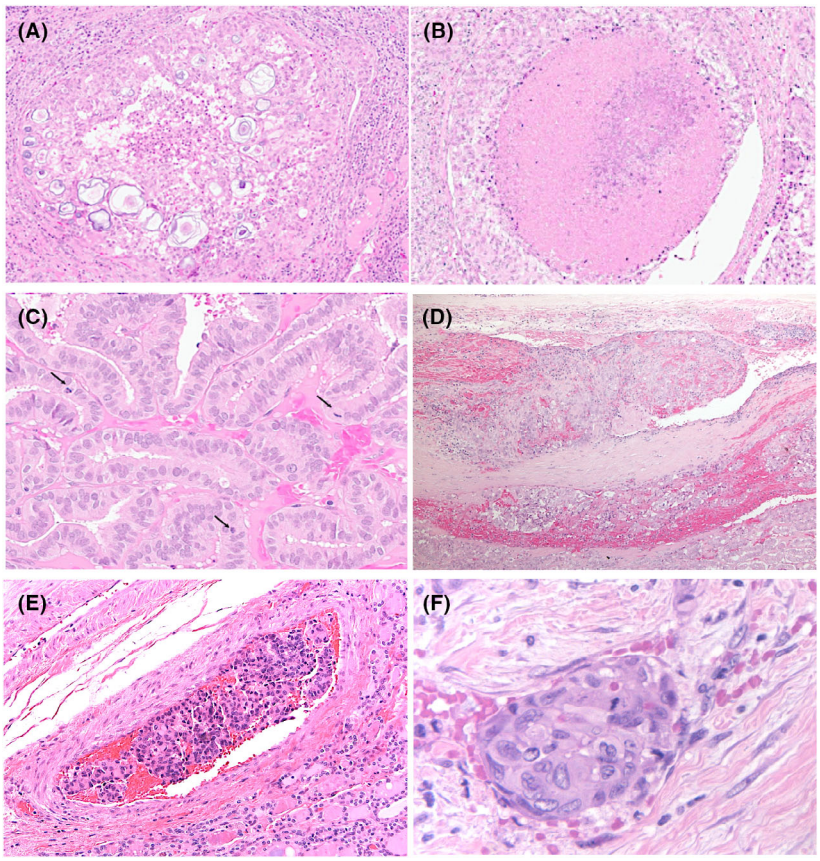


Figure 1. Histological features assessed: focal (**A**) and extensive (**B**) tumour necrosis characterised by devitalised apoptotic cellular debris with karyorrhexis and a 'dirty' appearance. Mitoses (**C**, arrows) were counted per 2 mm^2, starting from a hot-spot. Vascular invasion in large-sized (**D**), medium-sized (**E**) and small-sized vessels (**F**); intravascular tumour is attached to the vessel wall, and/or admixed with fibrin.

and a 'dirty' appearance, as found in colorectal adenocarcinoma. Infarct-like necrosis, frequently associated with fibroblastic stromal reaction or identifiable fine-needle aspiration tract, was not considered tumour necrosis.[18] Necrosis was considered extensive when at least one focus was visible at ×40, focal when it could be clearly identified only at higher magnification. Mitoses were counted per 2 mm^2 in areas showing the highest proliferative activity, so called 'hot-spot' counting.[18] Vascular (blood vessel) invasion was diagnosed according to conventional criteria for encapsulated follicular carcinoma (also applied to encapsulated oncocytic carcinoma of follicular cells[6,7]) when intravascular tumour was attached to the vessel wall or admixed with fibrin or covered by endothelium. Foci of tumour that only pushed up against vessels, tumour floating within a vessel lumen (without associated endothelium) and vascular alterations associated with the site of the prior fine-needle aspiration were excluded. Angioinvasive foci in adjacent vessels were counted separately, following current WHO recommendations.[6] Immunohistochemistry for CD31 and D2-40 was used to confirm blood vessel invasion in selected cases with doubtful features.

STATISTICAL ANALYSIS

The associations of clinical and pathological factors with the presence of metastases were assessed by means of a *t*-test for continuous variables and Fisher's test for categorical variables, using the SVY command on STATA/IC version 16. We report the frequencies and means of the variables of interest for all cases with metastases (DM-PTC), cases with synchronous metastases, cases with metachronous metastases and controls (N-DM-PTC). We weighted the controls to obtain equal frequency of cases and controls in each nodal status stratum; we applied probability weighting to maintain the original sample size in computing the variance and the precision of the estimates. Given its explorative nature, we did not fix a statistical threshold to refuse the null hypothesis and *P*-values should be interpreted as continuous variables. Receiver operating characteristic (ROC) curve and area under the curve (AUC) analyses were calculated. Cut-off values ensuring very high specificity, while maintaining good sensitivity, were considered optimal and used for univariate and multivariable analyses. Positive and negative predictive values (PPV, NPV) were computed, considering an expected 3% prevalence of metastases in PTC patients at our medical centre,[19] which is in line with historical data.[20] Logistic models were conducted to evaluate the association between clinical and pathological factors with the presence of metastases in terms of odds ratios (OR) with relative 95% confidence intervals (95% CI) using the SVY command on STATA/IC version 16. We built three models for each variable of interest: (a) univariate model; (b) multivariable model adjusted by age (as continuous variable) and pT (six classes); and (c) multivariable model adjusted by age, pT, necrosis and mitoses (fewer than five versus five or more).

