UNIVERSITÉ FRANCO ITALIENNE | UNIVERSITÀ ITALO FRANCESE

International Doctorate Program in Molecular Oncology and Endocrinology
Doctorate School in Molecular Medicine
University of Naples Federico II
XIX cycle - 2003–2007
Coordinator: Prof. Giancarlo Vecchio

"POORLY DIFFERENTIATED FOLLICULAR THYROID CARCINOMA: PROGNOSTIC FACTORS AND RELEVANCE OF HISTOLOGICAL CLASSIFICATION"

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“POORLY DIFFERENTIATED FOLLICULAR THYROID CARCINOMA: PROGNOSTIC FACTORS AND RELEVANCE OF HISTOLOGICAL CLASSIFICATION”

List of abbreviations

PDFC: Poorly differentiated follicular carcinomas

FTC: Follicular thyroid carcinoma

WDC: Well differentiated carcinoma

TPO: Thyroperoxidase

DUOX: Dual oxidase

CDK: Cyclin-dependent kinases

Rb: Retinoblastoma

VEGF: Vascular endothelial growth factor

EGF: Epidermal growth factor

PI3: phosphatidylinositol-3

PLC: phospholipase

PK: Protein kinase

NOS: Nitric oxide synthetase

TIS: trabecular, insular, solid

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Abstract

Background. Poorly differentiated follicular carcinomas (PDFC) of the thyroid represent an heterogeneous but distinct group of tumors, clinically and histopathogenetically intermediate between follicular-derived well-differentiated and anaplastic carcinomas. The existence of this group of tumors was first proposed independently by Sakamoto et al. in 1983 and Carcangiu et al. in 1984 according to substantially different diagnostic criteria. Indeed, although the number of articles written on the subject of PD carcinoma has grown exponentially during the last decade, at the present the diagnostic criteria differ between various authors. Moreover, there is no clear definition of the histological, immunohistochemical and genetic characteristics of PDFC. Furthermore, although the majority of studies show the aggressiveness of PDFC with a high propensity for local recurrence and distant metastasis, there is no consensus regarding the prognostic indicators for these tumors.
Objective: The work performed during my Doctorate thesis in France contributes to clarify the histological definition and to identify clinical, histological, immunohistochemical characteristics of PDFC. For this purpose, parallel clinical, histological and immunohistochemical investigations have been performed.
Methods: Forty patients affected by PDFC were identified on the basis of a trabecular, insular, or solid (TIS) growth pattern, and their clinical outcome was correlated with histological architecture, cytological characteristics and expression of various markers of cell proliferation and differentiation such as cyclin A, B1, D1 and E, Ki67, TPO, galectin 3, Duox, VEGF, EGFR, P53.
Results: The mean survival was 5.2 ± 5 years. At 5 years,the survival rate was 63% and the metastasis-free survival rate was 57%. An older age at the time of diagnosis and a larger tumor size were associated with an increased risk of distant metastases and of cancer related death whereas a high expression of Duox was associated with a reduced risk of death. In these patients with PDFC, no histological features or marker expression was prognostic. ***Conclusion:*** this study confirmed that PDFC has a more aggressive behavior than well differentiated carcinoma (WDC); prognosis is related to indicators that are relevant in patients with WDC, advanced age and larger tumor size.

1. INTRODUCTION

Follicular thyroid cells can give rise to both benign and malignant tumors. It is estimated that 5-10% of the population will develop a clinically significant thyroid nodule during their life time (Mazzaferri 1993). However, thyroid cancer is found in less than 5% of thyroid nodules.
A schematic classification of thyroid tumors is presented in Table 1.

Table 1: Classification of thyroid tumors (adapted from Hendinger et al. 1988)

EPITHELIAL THYROID CARCINOMA

Papillary Carcinoma:
evidence of follicular differentiation with papillary and follicular structures and characteristic nuclear changes

Follicular Carcinoma
evidence of follicular cell differentiation without the diagnostic features of papillary carcinoma

Undifferentiated (anaplastic) Carcinoma
A highly malignant tumor composed in part or wholly of undifferentiated cells.

C CELL-DERIVED THYROID CARCINOMA

Medullary carcinoma
evidence of C cell differentiation

MALIGNANT NON- EPITHELIAL TUMORS

Sarcoma

Hemangioendothelioma

Malignant Lymphoma

SECONDARY TUMORS

There are two histological types of epithelial differentiated thyroid cancer, papillary or follicular lesions. Papillary carcinomas are the most common, accounting for 85% of all thyroid cancers whereas follicular thyroid carcinoma (FTC) accounts for approximately 10% of all thyroid malignancies (Parkin et al. 1997) Unlike the papillary histologic type, follicular carcinoma usually presents as a solitary thyroid tumor. In epidemiological surveys, FTC tends to be more common in areas with iodine deficiency. Owing to a combination of changing diagnostic criteria and an increased incidence of papillary thyroid carcinoma associated with dietary iodine supplementation, the diagnosis of FTC is becoming less frequent. The female-male ratio for individuals with FTC is 3.3:1.0 and it occurs mostly after the age of 50 years (Grebe and Hay 1995).
FTC generally is considered to be a more aggressive tumor than PTC and lymph node metastases are uncommon in patients with FTC, occurring in 10% whereas 50% of patients with papillary thyroid carcinoma have at least microscopic lymph node metastases. Distant pulmonary or bone metastases, however, are more common in FTC patients (30%) compared with papillary thyroid carcinoma patients (15%) (Mazzaferri et al. 1977, Emerick et al. 1993). However, the cancer related mortality is low in well differentiated thyroid carcinoma as a consequence of the availability of accurate diagnostic techniques, effective treatment and specific protocol of follow up for detecting persistent or recurrent disease (Pacini et al 2006, Cooper et al 2006, Pagano et al 2004)

1.1 Follicular Thyroid Carcinoma: Definition And Histological Classification

1.1.1 Common Histologic type

FTC is defined as "a malignant epithelial tumor showing evidence of follicular cell differentiation but lacking the diagnostic features of papillary carcinoma."
Follicular carcinomas constitute a heterogeneous group of tumors that includes tumors with favorable prognosis and tumors with an aggressive behavior. The microscopic appearance of FTC varies from well-formed follicles to a predominantly solid growth pattern. Poorly formed follicles and atypical patterns (e.g., cribriform) may occur, and multiple architectural types may coexist. Mitotic activity is not a useful indicator of malignancy. The diagnosis of malignancy depends on the demonstration of blood vessel and/or capsular invasion (Brennan et al. 1991, Lang et al. 1986, D'Avanzo et al. 2004).
Indeed, FTCs are classified into two categories based on the degree of invasiveness :
1) minimally invasive or encapsulated
2) widely invasive.
This distinction has a significant prognostic impact, since the prognosis is more

severe in widely invasive tumors. There is minimal overlapping between these two types of FTC. Minimally invasive FTC is an encapsulated tumor whose growth pattern resembles that of a trabecular/solid, microfollicular, or atypical adenoma. Blood vessel invasion is almost never seen grossly. Microscopically the vessels "should be of venous caliber, be located in or immediately outside the capsule and contain one or more clusters of tumor cells attached to the wall and protruding into the lumen." Rupture of the capsule must concern the entire thickness to be considered as capsular invasion. The diagnosis of FTC cannot be made if there is penetration of only the inner half or if tumor cells are embedded in the capsule. Foci of capsular invasion must be distinguished from capsular rupture that can be caused by FNA. Fine-needle biopsy is incapable of differentiating benign from malignant lesions and frozen section analysis, even with multiple sampling of different areas of the nodule, may still lead to misdiagnosis. Examination of multiple blocks including the periphery of the nodule is often necessary to exclude or confirm invasion.
"Widely invasive" FTC may be partially encapsulated, but the margins are infiltrative even on gross examination, and vascular invasion is often extensive.
The structural features are variable with solid and trabecular areas, but a follicular element is always present.

1.1.2Hürthle cell carcinoma (oncocytic carcinoma or oxyphilic variant FTC)

It is a tumor composed of more than 75% of cells with oncocytic features. The correct classification of Hürthle cell carcinomas is controversial. The WHO committee has taken the stance that this tumor is an oxyphillic variant of FTC (Hedinger et al. 1988). The AFIP, on the other hand, considers that "the tumors made up of this cell type have gross, microscopic, behavioral, cytogenetic (and conceivably etiopathogenic) features that set them apart from all others and justify discussing them in a separate section" (Rosai et al. 1995). Macroscopically, the oxyphilic variant presents as a solitary thyroid tumor with complete or partial encapsulation. The same criteria of malignancy mentioned for follicular tumors (i.e. vascular and capsular invasion) also apply to these tumors. Oncocytic carcinomas occur at a mean age of about 60 years, are more frequently associated with extrathyroid extension and with both distant and lymph-node metastases than typical follicular carcinomas. In several series, the prognosis of Hürthle cell carcinoma is reported to be similar to that of FTC (Chen et al. 1998).

1.1.3 Poorly differentiated thyroid carcinoma

When follicular differentiation is poor or absent, the tumor is classified as a poorly-differentiated carcinoma (PDFC). WHO classification individualizes the poorly differentiated follicular carcinoma (PDFC) defined as "a tumor of follicular cell origin with morphological and biologic attributes intermediate between differentiated and anaplastic carcinomas of the thyroid" (DeLellis et al.

2004). It accounts for approximately 2-4% of all thyroid carcinomas and represents a heterogeneous group (Carcangiu et al. 1984, Ashfaq et al 1994, Pilotti et al. 1997).
Insular thyroid carcinoma usually maintains some of the functional characteristics of the follicular thyroid cells, such as iodine uptake and thyroglobulin production (Carcangiu et al. 1984, Papotti etal. 1993). The most distinctive histologic feature is the presence of small cells with round nuclei and scant cytoplasm with a diffuse solid pattern or organized in round or oval nests (insulae) or in trabeculae. The predominant pattern of growth is solid, but papillary structures or microfollicles are also seen. Foci of necrosis, extra-thyroid extension and blood vessel invasion are common.
However, the histological definition of PDFC is still controversial and various diagnostic criteria have been proposed (Sakamoto et al 1983, Carcangiu et al. 1984, Papotti M et al 1993, Hiltzik et al 2006, Volante et al. 2007). Indeed, the existence of poorly differentiated thyroid carcinoma was first proposed independently by Sakamoto (1983) and by Carcangiu (1984) according to substantially different criteria. Sakamoto (1983) relied exclusively on the pattern of growth, that is, they regarded as well-differentiated for those tumors that had a follicular or a papillary architecture, and as PD those that had (even focally) a solid, trabecular, or "scirrhous" architecture. The appearance of the nuclei, the mitotic rate, and the presence of necrosis played no role in this classification scheme. Conversely, Carcangiu (1984) adopted a much more restricted approach: these authors required an insular pattern of growth (hence the term "insular carcinoma" they proposed as a synonym), necrosis with formation of "peritheliomatous" areas, small round hyperchromatic nuclei, and mitotic activity. Following these descriptions, there is little uniformity in the criteria used by the various authors for inclusion of a given tumor into the poorly differentiated category. Indeed, some pathologists define these tumors on the basis of growth pattern (trabecular, insular, solid) (Sakamoto et al 1983, Carcangiu et al. 1984, Papotti M et al 1993, Pellegritti et al. 2002, Volante et al. 2004, Pulcrano et al 2007, Ruffini et al. 2007), others suggest the use of high-grade histology (mitotic counts and necrosis), rather than a specific growth pattern (Hiltzik et al. 2006) whereas still others attempted to combine both approaches (Volante et al. 2007).

1.2 Prognostic Indicators

Various prognostic factors have been identified and used to create the staging classification for well differentiated thyroid carcinoma but their value has not yet been validated for PDFC. Indeed, there is neither a clear identification nor an established definition of prognostic factors in PDFC.

1.2.1 The TNM staging system (established by the International Union Against Cancer)

The TNM staging system was introduced in 1987 and reviewed in 1992, 1997 and 2002 (Hermanek et al. 1992, American Joint Committee on Cancer 2002). Since 1988, it has been recognized as the international reference staging system. The pathological classification (pTNM), is based on intra-operative and surgical-pathology data. It takes into account the patient's age at diagnosis and three variables which are the extent of the primary tumor (T), the presence (N1) or absence (N0) of lymph-node metastases and the presence (M1) or absence (M0) of distant metastases. Tumor features taken into account are size and extension beyond the thyroid capsule. Table 2 present the criteria used in the 1992 and in the 2002 classifications.

