WHOLE TUMOR CAPSULE IS PROGNOSTIC OF A VERY GOOD OUTCOME IN THE CLASSICAL VARIANT OF PAPILLARY THYROID CANCER

Carlotta Giani[1], Liborio Torregrossa[2], Ramone Teresa[1], Romei Cristina[1], Antonio Matrone[1], Eleonora Molinaro[1], Laura Agate[1], Gabriele Materazzi[3], Paolo Piaggi[4], Clara Ugolini[2], Fulvio Basolo[2], Raffaele Ciampi[1], Rossella Elisei[1]

Carlotta Giani [1], MD - carlottagiani@hotmail.com (ORCID: 0000-0002-5248-4436)

Liborio Torregrossa [2], PhD - libo.torregrossa@gmail.com

Teresa Ramone [1], PhD - teresa.ramone@hotmail.it

Cristina Romei, PhD – cristina.romei@unipi.it

Antonio Matrone [1], PhD – anto.matrone@yahoo.com

Eleonora Molinaro [1], PhD - elemoli@hotmail.com

Laura Agate [1], MD – laura.agate@virgilio.it

Gabriele Materazzi [3], MD – gabriele.materazzi@unipi.it

Paolo Piaggi [4], PhD – paolo.piaggi@gmail.com

Clara Ugolini [2], PhD – clara.ugolini@gmail.com

Fulvio Basolo [2], MD - fulvio.basolo@unipi.it

Raffaele Ciampi[1], PhD - raffaele.ciampi@unipi.it

Rossella Elisei[1], MD – rossella.elisei@med.unipi.it (ORCID: 0000-0002-5333-9257)

[1] *Department of Clinical and Experimental Medicine, Unit of Endocrinology, University Hospital of Pisa – Via Paradisa 2, 56124, Pisa, Italy*

[2] *Department of Surgical, Medical, Molecular Pathology and Critical Area, Pathology Unit, University Hospital of Pisa – Via Savi 10, 56126, Pisa, Italy*

[3] *Department of Surgical, Medical, Molecular Pathology and Critical Area, Surgery Unit, University Hospital of Pisa – Via Paradisa 2, 56124, Pisa, Italy*

[4] *Department of Information Engineering, University of Pisa, 56122 Pisa, Italy*

Correspondence author and person to whom reprint request should be addressed:

Rossella Elisei MD, Department of Clinical and Experimental Medicine, Unit of Endocrinology

University of Pisa

Via Paradisa 2 - 56124 Pisa, Italy

e-mail: rossella.elisei@med.unipi.it

Tel. 0039-050-995120

Fax: 0039-050-578772

ORCID: 0000-0002-5333-9257

Funding: The study was partially supported by a grant of Ministero dell'Istruzione, dell'Università e della Ricerca Italiano (MIUR, investigator grant 2017 project code PRIN 2017YTWKWH) for the continuing research support.

Author disclosure: The authors have nothing to disclose.

ABSTRACT

Context: Tumour capsule integrity is becoming a relevant issue to predict the biological behaviour of human tumours, including thyroid cancer.

Aim: To verify if a whole tumour capsule in the classical variant of PTC (CVPTC) could have a predictive role of a good outcome as for follicular variant (FVPTC).

Methods: FVPTC (n=600) and CVPTC (n=554) cases, were analysed. We distinguished encapsulated-FVPTC (E-FVPTC) and encapsulated-CVPTC (E-CVPTC) and, thereafter, invasive (Ei-FVPTC and Ei-CVPTC) and non-invasive (En-FVPTC and En-CVPTC) tumours, according to the invasion or integrity of tumour capsule, respectively. Cases without tumour capsule were indicated as invasive-FVPTC (I-FVPTC) and invasive-CVPTC (I-CVPTC). Sub-group of each variant was evaluated for *BRAF* mutations.

Results: E-FVPTC was more frequent than E-CVPTC ($p<0.0001$). No differences were found between En-FVPTC and En-CVPTC or between Ei-FVPTC and Ei-CVPTC. After 18 years of follow-up, a greater number of not-cured cases were observed in Ei-CVPTC with respect to Ei-FVPTC, but not in En-CVPTC to En-FVPTC. Multivariate clustering analysis showed that En-FVPTC, En-CVPTC, and Ei-FVPTC have similar features but different from I-FVPTC and I-CVPTC and, to a lesser extent, from Ei-CVPTC. 177/614 (28.8%) cases were $BRAF^{V600E}$-mutated and 10/614(1.6%) carried *BRAF*-rare alterations. Significantly higher rate of En-CVPTC (22/49,44.9%) than En-FVPTC (15/195,7.7%) ($p<0.0001$) were $BRAF^{V600E}$-mutated.

Conclusions: En-CVPTC is less prevalent than En-FVPTC. However, they have a good clinical/ pathological behavior comparable to En-FVPTC. This finding confirms the good prognostic role of a whole tumour capsule also in CVPTC. New nomenclature for En-CVPTC, similar to that introduced for En-FVPTC (i.e, NIFTP) could be envisaged.

Key words: Whole tumour capsule, papillary thyroid cancer, follicular variant, classical variant.

Introduction

The increasing incidence of papillary thyroid carcinoma (PTC) over the last decades is due to many factors, and in particular, it is attributable to a more clinical surveillance which leads to improved detection of small PTCs (1–3). Nevertheless, this increasing incidence is also attributable to the evolution of histological criteria for certain variants, particularly the follicular variant of PTC (FVPTC) (4–8). The tumour capsule in FVPTC has been traditionally considered an important feature and has been used to classify FVPTC into two different sub-variants: the encapsulated FVPTC (E-FVPTC) and infiltrative FVPTC (I-FVPTC), according to the presence or absence of a fibrous capsule that surrounded the tumour, respectively (9–14). In agreement with the indolent behaviour of some E-FVPTCs to others with capsular and vascular invasion prone to metastasize, some physician proposed to further divide the E-FVPTC into two different groups: the noninvasive (En-FVPTC) and invasive E-FVPTC (Ei-FVPTC) based on the presence or absence of capsular and/or vascular invasion (15). Emerging data regarding the indolent nature of the En-FVPTC (9,10) led to the problem of the overtreatment of these patients. Based on this, the need to reclassify the En-FVPTC as an entity closer to the follicular adenoma is emerging and has brought an international working group of Endocrine Pathologists and other physicians to critically re-examine this entity. After an accurate revision of more than one-hundred cases of En-FVPTC, only surgically treated and with no evidence of disease after 13 years of follow-up, they decided to remove the term “carcinoma” and named these tumours as “Noninvasive follicular thyroid neoplasm with papillary like nuclear features” with the acronym of NIFTP (16). Over the last years, other criteria have been established to better define this new entity (17) that has been finally included in the 2017 World Health Organization Classification of Tumours of Endocrine Organs book (18). Therefore, nowadays the pathologists and clinicians paid a specific attention to the presence of a whole tumour capsule in the FVPTC to identify NIFTP cases that are now considered as ‘premalignant’ lesion and do not require further treatments. This new concept is going to have a great impact on the clinical scenario since it has been recently demonstrated that there is a relevant decline of FVPTC incidence after 20 years of continuous increase (19).

Recently, we demonstrated that, in a big monocentric series of PTC (FVPTC and CVPTC), the presence of an encapsulated tumour, without evidence of the invasion of the tumour capsule, was an independent prognostic factor for a good prognosis, independently from the histological variant (20). Based on this observation, we planned this study to verify if the presence of a whole tumour capsule could have a similar pathological and clinical significance also in a classical variant of PTC (CVPTC) and if the encapsulated noninvasive CVPTC (En-CVPTC) could be better considered a premalignant lesion like NIFTP.