Results

CLINICOPATHOLOGICAL FEATURES OF PAPILLARY CARCINOMA WITH AND WITHOUT DISTANT METASTASES

A total of 50 DM-PTC were identified in the study period (1979–2013). Among these, 16 (32.0%) presented with distant metastases (synchronous DM-PTC), while 34 (68.0%) developed distant metastases during FU (metachronous DM-PTC). Distant metastases mainly affected lung (30 cases), bone (three cases), lung and bone (four cases) and other sites (e.g. brain, liver) with or without lung or bone metastases (13 cases). The control group included 108 N-DM-PTC. Clinicopathological features of the entire cohort are summarised in Table 1.

Patients with DM-PTC were older than controls (mean age 54.8 versus 45.2 years; $P = 0.002$). There were no significant differences between cases and controls regarding sex and tumour site. Classic PTC (C-PTC) was the most common subtype, among both DM-PTC (48.0%) and N-DM-PTC (59.3%), followed by the tall-cell PTC subtype (TC-PTC). PTC subtyping was not associated with distant metastases. TC-PTC was slightly more represented in DM-PTC (34.0 versus 20.2%), especially in metachronous DM-PTC (47.1%), although the difference was not significant and compatible with random fluctuations. As expected, pathological stage was higher in DM-PTC than in controls. Similarly, DM-PTC had higher pT ($P < 0.001$) compared to controls. The correlation of necrosis (Figure 1A,B) with clinicopathological features was independent of its amount: focal versus extensive (data not shown). Therefore, it was considered a dichotomous variable (present versus absent) for statistical computation. Tumour necrosis was more frequent in the DM-PTC group (43.8 versus 5.0%, $P < 0.001$) in both synchronous (25.0%) and metachronous (53.1%) DM-PTC cases. Mitoses were more frequent in DM-PTC than in N-DM-PTC (83.7 versus 47.2%, $P < 0.001$). They were especially frequent in metachronous DM-PTC (90.1%), whereas

Table 1. Clinicopathological features of papillary carcinoma with distant metastases (all cases, synchronous and metachronous metastases) and without distant metastases

Clinicopathological features	No DM (n = 108) n (weighted %)	DM					
		All cases (n = 50)		Synchronous (n = 16)		Metachronous (n = 34)	
		n (%)	P-value	n (%)	P-value	n (%)	P-value
Age (years) mean (SD)	45.2 (16.3)	54.8 (19.7)	0.002[b]	38.1 (20.6)	0.121[b]	62.6 (13.6)	< 0.001[b]
Sex[a]	108	50		16		34	
Female	70 (63.3)	32 (64.0)	0.936[c]	9 (56.2)	0.593[c]	23 (67.6)	0.654[c]
Male	38 (36.7)	18 (36.0)		7 (43.8)		11 (32.4)	
Tumour site[a]	108	50		16		34	
Not assessed	2 (1.1)	1 (2.0)	0.908[c]	1 (6.25)	0.472[c]	0 (0)	0.778[c]
Right lobe	25 (24.2)	15 (30.0)		2 (12.5)		13 (38.2)	
Left lobe	36 (32.3)	14 (28.0)		4 (25.0)		10 (29.4)	
Isthmus	4 (2.6)	1 (2.0)		0 (0)		1 (2.9)	
Left lobe and isthmus	6 (7.0)	3 (6.0)		2 (12.5)		1 (2.9)	
Right lobe and isthmus	6 (6.1)	1 (2.0)		0 (0)		1 (2.9)	
Left and right lobes	21 (16.7)	11 (22.0)		5 (31.3)		6 (17.7)	
Left and right lobe and isthmus	8 (10.0)	4 (8)		2 (12.5)		2 (5.9)	
Histological diagnosis[a]	108	50		16		34	
C-PTC	64 (58.8)	24 (48.0)	0.394[c]	12 (75.0)	0.737[c]	12 (35.3)	0.083[c]
IFV-PTC	14 (11.7)	7 (14.0)		3 (18.8)		4 (11.8)	
TC-PTC	21 (20.2)	17 (34.0)		1 (6.3)		16 (47.1)	
ST-PTC	3 (2.3)	2 (4.0)		0 (0)		2 (5.9)	
DS-PTC	3 (3.8)	0 (0)		0 (0)		0 (0)	
ColC-PTC	1 (1.3)	0 (0)		0 (0)		0 (0)	
WL-PTC	2 (2.1)	0 (0)		0 (0)		0 (0)	
AJCC stage group[a]	108	50		16		34	
I	82 (73.4)	11 (22.0)	< 0.001	1 (6.25)	0.001	10 (29.4)	< 0.001
II	26 (26.6)	27 (54.0)		12 (75.0)		15 (44.1)	
III	0 (0)	8 (16.0)		0 (0)		8 (23.6)	
IVA	0 (0)	1 (2.0)		0 (0)		1 (2.9)	
IVB	0 (0)	3 (6.0)		3 (18.75)		0 (0)	
pT[a]	108	49		16		33	
1b	53 (44.9)	3 (6.1)	< 0.001	1 (6.25)	< 0.001	2 (6.1)	< 0.001
2	32 (29.6)	11 (22.4)		3 (18.75)		8 (24.2)	
3a	5 (6.3)	8 (16.3)		3 (18.75)		5 (15.2)	
3b	17 (18.0)	16 (32.7)		6 (37.5)		10 (30.3)	
4a	1 (1.2)	10 (20.4)		2 (12.5)		8 (24.2)	
4b	0 (0)	1 (2)		1 (6.25)		0 (0)	