Table 2: The Tumor, Node, Metastases (TNM) scoring system (Hermanek et al. 1992, American Joint Committee on Cancer 2002)

1992		2002
Primary Tumor (T):		
T0:	No evidence of primary tumor	No evidence of primary tumor
T1:	Tumor ≤1cm limited to the thyroid	Tumor ≤2cm limited to the thyroid
T2:	Tumor>1-≤4cm limited to the thyroid	Tumor>2-≤4cm limited to the thyroid
T3:	Tumor>4cm limited to the thyroid	Tumor>4cm limited to the thyroid or any Tumor with minimal extrathyroid extension
T4:	Any size extending beyond the thyroid Capsule.	**T4a:** Tumor of any size with extension beyond the thyroid capsule and invades any of the following: subcutaneous soft tissues,larynx, trachea, oesophagus, recurrent laryngeal nerve.
		T4b: Tumor invades prevertebral fascia, mediastinal vessels, or encases carotid artery
Regional Lymph Node (N):		
N0	No regional lymph node metastasis	No regional lymph node metastasis
N1	Regional Lymph Node metastasis	Regional Lymph Node metastasis
		N1a: Metastases in pretracheal and paratracheal, including prelaryngeal and delphian lymph nodes
		N1b: Metastases in other unilateral, bilateral or contralateral cervical or upper mediastinal lymph nodes
Distant metastasis (M):		
M0	No distant metastasis	No distant metastasis
M1	Distant metastasis	Distant metastasis

The 1992 (and 2002) classification system defines 4 stages with increasing risks of cancer-related death (Table 3). A comparison of different staging systems evidenced

that no other systems had statistically significant superiority over the 1992 TNM classification (Hermanek et al. 1992).
The 2002 TNM classification is more complicated, and the definition of minimal or more extensive thyroid tumor extension may be difficult to define retrospectively (Loh 1997).. Six lymph nodes need to be examined at histology to qualify for the definition of N0 and the prognostic difference between level VI lymph-node metastases and other regional metastases has yet to be validated.
However, the prognostic value of the pathological classification (pTNM) was established with studies performed on patients with well-differentiated thyroid carcinoma and has not yet been validated for PDFC (American Joint Committee on Cancer 2002).

Table 3: TNM Staging (Hermanek et al. 1992, American Joint Committee on Cancer 2002)

1992		2002
Age <45 years		
Stage I	Any T, any N, M0	Any T, any N, M0
Stage II	Any T, any N, M1	Any T, any N, M1
Stage III	None	None
Stage IV	None	None
Age >45 years		
Stage I	T1, N0, M0	T1, N0, M0
Stage II	T2-T3, N0, M0	T2, N0, M0
Stage III	T4,N0,M0 or any T,N1,M0	T3, N0, M0 or any T1-3, N1a, M0
Stage IV	Any T, any N, M1	None
		Stage IVA: T1-3, N1b, M0 or T4a, Any N, M0
		Stage IVB: T4b, Any N, M0
		Stage IVC: Any T, Any N, M1

1.2.2 Oncological markers

Recent studies in a number of other human malignancies indicate that the expression of certain cell cycle regulators has a prognostic utility (Takano et al. 2000, Del Pizzo et al. 1999). Indeed, in the papillary thyroid carcinoma, the expression of some subtypes of cyclins is correlated with clinical/histological stage at diagnosis and with clinical outcome. Moreover, the expression of thyroid functional genes is increased or decreased in well differentiated thyroid carcinoma. (Pickett et al. 2005, Ito Y et al. 2002), while their role in the PDFC is still unknown.

1.2.2.1 Ki-67

Ki-67 is a nuclear antigen present in all proliferating cells; it is present in the G_1, S, G_2 and M phases of the cell cycle but absent in G_0 cells. It is expressed in dividing tumor cells from patients with aggressive thyroid tumors to a larger extent than in benign adenomas and well differentiated carcinomas (Krohmn 2003). Therefore, by staining for this antigen, it is possible to measure the tumor growth fraction directly and in a simple way. Ki-67 staining also yields results that are more reproducible than those obtained by counting mitotic figures.

1.2.2.2 TPO, Galectin 3 and Dual Oxidase

Galectin-3 occurs both in the nucleus and cytoplasm of many cells and on the cell surface. The multiple locations of this galectin-3 have suggested that it may have multiple functions. For example, galectin-3 is located in the nucleus of some cells and may be functionally important in RNA synthesis by being a component of the splicing complex with pre-mRNA. Galectin-3 can also bind cytokeratin, a cytoskeletal protein.
Increased expression of galectins (especially galectin-3) has also been associated with tumor progression. The molecular significance of this relationship is proposed to be the interactions of galectins with polylactosamines on matrix proteins such as laminin, aiding cellular invasion. Since polylactosamines are also expressed on cancer mucins and enriched on the β1–6-branched glycans of tumor N-glycans, this molecular interaction could mediate homotypic adhesion of carcinoma cells as well. Galectin recognition may also explain how adding cell surface galactose to tumor mutants lacking the Golgi UDP-Gal transporter enhances metastasis (Cornil et al. 1990, Lotan et al. 1991, Inohara et al. 1996).
Thyroperoxidase (TPO) is the key enzyme in the synthesis of thyroid hormones and is involved in two important reactions in the biosynthesis of thyroid hormone: the iodination of tyrosine residues on Tg and the intramolecular coupling reaction of iodinated tyrosines (figure 1).

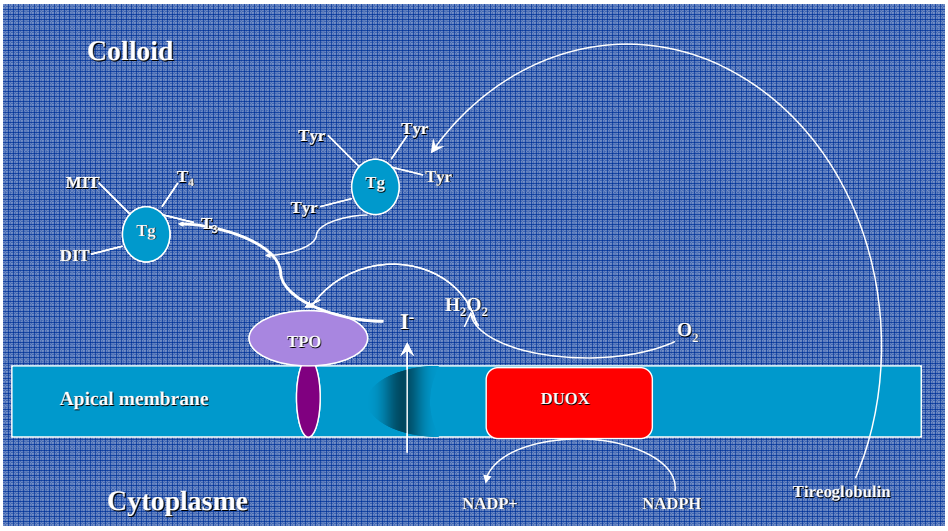


Fig. 1: Role of TPO and Dual oxidase in thyroid hormone synthesis.
The iodination of tyrosine residues on thyroglobulin and the intramolecular coupling reaction of iodinated tyrosines are catalyzed by thyroperoxidase (*TPO*) under oxidative conditions for which H2O2 is required. H2O2 production is catalyzed by an H2O2-generating system, for which *DUOX* is essential component.

A number of studies have been published demonstrating the value of a monoclonal antibody directed against TPO (monoclonal antibody 47) in the diagnosis of thyroid lesions, and although the specificities have ranged from 68% to 90%, the sensitivities have consistently been excellent, between 97% and 100%. (Weber et al. 2004, De Micco et al. 1994)

Immunohistochemistry with antibodies directed against TPO (monoclonal antibody 47) and Galectin-3 has been used to differentiate benign from malignant follicular tumors : typical cases are usually distinguished but in doubtful cases these antibodies are not reliable enough to be used in clinical practice (Weber et al. 2004). Galectin 3 is a positive marker of malignancy (expressed in cancer but not in benign lesions or normal tissue), while TPO is a negative marker (expressed in normal tissue and in benign lesions, but not in cancer).

In a retrospective study galectin-3 and TPO showed diagnostic and prognostic value for patients with papillary carcinoma (Weber et al. 2004) while their role in the PDFC carcinoma is still unknown.

Dual oxidase (Duox) is a glycoflavoprotein involved in thyroid hormone biosynthesis as the thyroid H_2O_2 generator (figure 1) (Caillou et al. 2001).

Duox expression is found in well differentiated thyroid carcinomas able to pick up radioiodine and with detectable expression of TPO, pendrin and sodium iodide symporter (Lacroix et al. 2001) but its expression has not been evaluated in PDFC

1.2.2.3 P53

Activation of p53 may lead to growth arrest at both G1 and G2 phases of the cell cycle. Arrest in G1 prevents replication of damaged DNA, while arrest in G2 prevents improper segregation of chromosomes. p53 may also arrest DNA replication in S phase, which is usually masked by the prior G1 arrest. The p53-target gene, p21 (waf-1/cip-1), is the key player in G1 arrest. p21 inhibits different complexes of cyclin/cyclin-dependent kinases (cdks) (Cyclin D-Cdk4/6 and Cyclin A, E-Cdk2) that sequentially phosphorylate the retinoblastoma (pRb) protein, and as a result release the S phase-promoting E2F-1 transcription factor (figure 2).

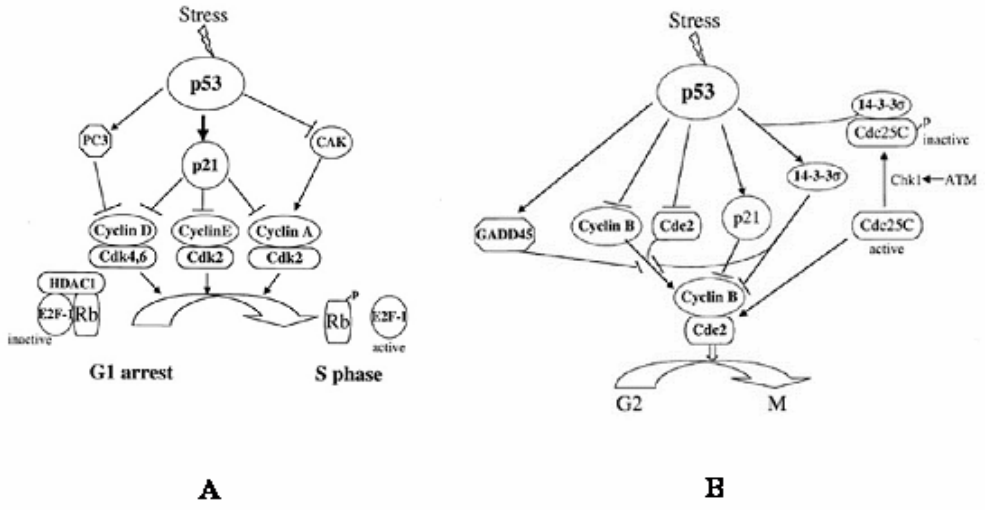


Fig. 2: Action of p53.

Activation of p53 may lead to growth arrest at both G1 and G2 phases of the cell cycle. The activation of p21 is central for mediating G1 arrest by inhibition of multiple cyclin/cyclin-dependent kinases (CDK complexes). As a result, retinoblastoma protein (Rb) is not phosphorylated, and it inactivates E2F-1transcription factor through recruitment of histone deacetylase (fig.1A). p53 triggers several parallel pathways that block the formation of the mitotic cyclin B/Cdc2 complex and inhibit its activity. This activity of p53 is essential for maintaining the G2 arrest. (fig.B) Defects in one of these pathways cause premature entry into M phase (adapted from Sionov et al. 2002)

The ability of p53 to arrest cells at multiple checkpoints is crucial for suppression of amplification of genetic alterations which otherwise can lead to cancer. Inactivation of p53 results in a loss of the DNA damage-induced G1/S checkpoint, impaired G2 arrest, and the appearance of aneuploid and polyploid cells.
In the thyroid tumors, point mutations of p53 occur in more than half of anaplastic carcinomas and in a significant proportion of poorly differentiated carcinoma but not in well-differentiated tumors, either benign or malignant. These data indicate that p53 inactivation is likely to play a direct role in triggering tumor dedifferentiation and progression to poorly differentiated and anaplastic carcinoma. Indeed p53 may serve as a prognostic factor in the poorly differentiated carcinoma (Nikiforov 2004)

1.2.2.4 Cyclins

Cyclins represent a heterogeneous group of proteins that are responsible for the regulation of the cell cycle (figure 3). Cyclin A, cyclin B and cdc2 are the G2-M regulators of the cell cycle and their expression was investigated in a variety of human tumors (Del Pizzo et al. 1999, Takano et al. 2000) but their relevance in differentiated thyroid tumors is unknown. A recent study investigated the expression of these 3 proteins in anaplastic thyroid carcinomas: cyclin A was overexpressed in all cases while cyclin B was overexpressed in only 19% of these tumors. These results suggest that cyclin A contributes to the aggressive character of thyroid carcinoma (Pickett et al. 2005, Ito et al. 2002).
Cyclin D1 and E2F-1 are essential for the regulation of the G1/S transition through the cell cycle. Over-expression of cyclin-D1 was found in the majority of the benign and malignant thyroid tumors (Lazzereschi et al. 1998), compared with normal thyroid tissue. Because cyclin D1 normally activates E2F-1, up-regulation of cyclin D1 may lead to E2F-1 overexpression in thyroid lesions (Saiz et al. 2002). In particular, papillary carcinomas showed the highest expression for cyclin-D1 (Basolo et al. 2000, Khoo et al 2002) and low expression of Cyclin-E, associated to poor prognosis while the expression of these cyclins in the follicular carcinoma has not been studied.