Patients and Methods

Patient Population

We reviewed the pathological data of 1154 consecutive patients with PTC who underwent total thyroidectomy between 1999 and 2006, divided into 2 different groups according to the histological variant: 600 follicular variant of PTC (FVPTC) and 554 classical variants of PTC (CVPTC). All patients signed informed consent to share personal data for research purposes. The study was approved by the local Ethical Committee (CEAVNO, Comitato Etico Area Vasta Nord Ovest) (Prot. n. 57880, December 7, 2020).

The epidemiological, clinical and pathological data of all patients were collected in an electronic database and reviewed for this study. To consider the outcome of patients we used the response to therapy criteria previously reported (20,21) with a slight modification since we merged those cases with a biochemical persistence or an indeterminate response in a single group named “biochemically affected”.

Histology revision

Cases were classified according the WHO classifications available in the time frame (1999-2006) during which patients were operated (22,23). The presence and the integrity of the tumor capsule was revised by two independent pathologists (L.T. and C. U.) to distinguish those cases with a whole tumour capsule and those with capsular invasion. The presence of a whole tumour capsule was defined as the

presence of a distinctive fibrous capsule around the lesion without evidence of capsular and/or vascular invasion (Fig. 1).

According to these criteria, cases with a tumour capsule were indicated as either encapsulated FVPTC (E-FVPTC) or encapsulated CVPTC (E-CVPTC). Both groups were further divided into two sub-groups named invasive (Ei-FVPTC and Ei-CVPTC) and noninvasive (En-FVPTC and En-CVPTC) tumours, according to the presence of a tumour capsule invasion or a whole tumour capsule, respectively. The remaining cases, those cases without tumour capsule, were indicated as invasive FVPTC (I-FVPTC) and invasive CVPTC (I-CVPTC).

Microdissection and DNA extraction

Serial 5-m sections were taken from formalin-fixed, paraffin-embedded (FFPE) tissues. The last section was stained with hematoxylin-eosin; the tumour area was marked, and the percentage of tumour cells was estimated by an experienced pathologist. The tumour tissue was manually microdissected to obtain tumour cell enrichment by excluding lymphocytic infiltration, hemorrhagic area, and fibrous tissue. Deparaffinized samples were lysed and digested with proteinase K. DNA was extracted from tumour tissues utilizing commercial kits (Puregene; Gentra Systems, Minneapolis, MN, USA) and its concentration was measured using an UV spectrophotometer (SmartSpec Plus Spectrophotometer, BioRad, Hercules, CA) and was stored at 4°C before use.

BRAF gene analysis

For *B-RAF* mutations analysis in exon 15 the DNA was PCR amplified using specific intronic primers according to the following protocol: 35 cycles of denaturation (94°C for 1 min), annealing (60 °C for 1 min) and extension (72 °C for 2 min) on a fast thermal cycler (Hybaid, Basingstoke, Middx, UK). The primer sequences were as follows: 5-TTCATGAAGACCTCACAGTAAAAA-3 (forward), and 5-CCACAAAATGGATCCAGACA-3 (reverse). After purification, PCR products were directly sequenced. An

aliquot of 3–10 ng/100 bp purified DNA and 3.2 pmol of either the forward or reverse primer was used in standard cycle sequencing reactions with ABI PRISM big dye terminators and run on an ABI PRISM 310 genetic analyser (PE Applied Biosystems, Foster City, CA, USA). The cycle-sequencing conditions consist of 25 cycles of 96 °C for 30 s, 50 °C for 15 s and 60 °C for 4 min.

Statistical analysis

Categorical variables are presented as frequencies and rates. Continuous variables are expressed as mean±SD if normally distributed or as median and interquartile range if not. The Kolmogorov-Smirnov test was used to assess normality distribution.

Statistical tests used to assess differences between groups included Student's t-test for Gaussian variables, χ^2 test for categorical variables and Wilcoxon rank-sum test for quantitative variables with skewed distribution.

Multiple correspondence analysis (MCA) was conducted to identify groups of correlated variables and to visualize clusters of patients with similar clinical characteristics by calculating the underlying dimensions based on the chi-square distance between variables. The following binary variables were included in this multivariate analysis: gender, age (< 55 vs. ≥ 55 years), tumour size (≤2 vs >2 cm), thyroid capsule invasion, extrathyroidal extension, focality, lymph node metastasis, and outcome, while the E-PTC classification was used as supplementary variable.

A p-value less than 0.05 was considered statistically significant. All statistical analysis was carried out with JMP 9.0.1 software package for Mac (SAS Institute, Inc, Cary, NC) and SPSS Statistics for Mac, Version 20.0 (IBM Corporation, Armonk, NY).

Results

Tumour capsule: overall prevalence and differences between CVPTC and FVPTC

As shown in Fig 2, we found that 475/1154 (41.2%) had a tumour capsule (E-PTC) while the remaining 679/1154 (58.8%) did not (I-PTC), with a statistically significant higher prevalence of encapsulated cases in FVPTC (E-FVPTC) respect to encapsulated CVPTC (E-CVPTC) (55.8% vs 25.3%, respectively; p<0.0001). Thereafter, we looked at the tumour capsule integrity or invasion in the 2 groups of E-FVPTC and E-CVPTC (i.e., En-FVPTC [82.4%] *vs* En-CVPTC [89.3%] and Ei-FVPTC [17.6%] *vs* Ei-CVPTC [10.7%]) and we did not find any difference of prevalence between the groups (p=0.06).

Different epidemiological, clinical and pathological features: En-FVPTC vs En-CVPTC and Ei-FVPTC vs Ei-CVPTC

As shown in Table 1, when we compared the epidemiological, clinical-pathological features of En-FVPTC vs En-CVPTC and Ei-FVPTC vs Ei-CVPTC we did not find any statistically significant difference.

After a median follow-up of 18 years (mean 17 ± 2 years, range 15-20) we observed a few cases of not cured patients in both En-PTC and Ei-PTC (Table 2), mostly with the persistent biochemical disease (12/16) and only 4/16 with the structural disease. While no difference was observed in the outcome of patients with En-FVPTC and En-CVPTC, a greater and statistically significant number of not cured cases in the Ei-CVPTC with respect to Ei-FVPTC were found (Table 1, last line).

Invasive FVPTC (I-FVPTC) vs invasive CVPTC (I-CVPTC)

As shown in Figure 2, 679/1154 (58.8%) of our PTC did not show any capsule and 74/475 (15.6%) of those with a capsule showed its infiltration. All together 65.3% of our PTC, which include only classical and follicular variant, but not the other aggressive variants, are invasive.

According to the different variant, 265/600 (44.2%) FVPTC and 414/554 (74.7%) CVPTC did not have a tumour capsule and they were named I-FVPTC and I-CVPTC, respectively.

Eleven out of 414 (2.6%) patients belonging to the I-CVPTC group were lost at follow-up after surgical treatment, and they were excluded from the statistical analysis for the outcome. No patients were lost at follow-up in the I-FVPTC group.

As shown in Table 3, when we analyzed the clinical, epidemiological and pathological features of I-FVPTC and I-CVPTC patients, we found several features that were significantly different in the two groups and particular I-FVPTC patients were older, more frequently females, tumours were smaller in size and showed less frequently a thyroid capsule invasion, extrathyroidal extension (ETE) and cervical lymph node metastases with respect to I-CVPTC.

When we analyzed the 18 years of follow-up of patients of the two groups of I-FVPTC and I-CVPTC we found that a statistically significant greater number of I-CVPTC patients were not cured respect to those of the I-FVPTC group (Table 3, last line).