Table 1. (*Continued*)

Clinicopathological features	No DM (n = 108) n (weighted %)	DM					
		All cases (n = 50)		Synchronous (n = 16)		Metachronous (n = 34)	
		n (%)	P-value	n (%)	P-value	n (%)	P-value
pN[a]	108	50		16		34	
0	22 (6.0)	3 (6)	–	0 (0)	–	3 (8.8)	–
1a	30 (24.0)	12 (24)		3 (18.75)		9 (26.5)	
1b	56 (70.0)	35 (70)		13 (81.25)		22 (64.7)	
Necrosis[a]	108	48		16		32	
No	104 (95.0)	27 (56.3)	< 0.001	12 (75.0)	0.0091	15 (46.9)	< 0.001
Yes	4 (5.0)	21 (43.8)		4 (25.0)		17 (53.1)	
Mitoses[a]	108	49		16		33	
No	54 (52.9)	8 (16.3)	< 0.001	5 (31.3)	0.114	3 (9.1)	< 0.001
Yes	54 (47.2)	41 (83.7)		11 (68.7)		30 (90.1)	
Angioinvasion[a]	108	50		16		34	
No	77 (67.3)	16 (32.0)	< 0.001	4 (25.0)	0.002	12 (35.3)	0.002
Yes	31 (32.7)	34 (68.0)		12 (75.0)		22 (64.7)	

Abbreviations: ColC-PTC, columnar cell papillary thyroid carcinoma (PTC); C-PTC, classic PTC; DM, distant metastases; DS-PTC, diffuse sclerosing PTC; IFV-PTC, infiltrative follicular variant PTC; ST-PTC, solid trabecular PTC; TC-PTC, tall-cell PTC; WL-PTC, warthin-like PTC; SD, standard deviation.

Age is reported as mean (± SD), and other data are reported as percentages. For N-DM-PTC controls only, weighted % refers to percentages statistically adjusted by nodal status (see the Statistical analysis section in Materials and methods). P-values are computed considering the weighted percentages in N-DM-PTC controls. Necrosis refers to tumour necrosis (see the Clinical and pathological data section in Materials and methods).

[a]Numbers of cases with available information for each category;
[b]t-test;
[c]Fisher's test.

the difference between synchronous DM-PTC and N-DM-PTC (68.7 versus 47.2%) was not significant. Mitotic counts ranged from 0 to 16, with median values of 4 in DM-PTC and 0.5 in N-DM-PTC (Figure 1C). Angioinvasion was present in most DM-PTC (68.0%), with both synchronous (75.0%) and metachronous (64.7%) metastases, and in a lower proportion (32.7%) of N-DM-PTC ($P < 0.001$; Figure 1D–F). Angioinvasive foci ranged from 0 to 21 in DM-PTC, with a median value of 3, whereas they ranged from 0 to 31 in N-DM-PTC, with a median value of 0.

DISEASE STATUS OF PAPILLARY CARCINOMA CASES WITH AND WITHOUT DISTANT METASTASES AT LAST FU

The FU period ranged from 1 to 40 years (mean = 10.8 years, median = 10.0 years) for DM-PTC, and from 5 to 34 years (mean = 15.3, median = 15.0 years) for N-DM-PTC. Of 16 synchronous DM-PTC patients, 10 (62.5%) were still alive: four alive with no evidence of disease (NED) and six alive with disease (AWD), one patient was DOC, while four patients were DOD, after a mean FU of 14.1 years (median FU = 14.4 years; FU range = 2–40 years). One patient with synchronous DM-PTC was lost to FU. Of 34 metachronous DM-PTC patients, 31 patients (91.2%) were DOD, while the remaining three were AWD, after a mean FU of 9.7 years (median FU = 8.5 years; FU range = 1–24 years). The latency of metastases (i.e. the time from PTC diagnosis to the development of distant metastases) in metachronous DM-PTC ranged from 1 to 21.8 years (mean = 6.0; median = 4.4 years). The likelihood of patients with metachronous DM-PTC to die of disease was higher than that of patients with synchronous DM-PTC ($P < 0.001$).