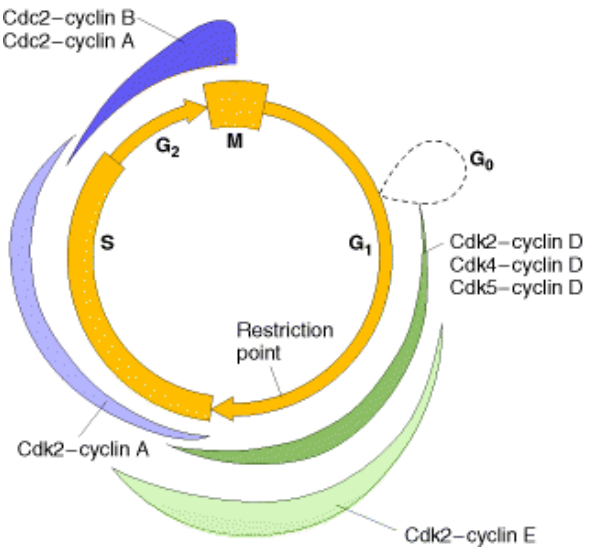


Fig. 3: A current view of the variations in cyclin–CDK activities throughout the cell cycle of a mammalian cell.

In animal cells, progression through the G_1 restriction point is controlled by complexes of Cdk2, Cdk4 and Cdk6 with D-type cyclins. Cdk2/cyclin E complexes function later in G_1 and are required for the G_1 to S transition. Cdk2/cyclin A complexes are then required for progression through S phase, and Cdc2/cyclin B complexes drive the G_2 to M transition (adapted from Lodish et al. 1995).

1.2.2.5 Vascular endothelial growth factor (VEGF) and Epidermal growth factor receptor (EGF-R)

Angiogenesis, the formation of new blood vessels, plays an integral part in the pathogenesis and spread of differentiated thyroid cancer (Soh et al. 1997). FTC is the most angiogenesis-dependent tumor of the thyroid. One of the major pro-angiogenic factors in thyroid tumors is vascular endothelial growth factor (**VEGF**) (figure 4) and the inhibition of VEGF production or VEGFR phosphorylation has been shown to reduce the growth of FTC xenografts (Younes et al. 2005).

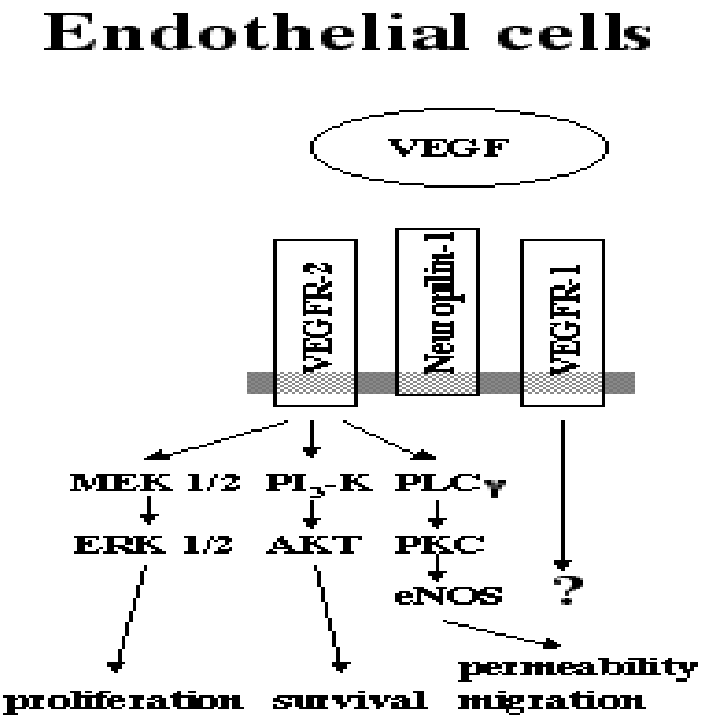


Fig. 4: Major signal transduction pathways induced by VEGF.

In endothelial cells, VEGF signaling through activated VEGFR-2 involves the MAP-kinase pathway (MEK-ERK) as well as the PI_3 kinase-AKT pathway. In endothelial cells, activation of the MAP-kinase pathway mediates cell proliferation, while signaling through the PI_3 kinase-AKT pathway leads to cell survival. Recruitment of phospholipase C γ (PLC-γ) followed by activation of protein kinase C (PKC) and endothelial nitric oxide synthetase (eNOS), finally, is involved in induction of vascular permeability. (Adapted from Clauss 2000)

Epidermal growth factor receptor **(EGFR)** is a trans-membrane cell-surface glycoprotein that stimulates cellular growth and angiogenesis (figure 5). In follicular cell EGFR overexpression increases cellular proliferation and enhances thyroid cancer invasiveness (Carpenter and Cohen 1990, Ullrich and Schlessinger 1990, Aaronson 1991).

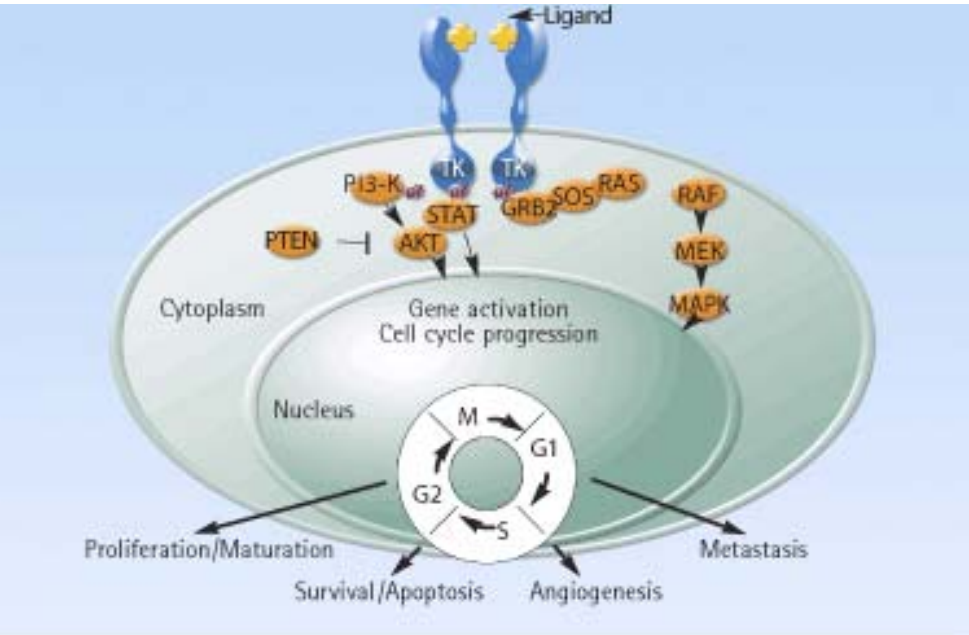


Fig. 5: EGFR signal trasduction.

Ligand binding with EGFR results in receptor homo- or heterodimerization at the cell surface (i.e. either with another EGFR or with another member of the erbB family, respectively), followed by internalization of the dimerized receptor. After dimerization, autophosphorylation of the intracytoplasmic EGFR tyrosine kinase domains occurs. Phosphorylated tyrosine kinase residues serve as binding sites for the recruitment of signaling molecules, such as Ras. These signaling molecules have the ability to phosphorylate other "downstream" molecules, leading to a chain of chemical reactions ultimately leading to the cell nucleus which ultimately leads to gene activation (Adapted from Salomon et al. 1995).

An increased expression of EGFR has been found in papillary and undifferentiated thyroid carcinomas (Hoelting et al.1994, Aasland et al. 1990, Schiff et al. 2004, van der Laan et al. 1995, Westermark et al. 1996, Akslen et al. 1993) and seems to be related to the dedifferentiation of the tumor (ref: Schiff et al. 2004). Moreover, the coexpression of EGF and EGFR is associated with bone metastases of follicular thyroid carcinoma and the blockade of EGFR signaling decreases the growth and invasion of FTC cells in vitro (Hoelting et al. 1995): However its role has not yet been demonstrated in PDFC.

2. AIM OF THE STUDY

PDFC are a heterogeneous group of tumors which show peculiar histological and clinical features with more aggressive behavior than WDTC.
PDFC are a heterogeneous group of tumors which show peculiar histological and clinical features with more aggressive behavior than WDTC. The definition of histology, epidemiology, clinical presentation and natural history of PDFC represents the key point to understand the molecular bases of these tumors and, by a clinical point of view, to recognize, treat and manage these tumors.

Therefore, the main objectives of my doctorate thesis were:

- To analyze initial presentation, treatment, outcome and prognostic factors according to the TNM classification in patients with PDFC, identified on the basis of a trabecular, insular, or solid (TIS) growth pattern.

- To define the histological characteristics of PDFC, searching for relationships between the clinical presentation, outcome and histological features of this thyroid tumor.

- To evaluate the expression of markers of proliferation and of differentiation by immunohistochemistry.

- To identify prognostic factors of PDFC correlating the histological architecture, cytological characteristics, the expression of markers of proliferation and of differentiation with survival and clinical outcome.

3. MATERIALS AND METHODS

3.1 Clinical Study

3.1.1 Patients

Clinical charts were retrieved from the archives, in according to a list obtained from the central computer system of Institut Gustave Roussy (IGR) of cases treated from the start of their illness at Institut Gustave Roussy, Villejuif, between 1975 and 2000. This search was conducted using the terms "thyroid", "carcinoma" and "follicular carcinomas". The patients affected by papillary, medullary, well differentiated follicular or undifferentiated carcinoma were not included in the study.

In all, 40 patients with PD carcinomas were selected from series of 200 patients with follicular thyroid carcinoma. For the 200 patients, all histologic hematoxylin and eosin-stained slides were reviewed by a single pathologist (Dr B. Caillou) who was unaware of clinical data, for confirmation of the diagnosis. The follicular cell origin of the tumors is immunohistochemically confirmed with positive thyroglobulin immunoreactivity and no stain for calcitonin.

Inclusion criteria were the presence of either focal, extensive or predominant TIS growth patterns in follicular thyroid carcinomas. Oxyphilic (Hürthle cell) tumors, that differed from classical follicular carcinomas for their mitochondrion-rich cytoplasm, were included in this study.

For all 40 patients, information regarding gender, age, tumor size and stage, therapy administered and outcomes were collected.

Stage for follicular FTC was assigned using the TNM system of American Joint Committee on Cancer (tab.2-3)American Joint Committee on Cancer 2002, Hermanek and Sobin 1992). Follow up data regarding recurrences and survival were obtained from the clinical chart of IGR. Tumor progression and/or disease recurrence was established on the basis of clinical examination and new findings on radiological and nuclear scanning. Dates of disease recurrence, newly found metastases and death were recorded. The end point was June 2005, or the date of death or loss to follow-up.

3.2 Histological Study

Four to 15 hematoxylin and eosin-stained slides were available from each patient. Histopatologic review was conducted by a pathologist, who was unaware of clinical characteristics or outcome of patients. On review, the following pathologic parameters were evaluated and recorded:

1. *Tumoral architecture*, defined as the presence of a trabecular pattern and/or insular and/or solid and/or polymorph pattern
2. *Presence of Hürthle cell features*, defined as the presence in the majority (>75%) of tumor cells of eosinophilic, granular cytoplasm
3. *Presence and grade of atypical nuclear pattern*: we used a score between 1 and 3 (1: <1% positive cells, 2: between 1-5% positive cells, 3: between 6-10% positive cells)

3.3 Immunohistochemistry

3.3.1 Methods

All slides were deparaffinized in xylene and rehydrated in a graded series of ethanol. To retrieve the antigenicity, the sections were then placed in a preheated 10mM citrate buffer (pH 6.0) and heated in various conditions. The sections were then immersed in methanol containing 0.3% hydrogen peroxidase for 5 min to block the endogenous peroxidase activity and were incubated during 30 min with 2.5-5 % blocking horse serum to reduce nonspecific binding. Sections were incubated with primary antibody according to previously validated conditions. Following several washes with PBS (phosphate-buffered saline), the slides were reacted for 30 min with an universal secondary biotinylated antibody, then with avidin-biotinylated horseradish peroxidase H complex (ABC kit, Vector Laboratories, Burlingame, CA). Diaminobenzidine was used as a chromogen (8 min), and commercial haematoxylin was used for counterstaining. For positive controls for each antibody, sections of thyroid previously validated by us to be strongly positive were used; to obtain a negative control for each antibody, incubation was performed without the primary antibody. Indeed, the expression of these markers in the tumor and in the normal thyroid tissue was compared in the 33 samples for which non tumoral tissue was available.
Specific antibodies used in the study were as follows: anti-cyclin A, anti-cyclin B, anti-cyclin E (1/25 dilution; monoclonal antibody; Novocastra, Newcastle upon Tyne, UK), anti-cyclin D1 (1/100 dilution; monoclonal antibody; Neomarkers, Fremont, USA), anti-Ki67 (1/25 dilution; monoclonal antibody; Dako, Glostrup, Denmark), anti-TPO (1/4 dilution; monoclonal antibody; BioCytex, Marseille, France), anti-galectin-3 (1/25 dilution; monoclonal antibody; Novocastra, Newcastle upon Tyne, UK) anti-Tg (1/100 dilution;

monoclonal antibody; Dako, Glostrup, Denmark), anti-VEGF (1/50 dilution; polyclonal antibody; SantaCruz, Heidelberg, Germany), anti-p53 (1/25 dilution; monoclonal antibody; Dako, Glostrup, Denmark), anti-Duox (1/25 dilution; polyclonal antibody raised at IGR (21)), anti-EGFR (monoclonal antibody; Ventana, Illkirch Cedex, France) antiserum.