Multivariate clustering analysis

Because data analysis showed that the En-FVPTC, the Ei-FVPTC and the En-CVPTC had similar features and outcomes, we performed a multivariate clustering analysis (Fig 3) to confirm our hypothesis. The first MCA dimension (x-axis, 30% of explained variance) was characterized by thyroid capsule invasion (loading=0.83) and extrathyroidal extension (loading=0.83) and, to a lesser extent, lymph node metastasis (loading=0.47) (Fig. 3, panel A). This first MCA dimension clearly separate the three E-PTC groups (En-FVPTC, En-CVPTC, Ei-FVPTC) from the I-CVPTC group. The second MCA dimension (y-axis, 14%) was mostly dependent on tumour size (loading=0.44) (Fig. 3, panel A) and further separates I-FVPTC from Ei-CVPTC. The clinical interpretation of the results obtained by multivariate analysis is that, as shown in Fig 3, panel B, the En-FVPTC, the En-CVPTC and the Ei-FVPTC have similar clinical and pathological features (as shown by the cluster made by these three groups on the right side of Figure 3, panel B), which are significantly

different from I-FVPTC and I-CVPTC on Dimension 1 (x-axis of Fig. 3, panel B) and, to a lesser extent, also from Ei-CVPTC (y-axis of Fig. 3 panel B).

BRAF mutational status and pathological features

Among the entire group of PTC (n=1154) we assessed the *BRAF* molecular status on 614 cases, in particular on 384 FVPTC and 230 CVPTC cases. We found $BRAF^{V600E}$ in 177 cases (28.8%) and *BRAF* rare alterations in 10 cases (1.6%). The most frequent rare *BRAF* mutation was a point c.1801A>G mutation (p.K601E) that was present in 5 cases, followed by 1-bp insertion c.1794_1795insGTT (p.A598-T599insV) that was relived in 2 cases, an in-frame deletion of a triplet c.1799_1801delTGA (p.V600_K601delinsE) in 2 cases and a 14-bp deletion c.1798_1811del with a concomitant 2-bp insertion c.1798_1799ins (p. T599I-V600_R603del) in one case. All these rare variants were found in the group of FVPTC. Five out of 10 rare mutations were in the group of En-FVPTC, 3/10 were in the group of Ei-FVPTC, and 2 in the group of I-FVPTC. Due to the very low number of *rare-BRAF* mutations in our series and being associated with a good outcome as previously reported by Torregrossa et al. (24) in the subsequent analysis we decided to merge the 10 *rare-BRAF mutations* FVPTC mutated with *BRAF* wild type cases.

According to the histological variant, $BRAF^{V600E}$ mutation was present in 116/230 (50.4%) CVPTC and 61/384 (15.9%) FVPTC. According to the presence or absence of the tumour capsule or its invasion we found that $BRAF^{V600E}$ mutation was present in 37/244 (15.2 %) En-PTC, in 7/42 (16.7 %) Ei-PTC cases, and in 133/328 (40.4 %) I-PTC ($p < 0.0001$).

As shown in Fig 4, when the groups were divided also considering the histological variant, we found a statistically significantly higher rate of mutated cases in En-CVPTC (22/49, 44.9%) than in En-FVPTC (15/195, 7.7%) ($p<0.0001$). Similarly, a significantly higher rate of mutated cases was found in Ei-CVPTC (4/4, 100%) than in Ei-FVPTC (3/38, 7.9%) ($p=0.0003$) as well as in I-CVPTC (90/177, 50.8%) with respect to I-

FVPTC (43/152, 28.3%) ($p<0.0001$). No differences in the morphological features and clinical manifestation of the sub-groups of *BRAF*^{V600E} and *BRAF* wild-type E-FVPTC were observed.

Discussion

The importance of the tumour capsule in PTC has already been questioned as far back as 1984 by Schroder and colleagues (25) and by Carcangiu M.L. et al. in 1985 (26). In this regard, it is worth noting that the presence of a whole tumour capsule is a strong predictor of the better outcome not only in follicular cell-derived thyroid tumours but also in medullary thyroid cancer (27).

In our series, we found a high prevalence of E-PTCs (41%) that is much higher than that reported in the previous series by Schroder S. et al. (14%) (25) and, more recently, by Rivera M. et al. in 2009 (12.8%) (28). There are at least two possible explanations for this discrepancy. The first could be related to the high prevalence of FVPTC in our series, likely also including cases that nowadays could be renamed as NIFTP and eventually excluded from this series. Since this was a retrospective study, our pathologists could not apply all the criteria that nowadays are used to define the NIFTP (17) and we cannot exclude the presence of several cases of them. The other possible explanation of this difference can be due to the fact that, at variance with previous studies, we analysed only CVPTC and FVPTC that is, in general, characterized by less aggressive behaviour with respect to the other variants (i.e., tall cells, columnar, solid ecc). We do not have the data regarding the prevalence of the tumour capsule in these other more aggressive variants but, we can suppose that they are less frequently capsulated in agreement with the evidence that the prevalence of the tumour capsule in our series was lower in CVPTC (25.3%) with respect to FVPTC (55.8%) and it is known that CVPTC is more aggressive than FVPTC (29). Thus, including more aggressive variants as done in other studies, the prevalence of tumour capsule could be lower.

When we looked at the tumour capsule invasion (Ei-PTC), we found that it was more frequent, but not statistically significant, in E-FVPTC than in E-CVPTC. At variance, Rivera M. et al demonstrated that E-

CVPTC had a significantly higher rate of tumour capsule invasion than E-FVPTC (65% vs 38%) (28), similarly to that reported by other authors from the University of Pennsylvania (30). Nevertheless, other series showed results similar to ours with a higher prevalence of capsular invasion in E-FVPTC with respect to E-CVPTC, even statistically significant (31,32). One possible explanation of this discrepancy between ours and other studies can rely on the retrospective nature of our study. Although all our thyroid tumours samples were accurately reanalysed by our pathologists, it is worth to note that they revised tumour which were sampled more than 10 years before when they performed one capsule sample every 8-10 mm. Probably the problem of the different rate in tumour capsule invasion lies in the frequency of tumour capsule sampling, used in different studies. The complete sampling of the tumour capsule interface is still not the standard of practice in most laboratories and in particular, it was not the standard of practice in our lab until the definition of NIFTP.

When we compared the En-CVPTC vs En-FVPTC, no epidemiological, clinical and pathological difference was found, showing a similarly good outcome after a long median time follow up of 18 years. Instead, a significantly lower rate of Ei-CVPTC patients was cured with respect to the Ei-FVPTC patients, even if they showed similar aggressiveness features.

At variance, a statistically significant difference was found in all epidemiological, clinical, and pathological features between I-CVPTC and I-FVPTC, except for the tumour focality, with a higher prevalence of the features of aggressiveness in I-CVPTC. This result was in agreement with the already well demonstrated greater aggressiveness of CVPTC with respect to FVPTC, although this was demonstrated in all cases independently from the presence/absence of the tumour capsule (29). Since the prevalence of CVPTC without a tumour capsule is high (almost 75% in our series), it is conceivable that they play a major role in determining both the rate of survival and the rate of recurrence when pulled together with those with a tumour capsule (29).

The truthfulness of our results was supported by the multivariate clustering analysis that confirmed that the En-FVPTC, the En-CVPTC, and the Ei-FVPTC have similar clinical and pathological features which are

significantly different from those of I-FVPTC and I-CVPTC and even if to a lesser extent, also from those of Ei-CVPTC.

To the best of our knowledge, this is the first study that compared CVPTC and FVPTC with a whole tumour capsule (En-CVPTC vs En-FVPTC). Taking into account these results and the very good behaviour of En-CVPTC, we believed that this pathological entity could be considered similar to En-FVPTC, that is NIFTP per new nomenclature (16). If this would be the case, En-CVPTC could also be considered a "premalignant lesion" without the necessity of aggressive treatment. A new nomenclature, similar to NIFPT, could be proposed by pathologists also for the En-CVPTC. As matter of fact, a quite similar concept has been already introduced by Ohba et al. (33) and Rosario P.W. (34) who, by describing singular cases, suggested to use the acronymous NEPRA (noninvasive encapsulated papillary RAS-like thyroid tumor) to indicate the encapsulated thyroid tumor with papillary architecture and RAS type nuclear features. Moreover, Kakudo et al. (35,36) had already proposed to downgrade noninvasive E-PTC (both classic and follicular variant) from malignant to borderline tumor category in 2012. However, so far, only encapsulated follicular variant of PTC with well-defined features (16,17) have been officially reclassified as NIFTP (18).