STATISTICAL PERFORMANCE OF TUMOUR NECROSIS, MITOSES AND ANGIOINVASION IN PREDICTING DISTANT METASTASES

The performance of tumour necrosis, mitoses and angioinvasion in predicting distant metastases is reported in Table 2. To identify the optimal cut-off value for mitoses and angioinvasion, ROC curves were computed and the accuracy in predicting distant metastases at different cut-offs was evaluated (Figures 2 and 3).

Tumour necrosis

Necrosis showed a sensitivity of 43.8% for DM-PTC overall; 25.0% for synchronous DM-PTC and 53.1% for metachronous DM-PTC. Specificity was 95.4%. Assuming a 3% prevalence of distant metastases,[19,20] other diagnostic performance metrics were as follows: PPV of 22.6%, NPV of 98.2% for DM-PTC overall; PPV of 14.3%, NPV of 97.6% for synchronous DM-PTC; and PPV of 26.2%, NPV of 98.5% for metachronous DM-PTC (Table 2).

Mitoses

The ROC curves demonstrated values of AUC of 0.78 for DM-PTC overall (Figure 2A), 0.63 and 0.85 for synchronous (Figure 2B) and metachronous (Figure 2C), respectively. An optimal cut-off value of five or more mitoses/2 mm^2 was determined to effectively predict distant metastases. At this threshold, sensitivity was 42.9% and specificity of 97.2% for DM-PTC overall. Indeed, a threshold of four mitoses showed low specificity (93.5 versus 97.2%), whereas a threshold of six ensured too low a sensitivity (38.8 versus 42.9%), with little gain of specificity (98.2 versus 97.2%). The five mitoses cut-off allowed correct classification in 80.3% of DM-PTC overall, in 87.1% of synchronous DM-PTC and 87.2% of metachronous DM-PTC (Figure 2). Assuming a 3% prevalence of distant metastases,[19,20] we estimated a PPV of 32.3% and NPV of 98.2% for DM-PTC overall (Table 2). With the same cut-off, considering only synchronous DM-PTC, sensitivity was 18.8%, PPV 17.3% and NPV 97.5%; considering only metachronous DM-PTC, sensitivity was 54.6%, PPV 37.8% and NPV 98.6% (Table 2).

Tumour necrosis and mitoses

When combining the information regarding necrosis and mitoses and considering as positive cases with necrosis OR of five or more mitoses, sensitivity increased for DM-PTC overall (56.6%), as well as for both synchronous (31.3%) and metachronous (68.8%) DM-PTC. NPV also increased for DM-PTC overall (98.6%), as well as for synchronous (97.8%) and metachronous (99.0%) DM-PTC. In contrast, specificity decreased (to 93.51%) as well as PPV (DM-PTC overall = 21.1%; synchronous DM-PTC = 13.0%; metachronous DM-PTC = 24.7%; Table 2).

Angioinvasion

The AUC value was 0.75 for DM-PTC overall (Figure 3A), 0.79 and 0.73 for synchronous (Figure 3B) and metachronous (Figure 3C) DM-PTC, respectively. An optimal cut-off value of five or more foci of vascular invasion was determined to effectively predict distant metastases. At this cut-off value, sensitivity and specificity were 36.2 and 91.7% for DM-PTC overall. Indeed, a threshold of four angioinvasive foci showed low specificity (88.0 versus 91.7%), whereas a threshold of six ensured too low a sensitivity (27.7 versus 36.2%), with little gain of specificity (92.6%). The five foci of vascular invasion cut-off allowed correct classification in 74.8% of DM-PTC overall, in 83.1% of synchronous DM-PTC and 80.6% of metachronous DM-PTC (Figure 3). Assuming a 3% prevalence of distant metastases,[19,20] we estimated a PPV of 11.8% and NPV of 97.9% for DM-PTC overall (Table 2). With the same cut-off, considering only synchronous DM-PTC, sensitivity was 25.0%, PPV 8.5% and NPV 97.5%; considering only metachronous DM-PTC, sensitivity was 41.9%, PPV 13.5% and NPV 98.1% (Table 2).

PROGNOSTIC IMPACT OF TUMOUR NECROSIS, MITOTIC ACTIVITY AND ANGIOINVASION AFTER UNIVARIATE AND MULTIVARIABLE ANALYSES

Tumour necrosis, considered as a dichotomous variable, remained more frequent in DM-PTC, both synchronous and metachronous, than in N-DM-PTC, also after adjustment for pT and age (Table 3). With a cut-off value at five or more, mitoses were more frequent in DM-PTC than in controls [42.9 versus 2.3%; OR = 31.5 (95% CI = 7.6–131.0), $P < 0.001$], both in synchronous [18.8%, OR = 9.7 (95% CI = 1.6–60.2), $P = 0.015$] and metachronous [54.6%, OR = 50.5 (95% CI = 11.5–221.4), $P < 0.001$] DM-PTC, and also remained significant after adjustment for pT and age (Table 3). Angioinvasion was more frequent in DM-PTC than in controls (36.2 versus 9.4%), in bothsynchronous (25.0%) and metachronous (41.9%) DM-PTC, but the difference was strong only in the univariate model [OR = 5.5 (95% CI = 2.1–14.1), $P < 0.001$] and after adjustment by

