ABSTRACT

The incidence of papillary thyroid carcinoma (PTC) has been rising in the last years while follicular thyroid carcinoma (FTC) is being diagnosed less and less frequently everywhere.
PTC gives frequently rise to nodal metastases *via* lymphatic vessels while FTC metastasizes mainly *via* blood vessels to lung and bones. The follicular variant of PTC (FVPTC) encompasses the poorly circumscribed/infiltrative subtype (PC-FVPTC), which shares most of the features of classic PTC (CPTC), and the encapsulated subtype (E-FVPTC) which appears to be related to minimally invasive FTC.
Prognostic factors indicative of PTC clinico-pathological aggressive behaviour remain incompletely established in part due to the different composition of the series on record regarding the relative proportion of CPTC and FVPTC subtypes.
Several clinico-pathological features of PTC, together with the occurrence of *BRAF* and *RAS* mutations, are still not fully accepted as markers of aggressiveness. On the other hand, TGF-beta/Smad-dependent pathway activity has been recently associated with local invasion, nodal metastization and *BRAF* mutated PTCs.
In the present clinico-pathological study we collected all cases of thyroid tumours (malignant tumours and tumours of uncertain malignant potential) from the Cancer Registry of the Pathology Department of Centro Hospitalar de São João, from 1978 to 2006, and selected a series of 75 CPTC and FVPTC cases, including E-FVPTC and PC-FVPTC, which were divided into cases of well circumscribed PTC (WC-PTC) (n=22) and poorly circumscribed PTC (PC-PTC) (n=53), according to the characteristics of their borders. We evaluated the morphological features of the tumours, namely intratumoural and peritumoural lymph vessels density (LVD) determined by D2-40 immunoexpression, the immunoexpression of TGF-beta and TGF-beta/Smad-dependent pathway elements (phosphorylated Smad2/Smad3, Smad4 and Smad7) and the *BRAF* and *N-RAS* mutational *status* of the tumours, in an attempt to clarify the contribution of these features for the occurrence of nodal metastases.
The number of cases with a diagnosis of well differentiated carcinoma (PTC and FTC) recorded in the Cancer Registry of the Pathology Department of Centro Hospitalar de São João has increased over the years due to an increased number of PTC cases, while the number of FTC cases remained persistently low over the years. We identified 1043 PTC cases that were sub-classified, irrespective of size, accordind to the WHO criteria. The FVPTC (n=411) was the most frequent variant followed by CPTC (n=377). From the CPTC cases, 23% developed lymph node metastases, 2% developed distant metastases and 6% had recurrences. From the FVPTC cases, 3% developed lymph node metastases, 1% developed distant metastases and 3% had recurrences.
In the 29 year period (1978 – 2006), we identified five cases of PTC with a poorly differentiated component that followed an aggressive clinical course and two cases of PTC coexisting with a particular basaloid component with unknown impact over the patients' long term outcome.
In our series of 75 PTCs, *BRAF* V600E mutation was detected in 29% of tumours, 41% of CPTC and 16% of FVPTC, whereas *NRAS* Q61R mutation was detected in 6% of tumours, 3% of CPTC and 10% of FVPTC. The *BRAF* V600E mutation was detected in 7% of E-FVPTC and 25% of PC-FVPTC. *BRAF* mutation was significantly more frequent in the CPTC group and in females, and it was detected only in patients older than 20 years, suggesting a late tumourigenic effect in the development of PTC. *BRAF* mutation was not significantly associated to any of the other studied features related to aggressiveness.
The morphological features most closely related to the occurrence of nodal metastases were extra-thyroid extension and poorly circumscribed growth pattern, in both CPTC and FVPTC. Additional features significantly associated to nodal metastases were multicentricity in CPTC and vascular invasion in FVPTC. None of the E-FVPTC cases presented extra-thyroid extension, lymph vessel invasion or nodal metastases, at variance with CPTC and PC-FVPTC cases. Only one case of E-FVPTC (8%) had intratumoural D2-40-stained vessels in contrast to

their presence in 77% of the cases of PC-FVPTC. Intratumoural LVD determined by D2-40 expression correlated with the occurrence of extra-thyroid extension, lymph vessel invasion and lymph node metastases in PTC cases. At variance with intratumoural LVD, peritumoural LVD was not associated with any clinico-pathological or molecular feature, being similar in CPTC, E-FVPTC and PC-FVPTC.

Nodal metastases were not detected in any WC-PTC regardless the presence of immunoexpression for TGF-beta, Smad2/Smad3, Smad4 and Smad7, and of the occurrence of *BRAF* mutation.

Increased cytoplasmatic expression of TGF-beta at the periphery of PC-PTC was associated to morphological features of invasiveness, featuring the so-called epithelial-to-mesenchymal transition (EMT), and presence of nodal metastases, as well as to the occurrence of *BRAF* mutation which did not significantly alter, *per se*, the frequency of nodal metastases.

The nuclear expression of Smad7 was more frequent in WC-PTCs than in PC-PTCs and was associated with unicentricity and absence of extra-thyroid extension, vascular invasion and nodal metastases.

Our results emphasize the importance of an extensive sampling and detailed observation of PTC surgical specimens; they also highlight the importance of infiltrative growth pattern and invasiveness over the presence of *BRAF* mutation in CPTC and FVPTC for the development of nodal metastases.

The results on the lymph vessels profile of PTC stress the role of intratumoural lymph vessels in PTC nodal metastization and reinforce the importance of distinguishing E-FVPTC from PC-FVPTC regarding invasiveness, metastatic pattern and molecular profile.

Finally, we conclude that nodal metastases are associated to poorly circumscribed, locally invasive PTCs with high intratumoural lymph vessel density; such metastatic PTCs exhibit low levels of nuclear Smad7 and a peripheral EMT phenotype displaying TGF-beta overexpression, regardless of the occurrence of *BRAF* mutation.