As far as the molecular status is concerned, it is well established that *BRAF* alterations are the most common genetic events in PTC (37) and among these, the $BRAF^{V600E}$ mutation is the most frequent and best described (37–40) due to its known prognostic role. However, several different rare *BRAF* alterations have been described in a significantly smaller number of cases with a rather indolent prognostic role as already demonstrated by Torregrossa et al. (24). In our series, the $BRAF^{V600E}$ mutation was the most frequent genetic alteration, occurring in 28.8% of the entire series (i.e, CVPTC + FVPTC) with a lower rate than that reported in the literature. The possible explanation is that our study evaluates a predominant number of FVPTC that, as far as we know, are more RAS-like than BRAF-like tumours.

In agreement with the data already reported (24,41), our study confirmed the higher prevalence of $BRAF^{V600E}$ mutation in CVPTC (50.4%) with respect to the FVPTC (15.9), and this difference of $BRAF^{V600E}$ prevalence was confirmed also in the subgroups of En-PTC, Ei-PTC, and I-PTC, thus indicating that the

relationship of the mutation with the histological variant is stronger than with the presence/absence of the capsule. Consistently with data reported in other studies (24,42–44), we found that 10/614 (1.6%) PTCs carried a rare *BRAF* mutation and all of them were present in FVPTC, independently from the presence or absence of the tumour capsule and its integrity. Our finding confirmed that the rare *BRAF* mutations are almost exclusive of the FVPTC and thus related to lower aggressive behaviour.

In our series, the outcome of En-FVPTC and En-CVPTC was similar and, after 18 years of follow-up, only a very low percentage of cases were not cured (2% and 3%, respectively). One could argue that in both groups the outcome of the disease is not positive in all cases but we have to consider that 8/10 cases (4/4 En-CVPTC and 4/6 En-FVPTC) have been considered not cured for very low levels of serum Tg whose clinical meaning is unknown (45,46). For the other 2 cases with a structural disease (2/6 En-FVPTC) it is conceivable that, if we could have more information regarding the presence of $BRAF^{V600E}$ or a more appropriate description of the invasion of the tumour capsule they might be excluded. In this regard, it is useful to recall, three studies (47–49) have found up to a 6% rate of metastasis (predominantly nodal) in NIFTP patients, particularly in those cases containing a small percentage (< 1%) of true papillae. Because of these studies, members of the NIFTP consensus group proposed to revise the diagnostic criteria from "less than 1% papillae" to "no well-formed papillae" (17). Moreover, since some studies showed that $BRAF^{V600E}$ mutation is correlated with the percentage of papillae and risk of nodal metastases (41,50) it was suggested to exclude from NIFTP definition those cases with a $BRAF^{V600E}$mutation. If we applied these criteria in En-CVPTCs, which represent in our series about 23% of all CVPTC, and we excluded the $BRAF^{V600E}$ mutated cases, still, approximately 12.5% of all CVPTC could be considered similar to NIFTP from a clinical, pathological, and molecular point of view.

Other than the retrospective nature, another limitation in our study is that most of our patients were treated, as per the standard clinical practice of those years, with total thyroidectomy and radioiodine thyroid remnant ablation. We cannot exclude that the excellent course of these cases was due to this initial treatment that nowadays is no more indistinctly performed to all patients. However, about 10% of our patients did not undergo 131I ablation therapy because unifocal micro-PTC and all of them were cured

after a median follow-up of 18 years. Nevertheless, a prospective study in a consecutive series of En-CVPTC treated only by surgery could answer the question of whether thyroidectomy alone, mostly lobectomy, can be safe in the En-CVPTC as well as in En-FVPTC.

In conclusion, we demonstrated that the En-CVPTC are less frequent than the En-FVPTC but still they represent about a quarter of all CVPTC. They have a clinical and pathological behavior similar to that of En-FVPTC that is NIFTP per new nomenclature. Probably, in the near future, we might consider En-CVPTC as a "pre-malignant lesion" just like NIFTP. If this would be the case, about 15% of CVPTC will no more be considered as malignant lesions and treated less aggressively. However, these data need to be confirmed in a prospective study with En-CVPTC cases selected according to criteria similar to those already established for NIFTP and treated only by surgery, which is nowadays the standard of care for nonmalignant thyroid lesions.

Acknowledgments

C.G. contributed to this paper as recipient of the PhD program in Clinical and Translational Science of the University of Pisa.

Data Availability statement

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References

1. **Vaccarella S, Franceschi S, Bray F, Wild CP, Plummer M, Dal Maso L.** Worldwide Thyroid-Cancer Epidemic? The Increasing Impact of Overdiagnosis. *N. Engl. J. Med.* 2016;375(7):614–617.

2. **Davies L, Morris LGT, Haymart M, Chen AY, Goldenberg D, Morris J, Ogilvie JB, Terris DJ, Netterville J, Wong RJ, Randolph G.** American Association of Clinical Endocrinologists and American College of Endocrinology Disease State Clinical Review: the Increasing Incidence of Thyroid Cancer. *Endocr. Pract.* 2015;21(6):686–696.

3. **Powers AE, Marcadis AR, Lee M, Morris LGT, Marti JL.** Changes in Trends in Thyroid Cancer Incidence in the United States, 1992 to 2016. *JAMA* 2019;322(24):2440–2441.

4. **Renshaw AA, Gould EW.** Why there is the tendency to "overdiagnose" the follicular variant of papillary thyroid carcinoma. *Am. J. Clin. Pathol.* 2002;117(1):19–21.

5. **Lloyd R V., Erickson LA, Casey MB, Lam KY, Lohse CM, Asa SL, Chan JKC, DeLellis RA, Harach HR, Kakudo K, LiVolsi VA, Rosai J, Sebo TJ, Sobrinho-Simoes M, Wenig BM, Lae ME.** Observer variation in the diagnosis of follicular variant of papillary thyroid carcinoma. *Am. J. Surg. Pathol.* 2004;28(10):1336–1340.

6. **Rosai J.** The encapsulated follicular variant of papillary thyroid carcinoma: Back to the drawing board. *Endocr. Pathol.* 2010;21(1):7–11.

7. **Xu B, Tallini G, Scognamiglio T, Roman BR, Tuttle RM, Ghossein RA.** Outcome of Large Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features. *Thyroid* 2017;27(4):512–517.

8. **Tallini G, Tuttle RM, Ghossein RA.** The history of the follicular variant of papillary thyroid carcinoma. *J. Clin. Endocrinol. Metab.* 2017;102(1):15–22.

9. **Liu J, Singh B, Tallini G, Carlson DL, Katabi N, Shaha A, Tuttle RM, Ghossein RA.** Follicular variant of papillary thyroid carcinoma: A clinicopathologic study of a problematic entity. *Cancer* 2006. doi:10.1002/cncr.22138.

10. **Vivero M, Kraft S, Barletta JA.** Risk stratification of follicular variant of papillary thyroid carcinoma. *Thyroid* 2013;23(3):273–279.

11. **Xu B, Tallini G, Ghossein RA.** Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features: Historical Context, Diagnosis, and Future Challenges. *Endocr. Pathol.* 2017;28(2):128–138.

12. **Gupta S, Ajise O, Dultz L, Wang B, Nonaka D, Ogilvie J, Heller KS, Patel KN.** Follicular variant of papillary thyroid cancer - Encapsulated, nonencapsulated, and diffuse: Distinct biologic and clinical entities. *Arch. Otolaryngol. - Head Neck Surg.* 2012;138(3):227–233.