Table 2. Statistical performance of tumour necrosis, mitoses and angioinvasion in predicting distant metastases

Histological features	Sensitivity (95% CI)			Specificity (95% CI)	PPV (95% CI)[c]			NPV (95% CI)[c]		
	All cases	Synchronous	Metachronous		All cases	Synchronous	Metachronous	All cases	Synchronous	Metachronous
Necrosis[a]	43.8 (29.5–58.8)	25.0 (7.3–52.4)	53.1 (34.7–71.0)	95.4 (89.5–98.5)	22.6 (10.5–42.2)	14.3 (4.8–35.8)	26.2 (12.4–47.0)	98.2 (97.7–98.6)	97.6 (96.9–98.2)	98.5 (97.8–99.0)
Mitoses[b]	42.9 (28.8–57.8)	18.8 (4.1–45.7)	54.6 (36.4–71.9)	97.2 (92.1–99.4)	32.3 (27.8–37.3)	17.3 (4.4–48.6)	37.8 (16.0–66.0)	98.2 (97.9–98.5)	97.5 (96.8–98.0)	98.6 (98.0–99.0)
Necrosis + mitoses[b]	56.3 (41.2–70.5)	31.3 (11.0–58.7)	68.8 (50.0–83.9)	93.5 (87.1–97.4)	21.1 (11.2–36.4)	13.0 (5.1–29.3)	24.7 (13.4–41.1)	98.6 (98.0–99.0)	97.8 (96.9–98.4)	99.0 (98.3–99.4)
Angioinvasion[b]	36.2 (22.7–51.5)	25.0 (7.3–52.4)	41.9 (24.6–60.9)	91.7 (84.7–96.1)	11.8 (9.9–14.2)	8.5 (3.1–21.0)	13.5 (6.9–24.8)	97.9 (97.6–98.2)	97.5 (96.7–98.1)	98.1 (97.4–98.64)

Abbreviations: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
[a]Necrosis refers to tumour necrosis (see the Clinical and pathological data section in Materials and methods);
[b]cut-offs for mitoses and angioinvasion are ≥ 5;
[c]positive and negative predictive values are calculated according to an expected 3% prevalence of metastases in papillary carcinoma patients.[18,19]

age and pT in metachronous DM-PTC cases [OR = 3.4 (95% CI = 1.0–11.0), $P = 0.044$], while it almost disappeared after adjustment for mitoses and tumour necrosis [OR = 1.2 (95% CI = 0.3–4.9), $P = 0.782$; Table 3].

DHGTCs in the study cohort

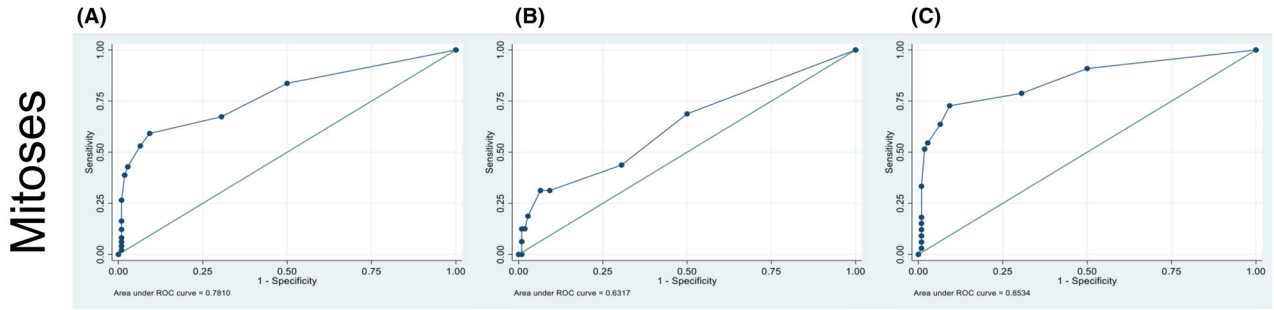
We demonstrate (see above) the relevance of tumour necrosis and/or mitoses with five or more mitoses per 2 mm^2 cut-off to predict distant metastases in PTC. These are the criteria proposed by the current WHO classification to identify tumours of follicular cells with intermediate prognosis, such as DHGTC. In our cohort, PTC reclassified as DHGTC were 27 among DM-PTC (seven cases with necrosis, four cases because of mitotic counts above cut-off, 14 with both) and six among N-DM-PTC (three cases with necrosis, two cases because of mitotic counts above cut-off, one with both) ($P < 0.001$).