3.3.2 Immunohistochemical analysis

Analysis of stained sections was performed with Dr Caillou at Institut Gustave Roussy- department of Pathology,. For each patient, all spots were analyzed and representative zones were chosen to count 500 cells. For each marker, the parameters evaluated were:

1. *localization of the marker:* in the nucleus, cytoplasm and membrane
2. *number of positive cells:* we used a score between 1 and 4 (1: <1% positive cells, 2: between 1-5% positive cells, 3: between 6-10% positive cells and 4: >10% positive cells)
3. *intensity of expression:* we used a score between 1 and 3 (1: low, 2: moderate, 3: high)

3.4 Statistical Analysis

The following clinical criteria were recorded for all patients: gender, age at diagnosis, TNM stage (American Joint Committee on Cancer 2002), histological characteristics of the thyroid tumor (microfollicular, trabecular, insular, solid, polymorph, Hürthle cells), and nuclear atypia. For tumor markers, we recorded staining location, number of positive cells and staining intensity, as described above.
Overall survival and metastases free survival were studied according to these parameters by using univariate and multivariate analysis. Multivariate analysis included variables for which p-value was less than 0.1 in the univariate analysis. For all analyses, two sided tests were employed and the 0.05 level of significance was used. Survival rates and their 95% confidence intervals were estimated by the Kaplan-Meier method (Kaplan and Meier 1958). The log-rank test (Peto et al. 1977) was used to compare overall and metastasis free survival rates (univariate analysis). The relative risk of death and of occurrence of metastases according to each variable were estimated using Cox's proportional hazards regression model (Cox 1972).
The analysis was performed using SAS® software (version 9.1, SAS Institut, Cary, NC, USA).

4. RESULTS

4.1 Patients

Forty patients with poorly differentiated follicular carcinomas (PDFC) were included in the analysis; there were 22 females and 18 males, with a mean age at diagnosis of 46.7 years [16-81 yrs]. No patient had a history of thyroid carcinoma in the family or of radiation exposure. The mean tumor size was 3.9 cm [0.5-12 cm]. Twenty patients were classified as T0,T1, or T2, 19 as T3 or T4 and one patient as Tx. Lymph-node metastases were present in 16 patients (Table 4). Nine patients had distant metastases at discovery of the disease, in lungs (5 patients), bones (1 patient) and in both lungs and bones (3 patients).
Median follow-up was 3.9 years [0-19.7 yrs], during which 9 patients developed distant metastases: in lungs (2 patients), bones (2 patients), in both lungs and bones (4 patients) and lungs, bones and brain (1 patient).
Radioiodine uptake was present in metastases on radioiodine (^{131}I) whole body scan performed 3 to 5 days after the administration of 3.7GBq in 6 and absent in 9 of the 15 patients with distant metastases who were treated with radioiodine; the other 3 patients did not receive any radioiodine treatment for poor clinical condition and the presence of uptake could not be determined. When present, radioiodine uptake in the metastases was low, and no significant clinical benefits were observed after treatment with radioiodine.
Twelve patients, all with distant metastases died after a mean follow-up of 2.6 years [0.02-6.5 yrs] after initial treatment.

Table 4: Clinical characteristics in 40 patients with PDFC treated and followed-up at IGR

Clinical characteristics	
Age	
≤ 45	18
> 45	22
Gender	
M	18
F	22
*T (TNM)**	
Stages 0,1,2	20
Stages 3,4	19
*Lymph Nodes (TNM)***	
Absent	20
Present	16

* One missing value for tumor size.
** One missing value for lymph nodes metastases.

4.2 Histological Characteristics

All patients had clear-cut signs of malignancy with different amounts of atypical nuclear pattern, mitosis and of necrosis. The characteristic histological architecture (TIS) represented the vast majority of the tumor areas in all tumor samples (figure 6) with the presence of trabeculae in 31 patients (77%), closely intermingled with insular architecture in 8 patients (20%) and solid architecture in 3 patients (8%). Microfollicles were found in variable amounts in 27 patients (67%). Polymorph architecture with a clearly separate distribution of various architectures was found in 14 patients (35%) and Hürthle cells were observed in 13 patients (33%) (Table 5).

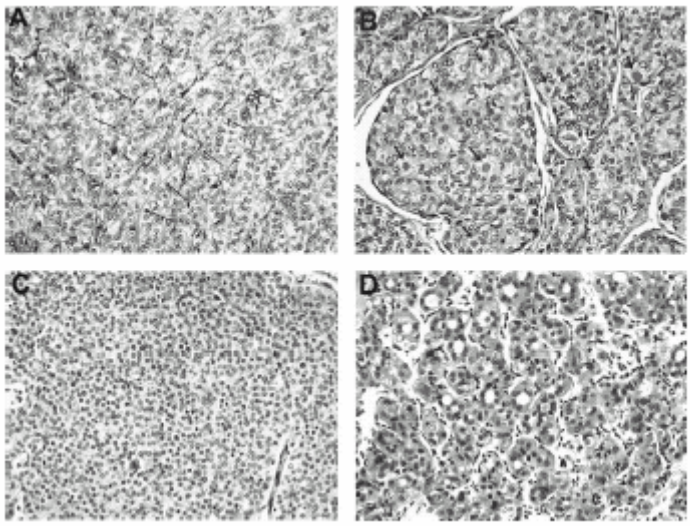


Fig. 6. Histological aspects of poorly differentiated follicular thyroid cancer (hematoxylin and eosin stain; original magnification: _100).
(A) Poorly differentiated trabecular carcinoma: dense proliferation of tumor cells without papillary or follicular structures. Note the absence of colloid formation and the presence of a trabecular pattern (arrows).
(B) Poorly differentiated insular carcinoma: presence of ‘‘islands’’ of tumor cells with well-defined limits and with ‘‘holes’’ filled up with colloid (arrows).
(C) Poorly differentiated solid carcinoma: diffuse solid pattern of tumor cells without any architecture. (D) Poorly differentiated microfollicular carcinoma:multiple microfollicles are seen.

Among the 18 patients with distant metastases, predominant trabecular architecture was found in 16 (89%). Insular pattern was found in 3 patients (17%), in association with a trabecular pattern in 1 and with a solid pattern in 2 (11%); microfollicles were observed in 12 patients (67%) and Hürthle cells in 6

(33%). Similar features were observed in patients without metastases. The polymorph architecture was found in 3 patients with metastases, less frequently than in 11 of the 22 patients without metastases (Table 5).

Table 5: Histological characteristics in 40 patients with PDFC treated and followed-up at IGR

Histological Characteristics	**Metastasis absent**	**Metastasis present**
Microfollicular		
Yes	15	12
No	7	6
Trabecular		
Yes	15	16
No	7	2
Insular		
Yes	5	3
No	17	15
Solid		
Yes	1	2
No	21	16
Polymorph		
Yes	11	3
No	11	15
*Hurthle**		
Yes	7	6
No	14	12

***** One missing value for the histological classification into Hurthle cells.

4.3 Immunohistochemical study

Twenty nine (72%) PDFC were positive for Cyclin A and 31 (77%) for Cyclin B1. A nuclear expression of Cyclin E was found in 31 PDFC (77%) (figure7A), whereas no expression of Cyclin D1 was found (figure 7B). Ki-67 staining was positive in 28 PDFC (72%) (figure 7C) with a percentage of positive cells of 1-5% in 10 tumors, of 6-10% in 6, and > 10% in 12. P53 staining was positive in 20 PDFC (50%).
Galectin 3 staining was positive in 21 PDFC (52%). Duox staining was positive in 17 PDFC (43%) and TPO in 26 (65%), and there was no clear relationship between staining for Duox and for TPO (both stainings being positive in 12 tumors, negative in 9, and either one positive in 19 tumors). In tumor positive for TPO, staining was located in the cytoplasm and not in the membrane (figure 7D). EGFR was positive in 29 PDC (72%) and VEGF in 38 (95%).

4.4 Prognostic Factors For The Occurrence Of Metastases And Thyroid Cancer Mortality

4.4.1 Occurrence of distant metastases

Distant metastases occurred in 4 of the 18 patients aged less than 45 years and in 14 of the 22 patients older than 45 years. Mean age at the first treatment was 58.7 years [32-81 yrs] in the 18 patients who developed metastases and 36.9 years [16-63 yrs] in the other 22 patients ($p < 0.0001$) (Table 6).

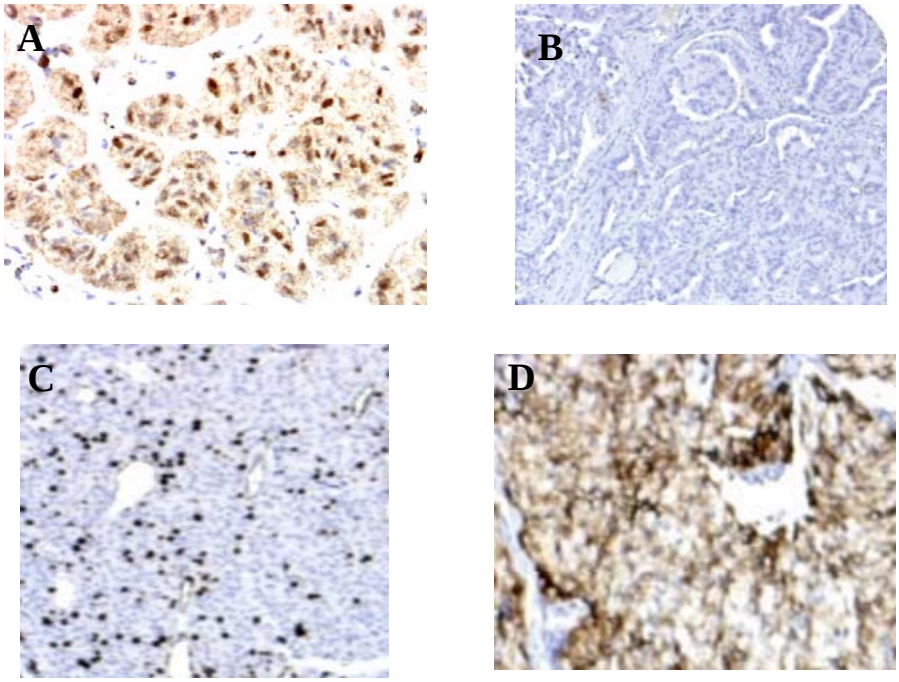


Fig.7 Immunohistochemical studies in poorly differentiated thyroid cancer

(A) Strong nuclear CyclinE-positive stanining in all tumor cells

(B) CyclinA negative staining in all tumor cells

(C) Ki-67 positive staining in the majority tumor cells

(D) TPO-positive staining in the cytoplasm in all tumor cells

Table 6: Clinical and histological factors of metastases occurrence in 40 patients with PDFC treated and followed-up at IGR - Univariate analysis

Clinical characteristics	Metastases	RR	95% CI	*P*
Age				
≤ 45	04/18			
> 45	14/22	1.08	1.04-1.12	< 0.0001
Gender				
M	07/18			
F	11/22	1.47	0.57-3.8	0.42
*T (TNM)**				
Stages 0,1,2	03/20			
Stages 3,4	14/19	3.50	1.84-6.64	0.0001
*Lymph Nodes (TNM)***				
Absent	05/20			
Present	09/16	2.50	0.84-7.55	0.1
Histological Characteristics				
Microfollicular				
Yes	12/27			
No	06/13	0.91	0.34-2.45	0.86
Trabecular				
Yes	16/31			
No	02/09	2.50	0.57-10.9	0.22
Insular				
Yes	03/08			
No	15/32	0.72	0.21-2.50	0.6
Solid				
Yes	02/03			
No	16/37	1.69	0.38-7.4	0.48
Polymorph				
Yes	03/14			
No	15/26	0.26	0.07-0.93	0.04
*Hurthle****				
Yes	06/13			
No	12//26	1.04	0.38-2.84	0.94
Nuclear Atypia				
Yes	09/24			
No	09/16	1.0	0.48-2.08	0.99

* One missing value for tumor size.
** One missing value for lymph nodes metastases.
*** One missing value for the histological classification into Hurthle cells.