13. **Xu B, Ghossein R.** Encapsulated Thyroid Carcinoma of Follicular Cell Origin. *Endocr. Pathol.* 2015;26(3):191–199.

14. **Zhu Y, Li Y, Jung CK, Song DE, Hang JF, Liu Z, Jain D, Lai CR, Hirokawa M, Kakudo K, Bychkov A.** Histopathologic Assessment of Capsular Invasion in Follicular Thyroid Neoplasms—an Observer Variation Study. *Endocr. Pathol.* 2020;31(2):132–140.

15. **Ganly I, Wang L, Tuttle RM, Katabi N, Ceballos GA, Harach HR, Ghossein R.** Invasion rather than nuclear features correlates with outcome in encapsulated follicular tumors: Further evidence for the reclassification of the encapsulated papillary thyroid carcinoma follicular variant. *Hum. Pathol.* 2015;46(5):657–664.

16. **Nikiforov YE, Seethala RR, Tallini G, Baloch ZW, Basolo F, Thompson LDR, Barletta JA, Wenig BM, Ghuzlan A Al, Kakudo K, Giordano TJ, Alves VA, Khanafshar E, Asa SL, El-Naggar AK, Gooding WE, Hodak SP, Lloyd R V., Maytal G, Mete O, Nikiforova MN, Nosé V, Papotti M, Poller DN, Sadow PM, Tischler AS, Michael TR, Wall KB, LiVolsi VA, Randolph GW, Ghossein RA.** Nomenclature revision for encapsulated follicular variant of papillary thyroid carcinoma a paradigm shift to reduce overtreatment of indolent tumors. *JAMA Oncol.* 2016;2(8):1023–1029.

17. **Nikiforov YE, Baloch ZW, Hodak SP, Giordano TJ, Lloyd R V., Seethala RR, Wenig BM.** Change in Diagnostic Criteria for Noninvasive Follicular Thyroid Neoplasm With Papillarylike Nuclear Features. *JAMA Oncol.* 2018;4(8):1125–1126.

18. **Lloyd RV, Osamura RY, Klöppel G RJ.** *WHO Classification of Tumours of Endocrine Organs. Fourth Edition - WHO - OMS* -.; 2017. Available at: https://apps.who.int/bookorders/anglais/detart1.jsp?codlan=1&codcol=70&codcch=4010#%0Ahttp://apps.who.int/bookorders/anglais/detart1.jsp?codlan=1&codcol=70&codcch=4010.

19. **Kitahara CM, Sosa JA, Shiels MS.** Influence of nomenclature changes on trends in papillary thyroid cancer incidence in the united states, 2000 to 2017. *J. Clin. Endocrinol. Metab.* 2020;105(12). doi:10.1210/clinem/dgaa690.

20. **Giani C, Torregrossa L, Piaggi P, Matrone A, Viola D, Molinaro E, Agate L, Romei C, Ugolini C, De Napoli L, Materazzi G, Basolo F, Elisei R.** Outcome of classical (CVPTC) and follicular (FVPTC) variants of papillary thyroid cancer: 15 years of follow-up. *Endocrine* 2020. doi:10.1007/s12020-020-02229-0.

21. **Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L.** 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid* 2016;26(1):1–133.

22. **Hedinger C, Willians ED, Sobin LH.** *Histological typing of thyroid tumours. Second Edition in: International Histological Classification of Tumours, Worl Health Organization.* Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong: Springer-Verlag; 1988.

23. **DeLellis RA, Lloyd R V., Heitz PU, Eng C.** *WHO Classification of Tumors. Pathology and Genetics. Tumors of Endocrine Organs*. (IARC Press, ed.). Lyon; 2004.

24. **Torregrossa L, Viola D, Sensi E, Giordano M, Piaggi P, Romei C, Materazzi G, Miccoli P, Elisei R, Basolo F.** Papillary thyroid carcinoma with rare exon 15 BRAF mutation has indolent behavior: A single-institution experience. *J. Clin. Endocrinol. Metab.* 2016;101(11):4413–4420.

25. **Schröder S, Böcker W, Dralle H, Kortmann K -B, Stern C.** The encapsulated papillary carcinoma of the thyroid a morphologic subtype of the papillary thyroid carcinoma. *Cancer* 1984;54(1):90–93.

26. **Carcangiu ML, Zampi G, Pupi A, Castagnoli A, Rosai J.** Papillary carcinoma of the thyroid. A clinicopathologic study of 241 cases treated at the University of Florence, Italy. *Cancer* 1985;55(4):805–828.

27. **Miccoli P, Minuto MN, Ugolini C, Molinaro E, Basolo F, Berti P, Pinchera A, Elisei R.** Clinically unpredictable prognostic factors in the outcome of medullary thyroid cancer. *Endocr. Relat. Cancer* 2007;14(4):1099–1105.

28. **Rivera M, Tuttle RM, Patel S, Shaha A, Shah JP, Ghossein RA.** Encapsulated papillary thyroid carcinoma: A clinico-pathologic study of 106 cases with emphasis on its morphologic subtypes (histologic growth pattern). *Thyroid* 2009;19(2):119–127.

29. **Shi X, Liu R, Basolo F, Giannini R, Shen X, Teng D, Guan H, Shan Z, Teng W, Musholt TJ, Al-Kuraya K, Fugazzola L, Colombo C, Kebebew E, Jarzab B, Czarniecka A, Bendlova B, Sykorova V, Sobrinho-Simães M, Soares P, Shong YK, Kim TY, Cheng S, Asa SL, Viola D, Elisei R, Yip L, Mian C, Vianello F, Wang Y, Zhao S, Oler G, Cerutti JM, Puxeddu E, Qu S, Wei Q, Xu H, O'Neill CJ, Sywak MS, Clifton-Bligh R, Lam AK, Riesco-Eizaguirre G, Santisteban P, Yu H, Tallini G, Holt EH, Vasko V, Xing M.** Differential clinicopathological risk and prognosis of major papillary thyroid cancer variants. *J. Clin. Endocrinol. Metab.* 2016;101(1):264–274.

30. **Baloch ZW, Shafique K, Flannagan M, LiVolsi VA.** Encapsulated classic and follicular variants of papillary thyroid carcinoma: Comparative clinicopathologic study. *Endocr. Pract.* 2010;16(6):952–959.

31. **Tielens ET, Sherman SI, Hruban RH, Ladenson PW.** Follicular variant of papillary thyroid carcinoma. A clinicopathologic study. *Cancer* 1994;73(2):424–431.

32. **Zidan J, Karen D, Stein M, Rosenblatt E, Basher W, Kuten A.** Pure versus follicular variant of papillary thyroid carcinoma: Clinical features, prognostic factors, treatment, and survival. *Cancer* 2003;97(5):1181–1185.

33. **Ohba K, Mitsutake N, Matsuse M, Rogounovitch T, Nishino N, Oki Y, Goto Y, Kakudo K.** Encapsulated papillary thyroid tumor with delicate nuclear changes and a KRAS mutation as a possible novel subtype of borderline tumor. *J. Pathol. Transl. Med.* 2019;53(2):136–141.

34. **Rosario PW.** Noninvasive encapsulated papillary RAS-like thyroid tumor (NEPRAS) or encapsulated papillary thyroid carcinoma (PTC). *J. Pathol. Transl. Med.* 2020;54(3):263–264.

35. **Kakudo K, Bai Y, Liu Z, Li Y, Ito Y, Ozaki T.** Classifcation of thyroid follicular cell tumors: With special reference to borderline lesions. *Endocr. J.* 2012;59(1):1–12.

36. **Kakudo K, Bai Y, Liu Z, Ozaki T.** Encapsulated papillary thyroid carcinoma, follicular variant: A misnomer. *Pathol. Int.* 2012;62(3):155–160.