Discussion

In this case–control study, we analysed the ability of tumour necrosis, mitotic activity and angioinvasion to predict distant metastases by comparing DM-PTC with a group of N-DM-PTC that did not develop distant metastases after a careful FU of approximately 15 years. Distant metastases are generally uncommon in PTC, but represent the main feature linked to fatal outcome.[1] They may be present at the time of initial diagnosis (synchronous distant metastases) or develop later (metachronous distant metastases). Based upon our results, patients with synchronous distant metastases are very different from those who develop them after initial diagnosis. Patients with synchronous metastases are younger than controls, usually have classic PTC and have better prognosis. Patients with metachronous distant metastases are older than controls, are more frequently diagnosed with tall-cell PTC and have a worse prognosis. These findings are supported by observations of other investigators showing that metachronous metastases are those with the greatest impact upon patient prognosis.[21,22]

In our study, tumour necrosis, mitotic activity and angioinvasion correlate with distant metastases while PTC subtyping does not, confirming the seminal observations of Akslen and LiVolsi in their study from 2000.[23] The optimal cut-off for mitotic count is five or more mitoses per 2 mm^2. It is the optimal value correlating with distant metastases in PTC. This

Mitoses

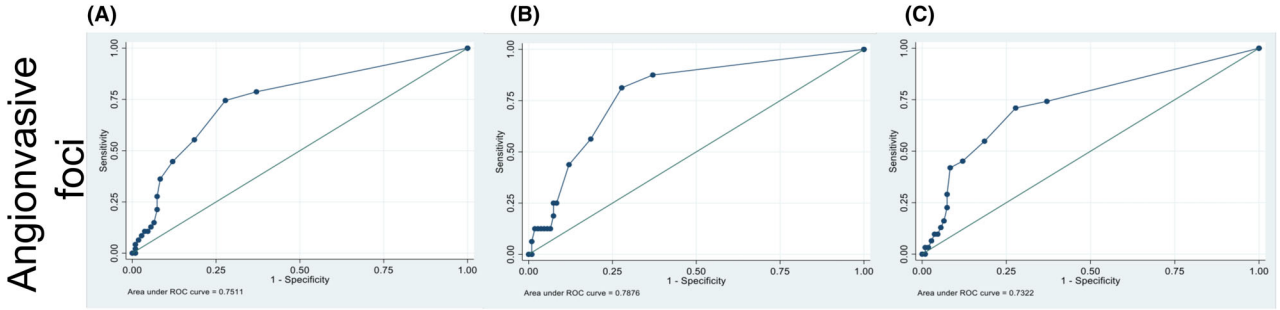


Cut off	Sensitivity	Specificity	Classified	Sensitivity	Specificity	Classified	Sensitivity	Specificity	Classified
≥ 0	100.00%	0.00%	32.21%	100.00%	0.00%	12.90%	100.00%	0.00%	23.40%
≥ 1	83.67%	50.00%	60.51%	68.75%	50.00%	52.42%	90.91%	50.00%	59.57%
≥ 2	67.35%	69.44%	68.79%	43.75%	69.44%	66.13%	78.79%	69.44%	71.63%
≥ 3	59.18%	90.74%	80.89%	31.25%	90.74%	83.06%	72.73%	90.74%	86.52%
≥ 4	53.06%	93.52%	80.89%	31.25%	93.52%	85.48%	63.64%	93.52%	86.52%
≥ 5	42.86%	97.22%	80.25%	18.75%	97.22%	87.10%	54.55%	97.22%	87.23%
≥ 6	38.78%	98.15%	79.62%	12.50%	98.15	87.10%	51.52%	98.15%	87.23%
≥ 7	26.53%	99.07%	76.43%	/	/	/	33.33%	99.07%	83.69%
≥ 8	16.33%	99.07%	73.25%	12.50%	99.07%	87.90%	18.18%	99.07%	80.14%
≥ 9	12.24%	99.07%	71.97%	6.25%	99.07%	87.10%	15.15%	99.07%	79.43%
≥ 10	8.16%	99.07%	70.70%	/	/	/	12.12%	99.07%	78.72%
≥ 13	6.12%	99.07%	70.06%	/	/	/	9.09%	99.07%	78.01%
≥ 14	4.08%	99.07%	69.43%	/	/	/	6.06%	99.07%	77.30%
≥ 16	2.04%	99.07%	68.79%	0.00%	99.07%	86.29%	3.03%	99.07%	76.60%
> 16	0.00%	100.00%	68.79%	0.00%	100.00%	87.10%	0.00%	100.00%	76.60%

Figure 2. Receiver operating characteristic (ROC) curves to define optimal cut-off value for mitotic activity. Areas under the curve (AUCs) for the performance of mitotic activity in predicting distant metastases: all cases with distant metastases (**A**), cases with synchronous (**B**) and metachronous distant metastases (**C**). The lower part of the figure shows the detailed report of sensitivity and specificity for each ROC curve. Optimal cut-off values, ensuring high specificity while maintaining good sensitivity, are highlighted in yellow.