Patients younger than 45 years had metastasis free survival significantly longer than patients older than 45 years (p = 0.002) (figure 8).

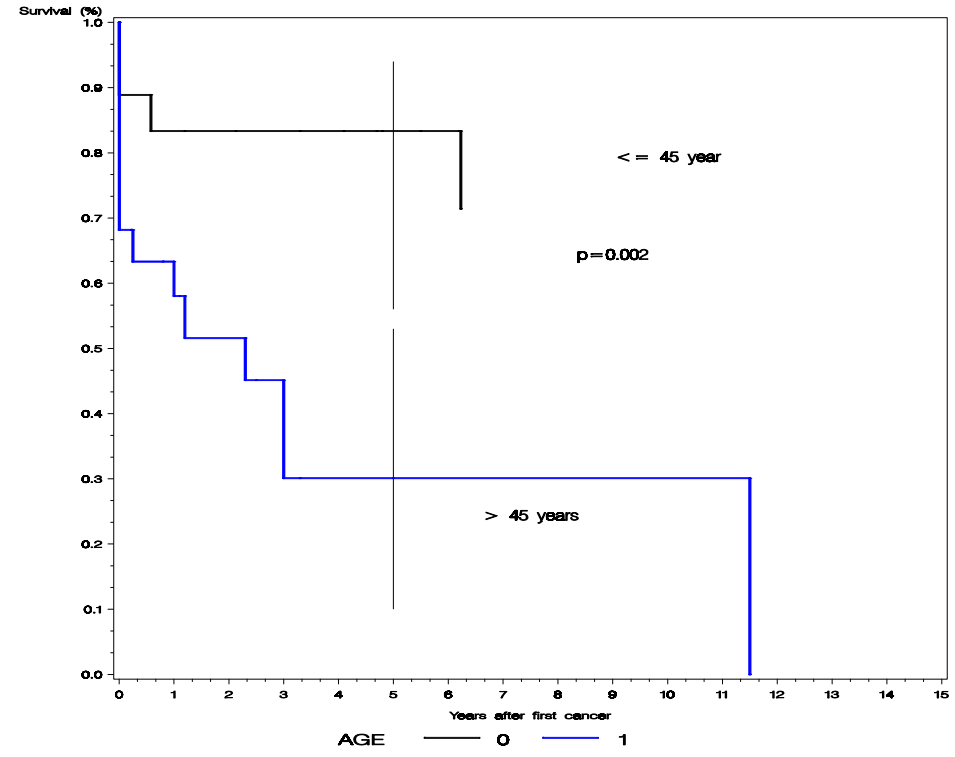


Fig. 8: Metastases free survival according to age at the time of diagnosis.

Among the 18 patients with metastases, 14 had a primary tumor larger than 4 cm and/or with an extension beyond the thyroid capsule, 3 had a primary tumor smaller than 4 cm and one was staged as Tx. Nine patients with distant metastases had lymph-node metastases at the time of diagnosis (Table 6).
The analyses of histological factors showed that only polymorph pattern was associated with a lower risk of metastases (RR : 0.26 ; 95% CI [0.07-0.93] ; p=0.04) (Table 6).

Univariate and multivariate analysis showed that an older age at the time of initial treatment and a larger extent of the primary tumor were significantly associated with an increased risk of metastases (Tables 1-4). Indeed, the RR of metastases was 1.08 in patients older than 45 years, [95% CI:1.02-1.14; p=0.003] and was 3.24, [95% CI:1.56-6.72; p=0.001] in patients classified as T3 and T4, as compared to patients classified as T0, T1 or T2 (Table 7).
Instead, a polymorph pattern was associated with a lower risk of metastases [RR : 0.26; 95% CI:0.07-0.93; p=0.04] (Table 7).

Table 7: Clinical and histological factors of metastases occurring and death in patients treated and followed-up at IGR - Multivariate analysis

Clinical characteristics	Metastases	RR	95% CI	*p*
Age				
≤ 45	04/18			
> 45	14/22	1.08	1.04-1.12	0.003
T (TNM)*				
0,1,2	03/20			
3,4	14/19	3.24	1.56-6.72	0.001
Histological Characteristics				
Polymorph				
Yes	03/14			
No	15/26	0.26	0.07-0.93	0.04

With reference to immunohistochemical factors, Cyclin A was more frequently expressed in patients with metastases, (p=0.1 at univariate analysis) (Table 8) whereas TPO expression was linked to a lower risk of metastases [RR : 0.74 95% CI:0.57-0.97; p=0.03] (Tables 5-11). The expression of the other markers studied was not different between patients with and without distant metastases.

Table 8 : Immunohistochimical factors of metastases occurrence in 40 patients with PDFC - Univariate analysis

	RR (for metastases)	95% CI	*p*
Cyclin A			
Cells with nuclear marking			
Per degree of positive cells			
Percentage	1.37	0.92-2.04	0.1
Cyclin B1			
Cells with cytoplasmic expression			
Per degree of positive cells			
Percentage	1.26	0.88-1.79	0.21
Cycline E			
Cells with nuclear expression			
Per degree of positive cells			
Percentage	0.91	0.67-1.22	0.53
Ki67			
Cells expression			
Per degree of positive cells			
Percentage	1.14	0.84-1.54	0.39
TPO			
Cells with cytoplasmic expression			
Per degree of positive cells			
Percentage	0.74	0.57-0.97	0.03
Galectine 3			
Cells with cytoplasmic expression			
Per degree of positive cells			
Percentage	1.01	0.78-1.29	0.93
Galectine 3			
Cells with nuclear expression			
Per degree of positive cells			
Percentage	0.95	0.74-1.22	0.69
VEGF			
Cells with cytoplasmic expression			
Per degree of positive cells			
Percentage	0.91	0.53-1.54	0.71
EGFR			
Cells with membrane expression			
Per degree of positive cells			
Percentage	0.86	0.67-1.13	0.28
Duox			
Per degree of positive cells			
Percentage	0.71	0.44-1.16	0.18
P53			
Cells with nuclear expression			
Per degree of positive cells			
Percentage	0.9	0.71-1.15	0.4

4.4.2 Survival

At 5 years, the overall survival rate was 63% and the metastases-free survival rate was 57% (figure 9).

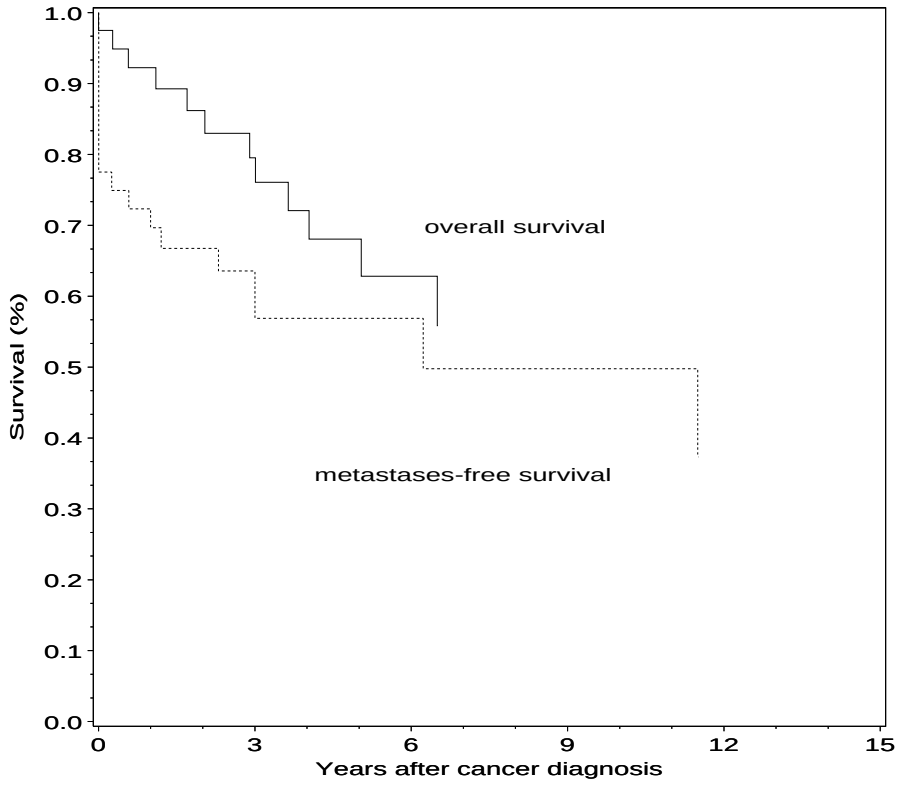
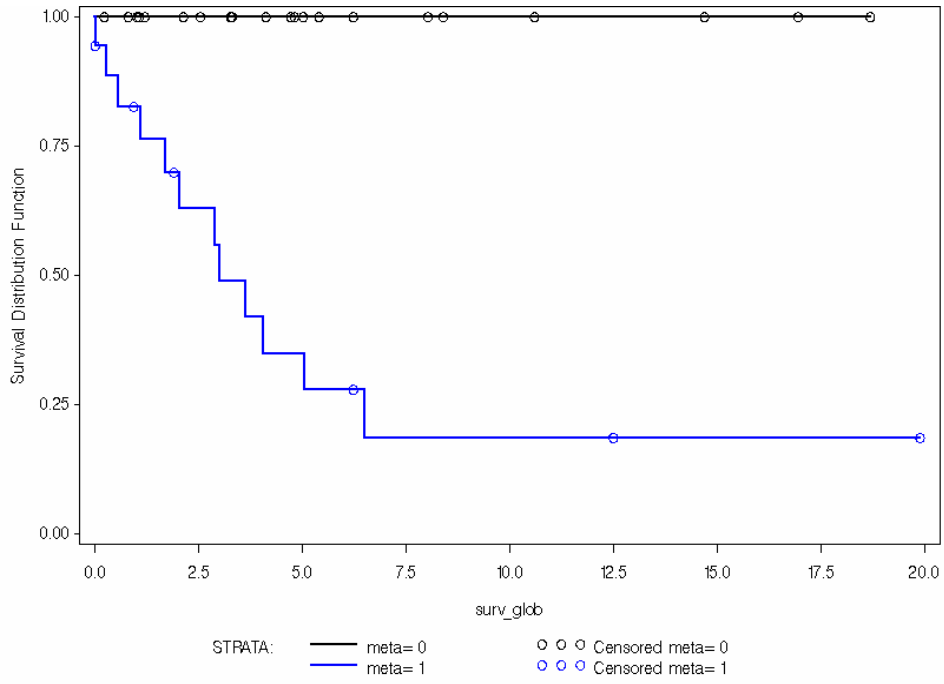


Fig. 9: Overall and metastases free survival in the whole series

Survival of patients without metastases was indeed significantly longer than for patients with metastases (p <0.001) (figure 10).



<u>Fig. 10</u>: Survival according to metastases occur (n= 40).

Univariate and multivariate analysis showed that an older age at the time of initial treatment and a larger extent of the primary tumor were associated with a higher risk of death (Tables 9-10). Indeed, the RR of death was 1.08 [95% CI:1.03-1.14; p=0.002] in patients older than 45 years and 3.14 [95% CI:1.02-9.7; p=0.04] in patients classified as T3 and T4 as compared to patients classified as T0, T1 or T2 (Table 10). No association was found between histological variables and survival (Table 9)

Table 9: Clinical and histological factors of death in 40 patients with PDFC treated and followed-up at IGR - Univariate analysis

Clinical characteristics	Deaths	RR	95% CI	*p*
Age				
≤ 45	0/18			
> 45	12/22	1.12	1.06-1.17	< 0.0001
Gender				
M	4/18			
F	8/22	1.76	0.53-5.87	0.36
*T (TNM)**				
Stages 0,1,2	1/20			
Stages 3,4	10/19	4.36	1.80-10.6	0.001
*Lymph Nodes (TNM)***				
Absent	3/20			
Present	6/16	2.28	0.57-9.20	0.29
Histological Characteristics				
Microfollicular				
Yes	8/27			
No	4/13	0.86	0.26-2.86	0.8
Trabecular				
Yes	10/31			
No	2/9	1.5	0.33-6.87	0.6
Insular				
Yes	3/8			
No	9/32	1.01	0.27-3.73	0.99
Solid				
Yes	1/3			
No	11/37	1.23	0.16-9.57	0.85
Polymorph				
Yes	3/14			
No	9/26	0.46	0.12-1.72	0.25
*Hurthle****				
Yes	4/13			
No	8/26	1.02	0.303.44	0.98
Nuclear Atypia				
Yes	9/24			
No	9/16	1.63	0.68-3.90	0.27

* One missing value for tumor size.
** One missing value for lymph nodes metastases.
*** One missing value for the histological classification into Hurthle cells

Table 10: Clinical and histological factors of death in patients treated and followed-up at IGR - Multivariate analysis

Clinical characteristics	Deaths	RR	95% CI	*p*
Age				
≤ 45	0/18			
> 45	12/22	1.08	1.03-1.14	0.002
T (TNM)*				
0,1,2	01/20			
3,4	10/19	3.14	1.02-9.7	0.04

The analysis of immunohistochemical factors showed that a high expression of Duox was associated with a lower risk of death [RR : 0.14; 95% CI:0.02-0.97; p=0.04](Tables 11-12) whereas the expression of the other markers studied was not statistically significant (Table 12).