37. **Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JWC, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Wooster R, Stratton MR, Futreal PA.** Mutations of the BRAF gene in human cancer. *Nature* 2002;417(6892):949–954.

38. **Xing M.** BRAF mutation in papillary thyroid cancer: Pathogenic role, molecular bases, and clinical implications. *Endocr. Rev.* 2007;28(7):742–762.

39. **Fugazzola L, Puxeddu E, Avenia N, Romei C, Cirello V, Cavaliere A, Faviana P, Mannavola D, Moretti S, Rossi S, Sculli M, Bottici V, Beck-Peccoz P, Pacini F, Pinchera A, Santeusanio F, Elisei R.**

Correlation between B-RAFV600E mutation and clinico-pathologic parameters in papillary thyroid carcinoma: Data from a multicentric Italian study and review of the literature. *Endocr. Relat. Cancer* 2006;13(2):455–464.

40. **Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, Elisei R, Bendlová B, Yip L, Mian C, Vianello F, Tuttle RM, Robenshtok E, Fagin JA, Puxeddu E, Fugazzola L, Czarniecka A, Jarzab B, O'Neill CJ, Sywak MS, Lam AK, Riesco-Eizaguirre G, Santisteban P, Nakayama H, Clifton-Bligh R, Tallini G, Holt EH, Sýkorová V.** Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. *J. Clin. Oncol.* 2015;33(1):42–50.

41. **Agrawal N, Akbani R, Aksoy BA, Ally A, Arachchi H, Asa SL, Auman JT, Balasundaram M, Balu S, Baylin SB, Behera M, Bernard B, Beroukhim R, Bishop JA, Black AD, Bodenheimer T, Boice L, Bootwalla MS, Bowen J, Bowlby R, Bristow CA, Brookens R, Brooks D, Bryant R, Buda E, Butterfield YSN, Carling T, Carlsen R, Carter SL, Carty SE, Chan TA, Chen AY, Cherniack AD, Cheung D, Chin L, Cho J, Chu A, Chuah E, Cibulskis K, Ciriello G, Clarke A, Clayman GL, Cope L, Copland JA, Covington K, Danilova L, Davidsen T, Demchok JA, DiCara D, Dhalla N, Dhir R, Dookran SS, Dresdner G, Eldridge J, Eley G, El-Naggar AK, Eng S, Fagin JA, Fennell T, Ferris RL, Fisher S, Frazer S, Frick J, Gabriel SB, Ganly I, Gao J, Garraway LA, Gastier-Foster JM, Getz G, Gehlenborg N, Ghossein R, Gibbs RA, Giordano TJ, Gomez-Hernandez K, Grimsby J, Gross B, Guin R, Hadjipanayis A, Harper HA, Hayes DN, Heiman DI, Herman JG, Hoadley KA, Hofree M, Holt RA, Hoyle AP, Huang FW, Huang M, Hutter CM, Ideker T, Iype L, Jacobsen A, Jefferys SR, Jones CD, Jones SJM, Kasaian K, Kebebew E, Khuri FR, Kim J, Kramer R, Kreisberg R, Kucherlapati R, Kwiatkowski DJ, Ladanyi M, Lai PH, Laird PW, Lander E, Lawrence MS, Lee D, Lee E, Lee S, Lee W, Leraas KM, Lichtenberg TM, Lichtenstein L, Lin P, Ling S, Liu J, Liu W, Liu Y, LiVolsi VA, Lu Y, Ma Y, Mahadeshwar HS, Marra MA, Mayo M, McFadden DG, Meng S, Meyerson M, Mieczkowski PA, Miller M, Mills G, Moore RA, Mose LE, Mungall AJ, Murray BA, Nikiforov YE, Noble MS, Ojesina AI, Owonikoko TK, Ozenberger BA, Pantazi A, Parfenov M, Park PJ, Parker JS, Paull EO, Pedamallu CS, Perou CM, Prins JF, Protopopov A, Ramalingam SS, Ramirez NC, Ramirez R, Raphael BJ, Rathmell WK, Ren X, Reynolds SM, Rheinbay E, Ringel MD, Rivera M, Roach J, Robertson AG, Rosenberg MW, Rosenthal M, Sadeghi S, Saksena G, Sander C, Santoso N, Schein JE, Schultz N, Schumacher SE, Seethala RR, Seidman J, Senbabaoglu Y, Seth S, Sharpe S, Shaw KRM, Shen JP, Shen R, Sherman S, Sheth M, Shi Y, Shmulevich I, Sica GL, Simons J V., Sinha R, Sipahimalani P, Smallridge RC, Sofia HJ, Soloway MG, Song X, Sougnez C, Stewart C, Stojanov P, Stuart JM, Sumer SO, Sun Y, Tabak B, Tam A, Tan D, Tang J, Tarnuzzer R, Taylor BS, Thiessen N, Thorne L, Thorsson V, Tuttle RM, Umbricht CB, Van Den Berg DJ, Vandin F, Veluvolu U, Verhaak RGW, Vinco M, Voet D, Walter V, Wang Z, Waring S, Weinberger PM, Weinhold N, Weinstein JN, Weisenberger DJ, Wheeler D, Wilkerson MD, Wilson J, Williams M, Winer DA, Wise L, Wu J, Xi L, Xu AW, Yang L, Yang L, Zack TI, Zeiger MA, Zeng D, Zenklusen JC, Zhao N, Zhang H, Zhang J, Zhang J, Zhang W, Zmuda E, Zou L.** Integrated Genomic Characterization of Papillary Thyroid Carcinoma. *Cell* 2014;159(3):676–690.

42. **Barollo S, Pezzani R, Cristiani A, Redaelli M, Zambonin L, Rubin B, Bertazza L, Zane M, Mucignat-Caretta C, Bulfone A, Pennelli G, Casal Ide E, Pelizzo MR, Mantero F, Moro S, Mian C.** Prevalence, tumorigenic role, and biochemical implications of rare BRAF alterations. *Thyroid* 2014;24(5):809–819.

43. **Afkhami M, Karunamurthy A, Chiosea S, Nikiforova MN, Seethala R, Nikiforov YE, Coyne C.** Histopathologic and clinical characterization of thyroid tumors carrying the BRAFK601E mutation. *Thyroid* 2016;26(2):242–247.

44. **Rossi ED, Martini M, Bizzarro T, Capodimonti S, Cenci T, Lombardi CP, Pontecorvi A, Fadda G, Larocca LM.** Uncommon BRAF mutations in the follicular variant of thyroid papillary carcinoma: New insights. *Cancer Cytopathol.* 2015;123(10):593–602.

45. **Tuttle RM, Tala H, Shah J, Leboeuf R, Ghossein R, Gonen M, Brokhin M, Omry G, Fagin JA, Shaha A.** Estimating risk of recurrence in differentiated thyroid cancer after total thyroidectomy and radioactive iodine remnant ablation: Using response to therapy variables to modify the initial risk estimates predicted by the new American thyroid association st. *Thyroid* 2010;20(12):1341–1349.

46. **Vaisman F, Momesso D, Bulzico DA, Pessoa CHCN, Dias F, Corbo R, Vaisman M, Tuttle RM.** Spontaneous remission in thyroid cancer patients after biochemical incomplete response to initial therapy. *Clin. Endocrinol. (Oxf).* 2012;77(1):132–138.

47. **Parente DN, Kluijfhout WP, Bongers PJ, Verzijl R, Devon KM, Rotstein LE, Goldstein DP, Asa SL, Mete O, Pasternak JD.** Clinical Safety of Renaming Encapsulated Follicular Variant of Papillary Thyroid Carcinoma: Is NIFTP Truly Benign? *World J. Surg.* 2018;42(2):321–326.

48. **Cho U, Mete O, Kim MH, Bae JS, Jung CK.** Molecular correlates and rate of lymph node metastasis of non-invasive follicular thyroid neoplasm with papillary-like nuclear features and invasive follicular variant papillary thyroid carcinoma: The impact of rigid criteria to distinguish non-invasive f. *Mod. Pathol.* 2017;30(6):810–825.