confirms the validity of the cut-off originally proposed by Hiltzik *et al.* at Memorial Sloan Kettering Cancer Center to define high-grade non-anaplastic carcinoma of follicular cells.[3,5] Interestingly, analysis of the ROC curves for mitotic activity shows how mitoses correlate much better with metachronous distant metastases (AUC = 0.85) than with synchronous metastases (AUC = 0.63). This is consistent with mitotic activity being an important grade parameter, as it provides a useful measure of the biological aggressiveness intrinsic to the tumour capable of predicting future metastatic spread. The optimal cut-off for angioinvasion is at five or more angioinvasive foci. This cut-off has high specificity with good sensitivity. It is the optimal value correlating with distant metastases in PTC. The cut-off value is little different from the four or more angioinvasive foci considered prognostically relevant for encapsulated angioinvasive follicular[6] and oncocytic[7] thyroid carcinoma. The first report demonstrating the importance of microscopic vascular invasion in thyroid cancer was by Graham in 1924.[24] Unlike the case of encapsulated carcinomas of follicular cells, relatively few studies have focused on the prognostic role of angioinvasion in PTC, with conflicting results. Some have shown that vascular invasion is of prognostic value,[11,14] others have shown the opposite,[12] some have shown that the prognostic value is not independent of other clinicopathological features[15] and some indicated that it is prognostically useful only if strict criteria are utilised.[13] Analysis of the ROC curves for vascular invasion shows how it correlates better with synchronous distant metastases (AUC = 0.79) than with metachronous metastases (AUC = 0.73). This is consistent with vascular invasion being primarily a stage parameter measuring the microscopic extent of tumour infiltration.

The definition of effective cut-offs for mitotic activity and angioinvasion has allowed us to examine statistical measures of performance. Necrosis, mitotic activity and angioinvasion have high statistical performance. Mitotic activity and tumour necrosis have high sensitivity and negative predictive power for all cases with metastases, including those with metachronous metastases. If tumour necrosis is combined with mitotic counts above the five or more per 2 mm^2 cut-off, sensitivity and negative predictive power are further increased, with values approaching



Cut off	Sensitivity	Specificity	Classified	Sensitivity	Specificity	Classified	Sensitivity	Specificity	Classified
≥ 0	100.00%	0.00%	30.32%	100.00%	0.00%	12.90%	100.00%	0.00%	22.30%
≥ 1	78.72%	62.96%	67.74%	87.50%	62.96%	66.13%	74.19%	62.96%	65.47%
≥ 2	74.47%	72.22%	72.90%	81.25%	72.22%	73.39%	70.97%	72.22%	71.94%
≥ 3	55.32%	81.48%	73.55%	56.25%	81.48%	78.23%	54.84%	81.48%	75.54%
≥ 4	44.68%	87.96%	74.84%	43.75%	87.96%	82.26%	45.16%	87.96%	78.42%
≥ 5	36.17%	91.67%	74.84%	25.00%	91.67%	83.06%	41.94%	91.67%	80.58%
≥ 6	27.66%	92.59%	72.90%	25.00%	92.59%	83.87%	29.03%	92.59%	78.42%
≥ 7	21.28%	92.59%	70.97%	18.75%	93.52%	83.06%	22.58%	92.59%	76.98%
≥ 8	14.89%	93.52%	69.68%	12.50%	94.44%	83.06%	16.13%	93.52%	76.26%
≥ 9	12.77%	94.44%	69.68%	12.50%	95.37%	83.87%	12.90%	94.44%	76.26%
≥ 10	10.64%	95.37%	69.68%	12.50%	96.30%	84.68%	9.68%	95.37%	76.26%
≥ 13	10.64%	96.30%	70.32%	12.50%	97.22%	85.48%	9.68%	96.30%	76.98%
≥ 14	8.51%	97.22%	70.32%	12.50%	98.15%	86.29%	6.45%	97.22%	76.98%
≥ 15	6.38%	98.15%	70.32%	12.50%	99.07	87.10%	3.23%	98.15%	76.98%
≥ 17	4.26%	99.07%	70.32%	/	/	/	3.23%	99.07%	77.70%
≥ 21	2.13%	99.07%	69.68%	6.25%	99.07%	87.10%	/	99.07%	/
≥ 31	0.00%	99.07%	69.03%	0.00%	99.07%	86.29%	0.00%	100.00%	76.98%
> 31	0.00%	100.00%	69.68%	0.00%	100.00%	87.10%	0.00%	100.00%	77.70%

Figure 3. Receiver operating characteristic (ROC) curves to define optimal cut-off value for angioinvasive foci. Areas under the curve (AUCs) for the performance of angioinvasion in predicting distant metastases: all cases with distant metastases (**A**), cases with synchronous (**B**) and metachronous distant metastases (**C**). The lower part of the figure shows the detailed report of sensitivity and specificity for each ROC curve. Optimal cut-off values, ensuring high specificity while maintaining good sensitivity, are highlighted in yellow.