Table 11: Immunohistochimical factors of metastases occurring and death in patients treated and followed-up at IGR - Multivariate analysis

	RR	95% CI	*p*
Metastases			
TPO			
Cells with cytoplasmic expression			
Per degree of positive cells percentage	0.74	0.57-0.97	0.03
Deaths			
Duox			
Per degree of positive cells percentage			
	0.14	0.02-0.97	0.04

A score from 1 to 4 was used to quantify the number of positive cells : 1: < 1% of positive cells ; 2: between 1 and 5% of positive cells ; 3: between 6 and 10% of positive cells ; 4: >10% of positive cells)

Table 12: Immunohistochimical factors of death in 40 patients with PDFC - Univariate analysis

	RR (for death)	95% CI	*p*
Cyclin A			
Cells with nuclear marking			
Per degree of positive cells			
Percentage	**1.23**	**0.78-1.95**	**0.36**
Cyclin B1			
Cells with cytoplasmic expression			
Per degree of positive cells			
Percentage	**1.13**	**0.74-1.73**	**0.56**
Cycline E			
Cells with nuclear expression			
Per degree of positive cells			
percentage	**0.83**	**0.58-1.18**	**0.31**
Ki67			
Cells expression			
Per degree of positive cells			
Percentage	**1.10**	**0.76-1.60**	**0.61**
TPO			
Cells with cytoplasmic expression			
Per degree of positive cells			
Percentage	**0.79**	**0.58-1.07**	**0.13**
Galectine 3			
Cells with cytoplasmic expression			
Per degree of positive cells			
Percentage	**0.96**	**0.71-1.31**	**0.80**
Galectine 3			
Cells with nuclear expression			
Per degree of positive cells			
Percentage	**0.95**	**0.70-1.29**	**0.76**
VEGF			
Cells with cytoplasmic expression			
Per degree of positive cells			
Percentage	**0.77**	**0.45-1.31**	**0.34**
EGFR			
Cells with membrane expression			
Per degree of positive cells			
Percentage	**0.79**	**0.58-1.07**	**0.13**
Duox			
Per degree of positive cells			
percentage	**0.14**	**0.02-0.97**	**0.04**
P53			
Cells with nuclear expression			
Per degree of positive cells			
Percentage	**0.86**	**0.62-1.16**	**0.32**

5. DISCUSSION

Poorly differentiated (PDFC) carcinomas of the thyroid represent an heterogeneous but distinct group of tumors, clinically and histopathogenetically intermediate between follicular-derived well-differentiated and anaplastic carcinomas. However, the criteria for the diagnosis of PD tumor are far from being well established. Indeed, although the number of the article written on the subject of PD carcinoma has grown exponentially during the last decade, the criteria of inclusion used by the various authors are very different. Some pathologists selected these tumors on the basis of growth pattern (trabecular, insular, solid) (Sakamoto et al 1983, Carcangiu et al. 1984, Papotti M et al 1993, Pellegritti et al. 2002, Volante et al. 2004, Pulcrano et al 2007, Ruffini et al. 2007), others used of high-grade histology (mitotic counts and necrosis) rather than a specific growth pattern (Hiltzik et al. 2006), whereas still others attempted to combine both approaches (Volante et al. 2007). Moreover, there are additional source of controversies on PD carcinomas. Some studies incorporated both follicular-derived tumors and papillary-derived tumors into PD carcinoma group (Nishida et al 1999, Papotti et al. 1993) whereas others classify the tumors with TIS growth pattern associated with characteristic nuclear features of papillary carcinoma as the solid variant of this tumor type (Nikiforov et al. 2001, Volante M et al. 2007).
The objective of the current study was to define the histological characteristics of PDFC, searching for relationships between the clinical presentation, outcome and histological features of this thyroid tumor. We also wanted to identify prognostic factors of PDFC correlating the histological architecture, cytological characteristics, the expression of markers of proliferation and of differentiation with survival and clinical outcome. Therefore, we identified 40 patients affected by PDFC, defined exclusively on the basis of growth pattern and we evaluated the prognostic significance of various clinical, histological and immunohistochemical characteristics. The small number of patients is due to the scarcity of PDFC and to the fact that we evaluated only those patients who were initially treated at Institut Gustave Roussy, and finally to the exclusion of tumors with a papillary component.
Our study confirms that PDFC had a clinical course intermediate between the indolent course observed in well differentiated thyroid carcinoma and the aggressive course of undifferentiated carcinoma (Sakamoto et al 1983, Volante et al. 2004, Hiltzik et al 2006, Pellegritti et al. 2002, Volante M and 2007). Indeed, at 5 years the overall survival rate was 63% and the metastasis free survival rate was 57%. In a recent retrospective study of PDC defined by the presence of mitosis and necrosis, the survival rate was similar (Hiltzik et al 2006). This similarity is probably due to the aggressive behavior of PDC, independently of whether they are defined on the basis of growth pattern and/or other criteria.
We did not find any relationship between the presence of a particular histological subtype and metastasis free survival. Only, a polymorph

architecture was more frequently observed in patients without distant metastases in the univariate analysis, and this may be related to the presence of a more differentiated component in these tumors. As reported in literature (Volante et al. 2004), the presence of Hurthle cells was not associated with a more aggressive behavior.

Our study confirms that prognostic factors identified for well differentiated thyroid cancers also apply to PDFC. An older age at the time of initial treatment was associated with a poor outcome (Volante et al. 2004, Baloch and LiVolsi 2001, d'Avanzo 2004, Papotti et Bussolati 1994), and in patients older than 45 years, metastases free survival and overall survival rates were lower (Fig.3). A larger tumor size (larger than 4 cm) and/or the invasion of the thyroid capsule was associated with a higher risk of distant metastases and with a poor survival (Hiltzik et al 2006, Baloch and LiVolsi 2001, d'Avanzo 2004, Papotti et Bussolati 1994). Finally, survival rate was lower among patients with distant metastases, and the 12 deaths were observed in the 18 patients who developed distant metastases.

Concerning the immunohistochemical study, an absence of detectable TPO expression has been reported in differentiated and in undifferentiated thyroid carcinomas (Weber 2004, De Micco 1994, De Micco 1994). In the current study, detectable TPO expression was associated with the absence of metastases. In positive cells, TPO was located intra-cellularly without membrane staining, indicating an abnormal processing of the protein.

We found a significant relationship between high Duox expression and a reduced thyroid cancer mortality. Similarly, in well differentiated thyroid carcinomas, Duox expression is related to tumor differentiation and is found in neoplastic tissues able to pick up radioiodine and with detectable expression of TPO, pendrin and sodium iodide symporter (De Micco 1994, Caillou 2001). These results suggest that persistent functional differentiation indicates a less aggressive behavior.

For cell cycle markers, expression of cyclins A, B1 and E was found in more than 70% of tumors, in accordance with previous reports. However, no significant differences were observed between patients with and without distant metastases. We do not confirm the low nuclear expression of cyclin E and p27 that was reported in papillary and undifferentiated carcinoma (Pickett 2005). P53 staining was positive in 50% of PDFC studied, in accordance with previous reports (Pollina et al 1996, Saltman 2006). However, no significant correlation between P53 expression and aggressiveness was found in our patients.

Ki 67, a nuclear protein expressed throughout the cell cycle, but not in the G0 or early G1 phase, is an important prognostic indicator in various types of tumors and is over-expressed in undifferentiated thyroid carcinoma (Saiz 2002). In our series of PDFC, Ki67 staining was positive in 72% of tumors but was relatively low in most positive tumors, being higher than 10% in only 30% of tumors. It was not different between patients with and without distant metastases, and high Ki67 labeling index was not associated with a shorter

survival rate.
In the multivariate analysis of immunohistochemical parameters, EGFR and VEGF expressions were frequently strongly expressed, but their expression had no prognostic significance. Vascular endothelial growth factor (VEGF) is one of the major pro-angiogenic factors in thyroid tumors and inhibitors of VEGF or of VEGFR reduce the growth of FTC xenografts (Soh 1997, Younes 2005). EGFR is a trans-membrane cell-surface glycoprotein that stimulates follicular cell proliferation and enhance thyroid cancer invasiveness (Carpenter and Cohen 1990, Ullrich and Schlessinger 1990, Aaronson 1991). An increased expression of EGFR has been found in poorly and undifferentiated thyroid carcinomas (Hoelting 1994, Aasland 1990, Schiff 2004, van der Laan 1995, Westermark 1996, Akslen 1993), and EGFR inhibitors inhibit the growth of anaplastic carcinoma (Schiff 2004).
The absence of a prognostic significance of these immunohistochemical parameters may be related to different reasons: first, a limited number of cases was included in our study because of our restricted selection criteria (the presence of TIS growth pattern and absence of distinctive nuclear features of papillary carcinoma); second, we selected a series of PDFC patients with a homogeneous phenotype. Therefore, an elevated number of evaluated parameters associated to a restricted sample of PDFC could have reduced the statistical significance, and further studies on a wider sample of PDFC patients are necessary.
It could be useful to assess other parameters by immunohistochemistry. Interestingly, in a recent study, this technique has helped shown an inverse relationship between TSH-receptor expression and proliferative activity in PDFC (Matsumoto et al 2008). Moreover, other studies have found a bcl-2 expression in advanced thyroid carcinoma (Pollina L 1996) with a very intense staining in insular areas (Pestereli HF 2001).
Therefore, since the absence of iodine uptake is a major prognostic indicator (Volante 2008) and our patients without metastasis showed a normal iodine uptake, it would be interesting to evaluate the expression of other proteins involved in iodine metabolism (NIS, Pendrin, hAIT) by immunohistochemistry.
Additionally, techniques of investigation other than immunohistochemistry should also be performed in PDFC. For example, although it is generally accepted that PDFC may arise either from pre-existing well differentiated thyroid carcinoma or de novo, specific genetic pathways in PDFC have not been clear; therefore, mutational analyses of different genes involved in thyroid tumorigenesis could be an interesting approach. Different studies suggest that FTCs develop through two distinct and virtually non overlapping molecular pathways initiated by either RAS point mutations or PAX8-PPARγ rearrangement.
The RAS gene family is comprised of three members (H-, K-, N-ras), which encode G-proteins involved in the signal transduction of various growth factor receptors. RAS gene point mutations were detected in 55-62% of PDFC (Pilotti

et al 1997, Garcia-Rostan et al 2003) with a significant link between RAS alterations and loss of thyroid tumor differentiation (Garcia-Rostan et al 2003) by the inhibition of thyroid-specific genes such as thyroid transcription factor-1 (TTF1), an activator of Tg promoter transcription (Dai et al 1996) and PAX-8, an activator of both TPO and Tg promoter transcriptions. Moreover, normal follicular cells transfected with a mutant ras gene showed reduced or abolished differentiation markers such as thyroglobulin, TPO and NIS (Francis-Lang et al 1992, Portella et al 1999).

A chromosomal translocation that fuses PAX-8 with PPARγ and causes expression of PAX8-PPARγ fusion protein (PPFP) was found in FTC (Cheung L et al 2003, Dwight T et al 2003, Lacroix L et al 2005). PAX8-PPARγ seemed to stimulate the transcription of TPO and NIS promoters but failed to stimulate the transcription from the thyroglobulin promoter and blocked the synergistic stimulation by wild-type PAX8 and thyroid transcription factor-1 (Au et al 2006). Moreover, both TTF-1 and PAX8 mRNA expressions were absent in undifferentiated thyroid carcinoma (Fabbro et al 1994). Therefore, these results may insinuate a role of mutant RAS or PAX-8- PPARγ in thyroid tumor dedifferentiation and development of PDFC and hypothesize a possible prognostic value of these genetic changes in PDFC.