49. **Kim MJ, Won JK, Jung KC, Kim JH, Cho SW, Park DJ, Park YJ.** Clinical Characteristics of Subtypes of Follicular Variant Papillary Thyroid Carcinoma. *Thyroid* 2018;28(3):311–318.

50. **Xu B, Serrette R, Tuttle RM, Alzumaili B, Ganly I, Katabi N, Tallini G, Ghossein R.** How many papillae in conventional papillary carcinoma? A clinical evidence-based pathology study of 235 unifocal encapsulated papillary thyroid carcinomas, with emphasis on the diagnosis of noninvasive follicular thyroid neoplasm with papillary-like nucle. *Thyroid* 2019;29(12):1792–1803.

Figure legends

Fig. 1: Representative histological images of CVPTC and FVPTC (hematoxylin and eosin stain). **Panel a:** CVPTC (magnification 4X): papillary architecture composed of branching papillae lined by neoplastic cells. **Panel a_1:** CVPTC (magnification 40X): at higher power, characteristic nuclear features of PTC in the neoplastic cells are observed (chromatin clearing, nuclear grooves, overlapping, and nuclear pseudoinclusion). **Panel a_2**: a thin tumour capsule that surrounds CVPTC with papillary architecture (magnification 10X). **Panel b:** FVPTC (magnification 4X): the lesion is entirely composed of variable-sized follicles. **Panel b_1:** FVPTC (magnification 40X): at higher power, the scalloping of the colloid is seen, as typically observed in this variant of PTC; moreover, there is nuclear overlapping and nuclear clearing. **Panel b_2**: a thin tumour capsule that surrounds FVPTC with follicular architecture (magnification 10X).

Fig. 2: Classification of the 1154 PTCs according to the presence/absence of a whole tumour capsule. In particular, 335/600 FVPTC and 140/554 CVPTC had a tumour capsule with a statistically significantly higher prevalence of encapsulated cases in FVPTC with respect to CVPTC. Among the encapsulated tumours (E-PTC, 475), 276/335 FVPTC, and 125/140 CVPTC had a whole tumour capsule, without capsular and/or vascular invasion with any difference between the groups.

Fig. 3: results of the multiple correspondence analysis (MCA) between En-CVPTC, Ei-CVPTC, I-CVPTC, En-FVPTC, Ei-FVPTC, and I-FVPTC. **Panel A**: the contribution (loading) of each clinical variable to the first two MCA dimensions. **Panel B**: the location of the six groups based on E-PTC classification.

Fig. 4: rate of *BRAF* mutations in FVPTC and CVPTC across different groups divided according to the presence/absence of a whole tumour capsule. **Panel A**: rate of *BRAF* mutations in En-PTC and comparison between En-CVPTC and En-FVPTC; **Panel B**: rate of *BRAF* mutations in Ei-PTC and comparison between Ei-CVPTC and Ei-FVPTC; **Panel C**: rate of *BRAF* mutations in I-PTC and comparison between I-CVPTC and I-FVPTC.

Table 1: comparison of the epidemiological, clinical and pathological features of the 4 groups of E-PTC: En-FVPTC *vs* En-CVPTC and Ei-FVPTC *vs* Ei-CVPTC.

	En-PTC n, 401			***Ei-PTC n, 74***		
	En-FVPTC	**En-CVPTC**	***p*[1*]**	**Ei-FVPTC**	**Ei-CVPTC**	***p*[2*]**
Total number, n	**276**	**125**		**59**	**15**	
Age at the diagnosis, years						
< 55	205 (74.3)	102 (81.6)	0.1	47 (79.7)	13 (86.7)	0.7
≥ 55	71 (25.7)	23 (18.4)		12 (20.3)	2 (13.3)	
Gender						
Female	201 (73)	92 (74)	0.87	46 (78)	8 (53)	0.06
Male	75 (27)	33 (26)		13 (22)	7 (47)	
Tumor size, cm						
≤ 2 cm	184 (66.7)	92 (73.6)	0.16	40 (67.8)	10 (66.7)	0.9
> 2 cm	92 (33.3)	33 (26.4)		19 (32.2)	5 (33.3)	
Thyroid capsule invasion						
Present	0 (0)	0 (0)	-	3 (5)	1 (6.7)	0.8
Extrathyroidal extension,						
Present	0 (0)	0 (0)	-	3 (5)	1 (6.7)	0.8
Focality, n						
Unifocal	207 (75)	86 (68.8)	0.19	49 (83)	10 (66.7)	0.15
Multifocal	69 (25)	39 (31.2)		10 (17)	5 (33.3)	
Lymph node metastasis, n						
Present	2/276 (0.7)	4/125 (3.2)	0.058	2 (3.4)	2 (13.3)	0.18
Outcome						
Cured	270 (98)	121 (97)	0.5	58 (98.3)	10 (66.7)	**<0.0001**
Not cured	6 (2)	4 (3)		1 (1.7)	5 (33.3)	

En-PTC, encapsulated noninvasive papillary thyroid cancer.
En-FVPTC, encapsulated noninvasive follicular variant of papillary thyroid cancer.
En-CVPTC, encapsulated noninvasive classical variant of papillary thyroid cancer.
Ei-PTC, encapsulated invasive papillary thyroid cancer.
Ei-FVPTC, encapsulated invasive follicular variant of papillary thyroid cancer.
Ei-CVPTC, encapsulated invasive classical variant of papillary thyroid cancer.
p^1, χ^2 test to compare the categorical variables of En-FVPTC *vs* En-CVPTC.
p^2, χ^2 test to compare the categorical variables of Ei-FVPTC *vs* Ei-CVPTC.
*value was corrected for Fisher's exact test.
In bold p value statistically significant.

Table 2: epidemiological, clinical and pathological features of patients with En-PTC and Ei-PTC persistently affected after a median of 18 years of follow-up.

En-PTC persistently affected										
Patient	**Age at diagnosis, years**	**Gender**	**Histology**	**TNM, 8th edition**	**Tumor size, cm**	**Focality**	**Lymph node metastases**	**Outcome**	**UTg, µg/L**	**Local or distant metastases**
1	24	Female	CVPTC	T2NxMx	2.2	Unifocal	Absent	BD	0.32	Absent
2	26	Female	CVPTC	T2NxMx	3.5	Unifocal	Absent	BD	1.82	Absent
3	40	Male	CVPTC	T3aNxMx	4.5	Unifocal	Absent	BD	0.48	Absent
4	65	Female	CVPTC	T1aNxMx	1.0	Multifocal	Absent	BD	1.0	Absent
5	24	Female	FVPTC	T1bN0Mx	1.6	Unifocal	Absent	BD	0.59	Absent
6	29	Female	FVPTC	T1aNxMx	1.0	Unifocal	Absent	BD	0.37	Absent
7	45	Female	FVPTC	T1aNxMx	0.7	Unifocal	Absent	BD	0.50	Absent
8	65	Male	FVPTC	T3aNxMx	4.8	Unifocal	Absent	BD	4.65	Absent
9	50	Female	FVPTC	T1bN0Mx	1.5	Unifocal	Absent	SD	<0.1 (TgAbs < 1 UI/ml)	Bone
10	68	Female	FVPTC	T2NxMx	2.2	Unifocal	Absent	SD	4.2	Lung metastases
Ei-PTC persistently affected										
Patient	**Age at diagnosis, years**	**Gender**	**Histology**	**TNM, 8th UICC/AJCC edition**	**Tumor size, cm**	**Focality**	**Lymph node metastases**	**Outcome**	**UTg, µg/L**	**Local or distant metastases**
1	24	Male	CVPTC	T2NxMx	2.7	Unifocal	Absent	BD	0.25	Absent
2	34	Female	CVPTC	T3bNxMx	1.2	Unifocal	Absent	BD	0.41	Absent
3	36	Male	CVPTC	T1aN1bMx	0.6	Unifocal	Present	BD	1.99	Absent
4	36	Male	CVPTC	T1bN0Mx	1.3	Unifocal	Absent	BD	<0.1 (TgAbs 75 U/ml)	Absent
5	32	Male	CVPTC	T1bNxMx	1.6	Unifocal	Absent	SD	0.63	Lymph node metastases
6	47	Female	FVPTC	T3aNxMx	4.1	Unifocal	Absent	SD	3.8	Lymph node metastases

En-PTC, encapsulated noninvasive papillary thyroid cancer.