100%. This indicates how the absence of necrosis and mitotic activity above cut-off is an extremely reliable indicator that a tumour without distant metastases at presentation will not develop in the future. As these metachronous metastases are those with the strongest link to tumour-related death, in this as well as in other series,[21,22] it is also therefore very likely that patients will not die of disease, if their tumors lack necrosis and mitotic activity above cut-off. Despite the excellent statistical performance of angioinvasion in correlating with distant metastases, the association is not independent of age, pT, tumour necrosis and mitoses above cut-off. These results are in line with the study of Wreesmann *et al.*, which showed how angioinvasion correlates with distant metastases and distant recurrence-free survival at univariate, but not after multivariable analysis.[15]

Our study has some limitations. It is monocentric and retrospective, although based upon a sufficiently large cohort of cases from a large medical centre in northern Italy. Systematic CD31 and D2-40 immunohistochemical analysis to identify vascular invasion and to distinguish blood vessel invasion from lymphatic invasion was not performed on all cases; it is therefore possible that some foci of vascular invasion were missed and that some angioinvasive foci represented lymphatic, as opposed to blood vessel, invasion. Indeed, it may be extremely difficult to distinguish between small-sized blood vessels and lymphatic vascular spaces, but Puga *et al.* have recently demonstrated that angioinvasion, including invasion of lymphatic vessels only, is associated with a higher risk of persistent/recurrent disease in otherwise low-risk PTC. In that study, multivariable analysis was not performed due to the low number of persistent/recurrent disease events.[14]

In conclusion, this study strongly supports PTC grading, validating the current WHO classification scheme proposal. To the best of our knowledge, we report here for the first time an analysis of the statistical performance of WHO grade parameters (tumour necrosis and mitoses) and of vascular invasion for prediction of distant metastases in PTC. This is also the first study to statistically define optimal cut-off

Table 3. Prognostic impact of tumour necrosis, mitotic activity and angioinvasion after univariate and multivariable logistic models (model A, univariate model; model B, multivariable model adjusted by age and pT; model C, multivariable model adjusted by age, pT, necrosis, and mitoses)

Histological features	No DM Weighted %	DM, all cases %	Model A		Model B		Model C	
			OR (95% IC)	*P*-value	OR (95% IC)	*P*-value	OR (95% IC)	*P*-value
Necrosis[a]	108	48						
No	95.0	56.3	14.8 (4.6–47.4)	< 0.001	11.1 (3.2–38.4)	< 0.001		
Yes (extensive or focal)	5.0	43.8						
Mitoses (2 mm^2)[a]	108	49						
<5	97.7	57.1	31.5 (7.6–131.0)	< 0.001	25.3 (4.3–149.2)	< 0.001		
≥5	2.3	42.9						
Mitoses and necrosis[a]	108	48						
Absent	93.9	43.8	19.9 (6.9–57.3)	< 0.001	19.8 (5.1–77.0)	< 0.001		
Any	6.1	56.3						
Foci of vascular invasion[a]	108	47						
<5	90.6	63.8	5.5 (2.1–14.1)	< 0.001	2.2 (0.7–6.7)	0.149	1.2 (0.3–4.9)	0.782
≥5	9.4	36.2						

Histological features	No DM Weighted %	Synchronous DM %	Model A		Model B		Model C	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Necrosis[a]	108	16						
No	95.1	75.0	6.3 (1.4–29.3)	0.019	12 (1.6–90.8)	0.017		
Yes (extensive or focal)	5.0	25.0						
Mitoses (2 mm^2)[a]	108	16						
< 5	97.7	81.3	9.7 (1.6–60.2)	0.015	29.5 (2.3–265.8)	0.009		
≥ 5	2.3	18.8						
Mitoses and necrosis[a]	108	16						
Absent	93.9	68.8	7 (1.7–28.3)	0.006	14.2 (2.0–103.7)	0.009		
Any	6.1	31.3						
Foci of vascular invasion[a]	108	16						

Table 3. (*Continued*)

Histological features	No DM Weighted %	Synchronous DM %	Model A		Model B		Model C	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
< 5	90.6	75.0	3.2 (0.8–12.5)	0.091	0.8 (0.2–4.3)	0.824	0.35 (0.04–2.7)	0.31
≥ 5	9.4	25.0						

Histological features	No DM Weighted %	Metachronous DM %	Model A		Model B		Model C	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Necrosis[a]	108	32						
No	95.1	46.9	21.5	< 0.001	13.6	< 0.001		
Yes (extensive or focal)	5.0	53.1	(6.3–74.0)		(3.3–55.6)			
Mitoses (2 mm^2)[a]	108	33						
< 5	97.7	45.5	50.5	< 0.001	20	0.001		
≥ 5	2.3	54.6	(11.5–221.4)		(3.2–124.1)			
Mitoses and necrosis[a]	108	32						
Absent	93.9	31.3	34.0	< 0.001	19.3	< 0.001		
Any	6.1	68.8	(10.6–109.3)		(5.0–74.4)			
Foci of vascular invasion[a]	108	31						
< 5	90.6	58.1	7.0	<0.001	3.4	0.044	2.2	0.305
≥ 5	9.4	41.9	(2.5–19.5)		(1.0–11.0)		(0.5–10.3)	

Data are reported as percentages. For non-metastatic PTC (N-DM-PTC) weighted % refers to percentages statistically adjusted by residual nodal status (see Statistical analysis section).

Abbreviations: DM, distant metastases; OR, odds ratio; CI, confidence interval.

[a]Numbers of cases with available information for each category.

values for mitoses and angioinvasion that can be specifically applied to PTC. Tumour necrosis and mitotic activity are excellent histological predictors of distant metastases, both synchronous and metachronous. Angioinvasion is also strongly associated with distant metastases, but the association is not independent after multivariable analysis.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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