6. CONCLUSIONS

Our study confirms that PDFC had has a more aggressive behavior than well differentiated thyroid carcinoma with a clinical course intermediate between the indolent course of well differentiated thyroid carcinoma and the aggressive course of undifferentiated carcinoma. Moreover, in our study the survival rate was similar with that of a retrospective study of PDFC defined by the presence of mitosis and necrosis (Hiltzik et al 2006). This similarity underlie the aggressive behavior of PDC independently if they are defined on basis of growth pattern and/or histological grade. These data also confirm that PDFC are a different entity with a clinical outcome worse than well-differentiated thyroid carcinomas suggesting the necessity of a new and clear classification of these tumors.

As expected, advanced age and larger tumor size are associated with an aggressive behaviour. Indeed, in patients older than 45 years, metastases free survival and overall survival rates were lower and a larger tumor size (larger than 4 cm) and/or the invasion of the thyroid capsule was associated with a higher risk of distant metastases and with a poor survival. Moreover, death occurred in patients with distant metastases.

Surprisingly, in our patients detectable TPO expression was associated with the absence of metastases. However, in positive cells TPO was located in the cytoplasm suggesting an abnormal processing of the protein.

Moreover, we found that a high expression of Duox was significantly associated with a reduced risk of thyroid cancer mortality, suggesting that the persistence of a functional differentiation indicates a less aggressive behaviour.

Therefore, the parallel evaluations of clinical, histological and immunohistochemical characteristics of PDFC allow to achieve the following conclusions:

- an accurate histological classification is mandatory to recognize and uniform PDFC diagnosis, to separate this category from well differentiated thyroid cancer and to regulate the treatment and follow up to tumor histology.
- in PDFC the thyroid-specific enzymatic pathway, responsible of iodine metabolism, is severely modified and, of consequence, radioiodine treatment is inefficient in the majority of these tumors. Immunohistochemistry is useful to define the degree of expression and localization of many thyroid-specific proteins. In our study, immunohistochemical data provide prognostic information showing a high expression of DUOX in PDFC without metastasis.
- immunohistochemistry is of limited usefulness to achieve a diagnosis of PDFC but, in future, it may have a role to predict response to receptor-targeted therapy through the availability of novel cellular markers.

7. ACKNOWLEDGMENTS

This study was done during a fellowship from the Italian-French University in collaboration with the Institut Gustave Roussy (Villejuif), University "Paris Sud", Departments of Pathology and Nuclear Medicine and Endocrine Oncology and with the University of Naples "Federico II", Department of Molecular and Clinical Endocrinology and Oncology.
My special thanks go to Prof. Martin Schlumberger, who gave me the opportunity to work with him and his research team in Villejuif. He supported me from the first time I met him in Naples and during all my doctorate.
I would like to express my gratitude at Dr. Bernard Caillou who helped me to know and appreciate the histological study of thyroid tumors.
I would like thank Dr Houda Boukeris who helped me to elaborate the statistical analysis.
My thanks to Prof. Giancarlo Vecchio, Coordinator of Doctorate School in Molecular Medicine, University of Naples Federico II.
My special thanks go to Prof. Bernadette Biondi for her support during the whole period of my work. She guided me in discovering and investigating the fascinating field of thyroid tumors. I would like also thank Prof. Gaetano Lombardi who has always trusted me and my work.
My thanks to Monique Talbot to train me laboratory and immunohistochemical procedures and all of the people, in Villejuif and in Naples, who offered me all of their experience and competence during my Doctorate.

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LIST OF PUBLICATIONS

This dissertation is based upon the following publications:

1. Pagano L, Klain M, Pulcrano M, Angellotti G, Fasano F, Salvatore M, Lombardi G, Biondi B. Follow-up of differentiated thyroid carcinoma. Minerva Endocrinol. 2004 Dec;29(4):161-74.

2. Pulcrano M Boukheris H, Talbot M, Caillou B, Dupuy C, Virion A, De Vathaire F, Schlumberger M. Poorly differentiated follicular thyroid carcinoma: prognostic factors and relevance of histological classification. Thyroid. 2007 Jul;17(7):639-46.

3. Biondi B, **Pulcrano M,** Pagano L, Lombardi G Adjuvant treatment with thyrotopin alfa for remnant ablation in thyroid cancer Biologics: Targets & Therapy *in press*

MINERVA ENDOCRINOL 2004;29:161-74

Follow-up of differentiated thyroid carcinoma

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Thyroid cancer is the most common endocrine malignancy. More than 90% of primary thyroid cancers are differentiated papillary or follicular types. The treatment of differentiated thyroid carcinoma (DTC) consists of total thyroidectomy and radioactive iodine ablation therapy, followed by L-thyroxine therapy. The extent of initial surgery, the indication for radioiodine ablation therapy and the degree of TSH-suppression are all issues that are still being debated in relation to the risk of recurrence. Total thyroidectomy reduces the risk of recurrence and facilitates ^{131}I ablation of thyroid remnants. The aim of radioiodine ablation is to destroy any normal or neoplastic residuals of thyroid tissue. These procedures also improve the sensitivity of thyroglobulin (Tg) as a marker of disease, and increase the sensitivity of ^{131}I total body scan (TBS) for the detection of persistent or recurrent disease. The aim of TSH-suppressive therapy is to restore euthyroidism and to decrease serum TSH levels, in order to reduce the growth and progression of thyroid cancer. After initial treatment, the objectives of the follow-up of DTC is to maintain adequate thyroxine therapy and to detect persistent or recurrent disease through the combined use of neck ultrasound (US) and serum Tg and ^{131}I TBS after TSH stimulation. The follow-up protocol should be adapted to the risk of recurrence. Recent advances in the follow-up of DTC are related to the use of recombinant human TSH (rhTSH) in order to stimulate Tg production and the ultrasensitive methods for Tg measurement. Undetectable serum Tg during TSH suppressive therapy with L-T4 does not exclude persistent disease, therefore serum Tg should be measured after TSH stimulation. The results of rhTSH administration and L-thyroxine therapy withdrawal are equivalent in detecting recurrent thyroid cancer, but the use of rhTSH helps to avoid the onset of hypothyroid symptoms and the negative effects of acute hypothyroidism on cardiovascular, hepatic, renal and neurological function. In low-risk DTC patients serum Tg after TSH stimulation, together with ultrasound of the neck, should be used to monitor persistent disease, avoiding diagnostic TBS which has a poor sensitivity. These recommendations do not apply when Tg antibodies are present in the serum, in patients with persistent or recurrent disease or limited thyroid surgery. Low-risk patients may be considered to be in remission when undetectable Tg after TSH stimulation and negative US evaluation of the neck are present. On the contrary, detectable Tg after TSH stimulation is an indicator in selecting patients who are candidates for further diagnostic procedures.

Key words: **Differentiated thyroideal neoplasms, therapy - Follow-up - Neoplasms, therapy.**

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Thyroid cancer is the most common endocrine malignancy. In Europe, thyroid cancer is diagnosed in approximately 20 000 people each year and more than 200 000 thyroid cancer patients are being followed-up.[1] In recent years, the follow-up of differentiated thyroid carcinoma (DTC) has changed in relation with the changes in the clinical spectrum of the disease, more effective treatment and the availability of more accurate diagnostic techniques. As a consequence, the risk of recurrence and mortality has decreased.[2]

The follow-up of DTC for detecting persistent or recurrent disease is based on the evaluation of the tumor marker thyroglobulin (Tg), neck ultrasonography (US) and eventually on total body scan (TBS) with ^{131}I.

A high level of thyroid stimulating hormone (TSH) improves the sensitivity of Tg monitoring and is required to stimulate sufficient radioiodine uptake for diagnostic imaging or therapy. This can be obtained following either prolonged withdrawal of thyroid hormone treatment or injections of recombinant human TSH (rhTSH).

Classification and staging

Malignant thyroid tumors are classified into epithelial tumors (well-differentiated, *i.e.* papillary and follicular cancer, poorly differentiated and undifferentiated cancer), C cell derived carcinoma (medullary thyroid cancer), non epithelial tumors (lymphoma, sarcoma, and hemangioendothelioma) and secondary tumors. More than 90% of primary thyroid cancers are papillary or follicular, papillary cancer being the most frequent type in countries where iodine deficiency has been corrected.[3] In the USA between 1985 and 1995, the ten-year mortality rates for DTC were about 7% for papillary, 15% for follicular and 25% for Hurthle cell cancer.[3]

There are various staging systems for DTC, among which the TNM system is the most widely used. It includes size and extent of the thyroid tumor, presence of lymph node or distant metastases, and patient's age.[4] Moreover, several other prognostic factors are important, such as the degree of differentiation, the presence or absence of multifocality, vascular invasion in follicular tumors and incomplete surgical resection.[5]

Initial treatment

The treatment of DTC consists in total thyroidectomy and radioactive iodine ablation therapy, followed by L-thyroxine therapy.[2, 6, 7]

The extent of initial surgery, the indication for radioiodine therapy and the degree of TSH suppression are issues that are still debated in relation to the risk of recurrence, particularly in low-risk patients.

Surgical therapy

Surgical treatment of DTC includes total thyroidectomy, because it reduces the risk of recurrence in the contralateral lobe, and facilitates ^{131}I ablation of thyroid remnants. This procedure also improves the sensitivity of Tg as a marker of disease, and increases the sensitivity of ^{131}I TBS for the detection of persistent or recurrent disease.[8] The benefit of total thyroidectomy compared to lobectomy on recurrence rate was demonstrated in low and high risk patients.[9, 10]

Papillary thyroid cancer (PTC) tends to be a multifocal disease, and histological studies have shown microscopic cancer foci in the contralateral lobe in 30-80% of cases.[11] Total thyroidectomy is recommended in patients with PTC of more than 1 cm in diameter and whatever the size in case of previous neck irradiation, of family history of thyroid cancer, contralateral nodularity at palpation or ultrasound, lymph node metastases and multiple foci of papillary cancer in the excised lobe.

However, in cases of solitary and completely excised papillary cancer less than 1 cm in diameter, the risk of recurrence is so small that there is no reason to perform total thyroidectomy or radioiodine treatment.[12]

There is some controversy concerning the extent of lymph node dissection at the time of initial surgery. The presence of metastatic lymph nodes at the time of surgery increases tumor recurrence rates, but its impact on

survival is controversial.[10, 13] In patients with a papillary thyroid carcinoma, lymph node metastases are found in 35-65% of cases and in up to 80% of childhood cases.[14] Lymph node metastases are less frequent in patients with follicular carcinoma, being observed in less than 20% of cases. Central lymph node dissection is recommended at initial surgery in patients with papillary thyroid cancer [15] because lymph node metastases are frequent and difficult to detect, ^{131}I therapy rarely eradicates metastases exceeding 1 cm, and Tg concentrations are undetectable on thyroxine therapy in 20% of patients with lymph node metastases. Moreover, additional surgery in the central neck compartment, is associated with higher complication rates.

Radioiodine therapy for remnant ablation

The aim of postoperative iodine ^{131}I therapy is to eradicate residual tumor and remnant thyroid tissue. The presence of residual thyroid tissue may prevent visualization of less active cancer sites and may reduce the usefulness of Tg as a tumor marker in the monitoring of thyroid cancer. A highly sensitive scan can be performed 2-5 days after the administration of a high dose of ^{131}I.

Radioiodine therapy is recommended for invasive follicular and papillary cancers >2 cm, or whatever the size for locally invasive tumors, or with an extensive regional involvement or incomplete resection.[12] Radioiodine ablation is performed 4-6 weeks after surgery during which any thyroid hormone treatment is withheld leading to TSH concentration above 25-30 mU/l.[16] There is not benefit of ablation in patients with tumor smaller than 1.5 cm, completely confined to the thyroid gland, in whom ablation is clearly not indicated.[17]

Also the optimal ^{131}I dose for thyroid ablation has yet to be established. The empiric dose used in most studies ranges from 30 mCi to 100 mCi, with successful ablation rates of 60% to 90%. An alternative approach is to evaluate the dose for ablation by quantitative dosimetry.[18] An uptake >5% in the thyroid bed is an indication for completion thyroidectomy. Some centers no longer use pre-therapy diagnostic scanning before ablation with ^{131}I. In fact, 2-5 mCi of ^{131}I may "stun" remnant tissue and reduce the uptake of the subsequent therapeutic ^{131}I dose,[19] and when necessary the use of ^{123}I has been advocated.[20]

The most frequent complication of radioiodine treatment is sialodenitis, which occurs in 5-40% of patients undergoing radioiodine therapy, and that can be prevented by increasing the salivary flow.[16] Also transient leucopenia and oligospermia in males have been reported. High cumulative radioiodine doses can induce secondary malignancies, solid tumors and leukaemias, suggesting the necessity to delineate the indications for ^{131}I treatment in thyroid cancer patients.[21]

TSH-suppressive therapy

The aim of TSH suppressive therapy is to restore euthyroidism and to decrease serum TSH to a level that reduces the growth and progression of thyroid cancer. TSH is an important growth factor for thyroid cells. Well differentiated epithelial thyroid cancer cells have TSH receptors [22] and there is clinical evidence of the progression of thyroid cancers during TSH stimulation.[23, 24] Retrospective clinical trials, without stratification for tumor stage and histology subtypes, demonstrated a lower rate of tumor recurrences for patients with TSH levels <0.05 mU/l compared with patients with lesser degrees of suppression.[10]

There is some controversy about the degree and duration of TSH suppressive therapy, and there is no evidence that complete TSH suppression, *e.g.*, <0.01 mU/l or <0.001 mU/l, is better than mild suppression, *e.g.*, 0.1 mU/l. TSH suppressive thyroid hormone therapy may induce iatrogenic subclinical hyperthyroidism, which will have detrimental effects on the skeletal and cardiac system.[25, 26] Thus, it appears important to evaluate the risk benefit ratio of TSH suppressive therapy in each patient and to take into consideration the tumor stage, the patient's age and the clinical status.