Ei-PTC, encapsulated invasive papillary thyroid cancer.

CVPTC, classical variant of papillary thyroid cancer; FVPTC, follicular variant of papillary thyroid cancer.

BD, biochemical disease (biochemical persistence or an indeterminate response according to ATA guidelines) (21).

SD, structural disease.

UICC/AJCC American Joint Committee in Cancer adopted by the Union for International Cancer Control.

Table 3: comparison of the epidemiological, clinical and pathological features of I-FVPTC and I-CVPTC cases.

	I-FVPTC n (%)	**I-CVPTC n (%)**	***p****
Total number, n	265	414[§]	
Age at the diagnosis, years			
< 55	210 (79.25)	354 (85.5)	**0.03**
≥ 55	55 (20.75)	60 (14.5)	
Gender			
Female	206 (77.7)	291 (70.3)	**0.03**
Male	59 (22.3)	123 (29.7)	
Tumor size, cm			
Mean ± SD (range), median	1.5±1.0 (0.1-7), 1.3	1.7±1.2 (0.2-10), 1.5	
≤ 2	214 (80.75)	304 (73.4)	**0.03**
> 2	51 (19.25)	110 (26.6)	
Thyroid capsule invasion, n			
Present	89 (33.6)	193 (46.6)	**0.0008**
Absent	176 (66.4)	221 (53.4)	
Extrathyroidal extension, n			
Present	76 (28.7)	176 (42.5)	**0.0003**
Absent	189 (71.3)	238 (57.5)	
Focality, n			
Unifocal	143 (54)	228 (55)	0.77
Multifocal	122 (46)	186 (45)	
Lymph node metastasis, n			
Present	42 (15.8)	127 (30.7)	**<0.0001**
Absent	223 (84.2)	287 (69.3)	
Outcome			
Cured	242 (91.3)	330 (81.9)	**0.0007**
Not cured	23 (8.7)	73 (18.1)	

I-FVPTC, invasive (without tumour capsule) follicular variant of papillary thyroid cancer.
I-CVPTC, invasive (without tumour capsule) classical variant of papillary thyroid cancer.
p, χ^2 test to compare the categorical variables of I-FVPTC *vs* I-CVPTC.
SD, standard deviation.
[§]The outcome data has been available in 403 patients because 11 patients were lost at follow-up.
In bold p value statistically significant.

Figure 1

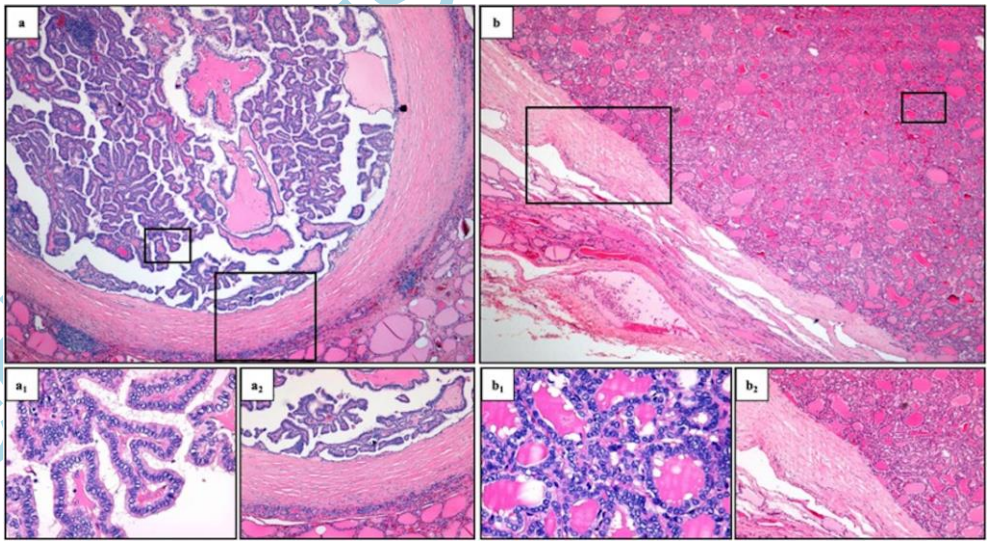

Figure 2

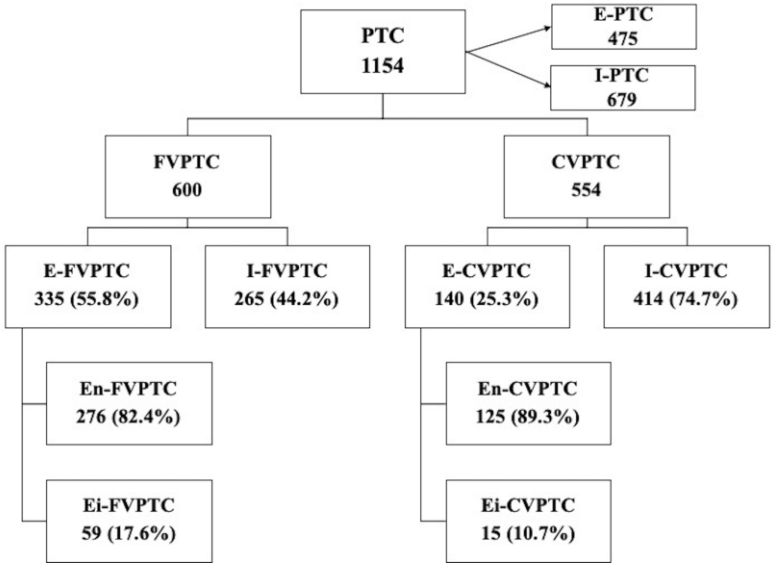

Figure 3

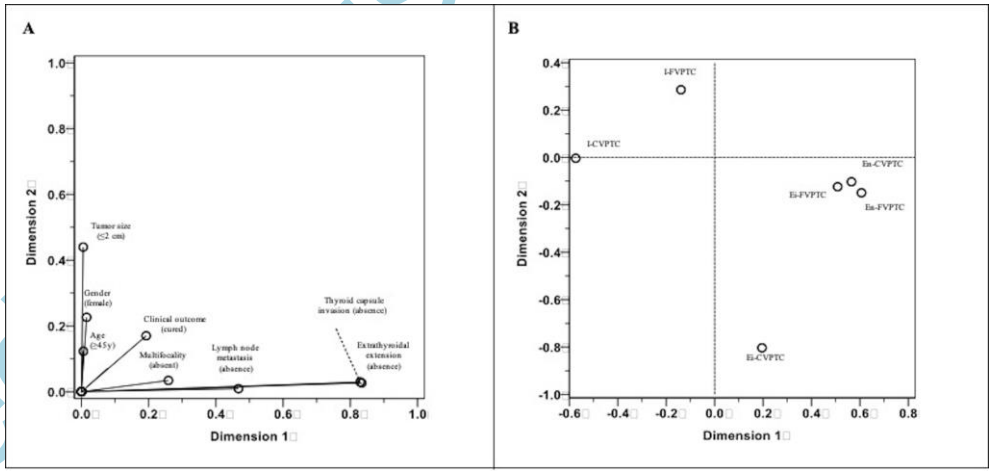

Figure 4