TABLE I.—*Low-risk patients.*

— Tumor <4 cm, no virulent subtype, no metastases
— Total thyroidectomy
— ^{131}I ablation of DTC
— No clinical evidence of disease
— Undetectable serum Tg levels during THST
— Negative anti-Tg antibodies

Follow-up of DTC

After initial treatment, the aims of follow-up of DTC is to maintain adequate L-T4 therapy and to detect persistent or recurrent tumor. The follow-up protocol should be adapted to the risk of recurrence. Monitoring of DTC consists of 4 stages: control at the moment of radioiodine ablation, evaluation after 3 months on L-T4 therapy, evaluation at 6-12 months after TSH stimulation and subsequent follow-up.[2]

The first control after ablation consists of Tg measurement during hypothyroidism and post therapy TBS 3-5 days after the administration of ^{131}I. At this stage, a low or undetectable Tg concentration is indicative of a favorable prognosis, whereas an elevated Tg concentration may be related to persistent disease or residual thyroid tissue.

At the three-month follow-up, TSH, FT3, FT4 and Tg levels are measured during L-T4 therapy to determine whether the L-T4 dose is correct. At this stage, a TSH concentration <0.1 mU/l with normal FT3 concentrations is indicative of an appropriate dose of L-T4. Serum Tg and neck ultrasound are performed for disease evaluation. These 2 steps are used to distinguish between patients with persistent disease requiring additional treatments, and patients without evidence of disease, in whom only a long-term follow-up is necessary.[2]

Low-risk patients are those submitted to complete tumor resection who have no clinical evidence of tumor, with absence of uptake outside the thyroid bed on post ablative TBS, undetectable serum Tg during TSH suppressive therapy in the absence of tg-antibodies (TgAb), and negative neck ultrasound (Table I). In these patients the aim of follow-up is the early detection of persistent or recurrent disease. More than 80% of the DTC patients are in this group.[2]

Two recent consensus reports [2, 27] emphasized that in low-risk DTC patients, serum Tg after TSH stimulation, together with ultrasound of the neck, should be used to monitor for persistent disease. The reports indicated that diagnostic TBS has a poor sensitivity in detecting disease. These recommendations do not apply to the case of TgAb in serum, to patients with persistent or recurrent disease and to patients with limited thyroid surgery.

The recent advances in the follow-up of DTC are the use of rhTSH to stimulate Tg production, ultrasensitive methods for Tg measurement, and innovative techniques of neck US and [^{18}F-^{2}Fluoro-^{2}Desossiglucosio] Positron Emission Tomography (FDG-PET).

rhTSH

Thyrogen is a heterodimeric glycoprotein produced by recombinant DNA technology. It is comprised of 2 non-covalently linked subunits. The amino acid sequence of rhTSH is identical to that of human pituitary TSH and it shares some of its biochemical properties. The binding of rhTSH to TSH receptors stimulates iodine uptake, iodine organification, synthesis and secretion of Tg, T3 and T4.[28]

After Food and Drug Administration approval of rhTSH (rhTSH; TSHα; Thyrogen; Genzyme Corp.,) for DTC monitoring, many studies compared the results of Tg testing and TBS obtained after thyroid hormone withdrawal and after rhTSH.[29-33]

Available data suggest that in clinical practice, the results of rhTSH administration and L-T4 therapy withdrawal are equivalent in detecting recurrent thyroid cancer, but the use of rhTSH permits to avoid the onset of hypothyroid symptoms and the negative effects of acute hypothyroidism on cardiovascular, hepatic, renal and neurologic function.[34] In particular, it avoids the acute effects induced by hypothyroidism on the cardiovascular system, *i.e.*, reduced heart rate in basal condition and during exercise, diastolic dysfunction, increased systemic vascular

resistance, with evidence of ECG abnormalities.[34] The greater cost of rhTSH is balanced by the negative economic and professional consequences due to the patient's absence from work during L-T4 withdrawal (mean 0.7 days of missed work after rhTSH *vs* 13.7 days after L-T4 withdrawal).[2] Side effects such as nausea, asthenia and fever are transient and mild, and occur in 20% of rhTSH-stimulated patients, and no patient has ever shown anti-rhTSH antibodies.

Measurement of serum Tg and Tg antibodies

TSH stimulates the production of Tg by normal and neoplastic thyroid tissue. Consequently, the clinical sensitivity of Tg testing is better when TSH levels are elevated or after rhTSH stimulation than during TSH-suppressive therapy.

The persistence or reappearance of elevated Tg values in the follow-up of patients with DTC is indicative of recurrence or metastases. During LT-4 treatment, serum Tg is undetectable in 98% of patients in remission after total thyroid ablation.[35] In about 20% of patients with isolated lymph-node metastases and in 5% of patients with small lung metastases, serum Tg is undetectable during TSH suppressive therapy, but may increase after withdrawal of hormone therapy. It remains however undetectable in about 5% of cases with isolated lymph-node metastases and in less than 1% of cases with distant metastases.[8] These false negative results may be due to small neoplastic areas in neck lymph nodes, which are identified with neck ultrasound. In fact, the amount of circulating Tg is correlated with tumor burden. It is estimated that 1 g of tumoral thyroid tissue increases serum Tg by about 0.5-1 ng/ml during therapy, and about 5-10 times after withdrawal of thyroxine.[36]

In patients in whom Tg is not detected after TSH stimulation, the risk of recurrence at 10 years is <1%. However, Tg may remain detectable after radioiodine treatment for up to 1 year, after which it will decrease in 1/3 to 2/3 of cases, in the absence of any further treatment due to the progressive disappearance of irradiated thyroid remnant. On the contrary, an increasing Tg value suggests tumor recurrence or metastases.[37, 38] The pattern of change in serial Tg measurements during follow-up of DTC patients is more important than an isolated Tg value.

In cases of Tg antibodies, the follow-up protocol includes the evaluation of TBS with neck ultrasound. In the absence of disease, Tg antibodies will decrease and disappear within the first 2 years of follow-up.[2] Tg is falsely lowered by anti-Tg antibodies when immunoradiometric or immunochemilumiscent assay are used.[39] Usually, an assay for Tg evaluation will be normalized to the international standard and should have a functional sensitivity of 0.5-1 ng/ml.[40] It is suggested that Tg antibodies be measured on the same serum sample as Tg. The recovery test assay is used to evaluate the degree of interference of anti-Tg antibodies.[12]

Tg alone vs Tg and whole body scan after TSH stimulation

In the absence of Tg antibodies, Tg monitoring alone is more sensitive than TBS in detecting recurrent disease. Diagnostic TBS only confirm the completeness of thyroid ablation. Endogenous TSH-stimulated serum Tg level produced by thyroid hormone withdrawal is in general higher than after rhTSH-stimulation, but the sensitivity for detecting persistent or recurrent disease is similar when using a sensitive assay and when any detectable level is taken into account. Moreover, in patients with undetectable levels of Tg during TSH suppressive therapy who have no clinically residual cancer, measurement of rhTSH-stimulated Tg concentration distinguishes patients who are disease-free from those with tumor who require further diagnostic testing, therapeutic procedures or both.[35, 41-43]

In conclusion, recent studies including 2 000 consecutive patients, after LT4 withdrawal, rhTSH or a combination of these methods have shown that TBS did not add any information to Tg testing in low-risk patients with undetectable rhTSH stimulated

Tg level. In these studies, no patients with negative Tg had positive TBS, defined as having uptake outside the thyroid bed and no more than a fraction of Tg-positive patients were also TBS positive.[2]

Role of neck US

In low risk patients, neck lymph nodes are the most frequent site of recurrence (60-70%), especially in papillary carcinoma. Therefore, neck US should be routinely performed during follow-up. Suspected lymph nodes are generally located in the lower part of the jugulo-carotid chains or in the central compartment. US can detect lymph node metastases as small as 2-3 mm in diameter.[36] If lymph-node metastases are suspicious, US-guided fine needle biopsy of the lymph node is performed for cytology and measurement of Tg in the aspirate.[44, 45] PCR-based technique by the amplification of thyroid specific transcripts TSH-receptor and Tg can detect thyroid cancer metastases in small lymph-node <1.5 cm.[46] Neck US can detect small neoplastic foci in case of undetectable serum Tg, and thus provides early evidence of disease recurrence.[47-49]

Other diagnostic procedures

In the presence of negative TBS, detectable Tg can select the patients who need further diagnostic procedures, such as computed tomography (CT) or magnetic resonance imaging (MRI). Non specific isotopic scan (Thallium-201, Technetium-99m tetrofosmin, ^{99m}Tc-sestamibi and Indium 111 Pentreotide) have little interest, if any. The limit of CT is the use of iodinated radiocontrast agent that can interfere with radioiodine treatment for 6 weeks. Helical chest CT is useful to detect lung metastases in patients with detectable Tg and negative TBS.

Using PET scanning in DTC

In recent years, PET scanning with 18-fluorodeoxyglucose (FDG) has become a useful test in the evaluation of patients with elevated Tg levels and negative TBS. ^{18}F-fluorodeoxyglucose is an indicator of poor functional tumor differentiation, and its uptake is indicative of a poor prognosis in thyroid cancer patients. In patients with increased Tg values and negative TBS, PET scan could be considered an important new non-iodine radionuclide imaging tool to detect recurrences and metastases.[50-52] The combination of PET/CT scan is particularly able to focus the disease on a specific area of the body thus facilitating radiological interpretation, improving the localization of the disease.[51] FDG-PET is useful mostly to detect mediastinal metastases without ^{131}I uptake. However FDG-PET is not tumor-specific. RhTSH may enhance FDG uptake because TSH increases glucose uptake and metabolism of thyroid cells with a consequent increase in GLUT1 glucose transporter expression.[53]

Conclusion

In conclusion, undetectable serum Tg during TSH suppressive therapy with L-T4 does not exclude persistent disease, therefore serum Tg should be measured after TSH stimulation. The results of Tg evaluation after rhTSH administration or L-T4 withdrawal are similar. Tg is detectable after rhTSH in 15-20% of patients with undetectable Tg during L-T4 treatment. Undetectable Tg does not exclude lymph node metastases in the neck. Therefore, neck ultrasound must be performed in the follow-up. Moreover, in patients with Tg <1 ng/ml (80% of cases) after stimulation (rhTSH or withdrawal), TBS does not provide additional information, whereas ultrasound may detect small recurrences in neck lymph nodes.

The follow-up of DTC in low-risk patients includes Tg measurement during TSH stimulation at 6-12 months after radioiodine ablation. When serum TSH is undetectable in basal condition and does not convert to detectable after endogenous or exogenous TSH stimulation, and if Tg antibodies are not present and ultrasound evaluation of the neck is negative, the patient may be considered in remission. After this first step of follow-up, these patients may be followed with a

periodic evaluation of neck US and of serum Tg during L-T4 therapy.

Differently, if stimulated Tg is elevated, there is the need of diagnostic procedure (US, TC, PET) to localize local or distant metastases. In some patients with elevated serum Tg, the diagnostic TBS is falsely negative but becomes positive after a therapeutic dose of radioiodine.[54-56] In patients with an elevated rhTSH-Tg concentration (>5-10 ng/ml) guidelines suggest that diagnostic scanning be avoided, and that patients be treated with a therapeutic dose of ^{131}I after L-T4 withdrawal.[15] If post-therapy WBS is negative, the PET scan may be useful to evaluate the presence of metastases in order to treat the patient with a different therapeutic approach.

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THYROID
Volume 17, Number 7, 2007
© Mary Ann Liebert, Inc.
DOI: 10.1089/thy.2007.0029

Clinical Research

Poorly Differentiated Follicular Thyroid Carcinoma: Prognostic Factors and Relevance of Histological Classification

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Objective: Poorly differentiated follicular thyroid carcinoma (PDFC) is a tumor of follicular cell origin with attributes intermediate between well-differentiated carcinomas and anaplastic carcinomas, but neither a clear histological description nor an established definition of prognostic indicators are available. *Design*: This study correlates the clinical outcome and survival of 40 PDFC patients with histological architecture, cytological characteristics, and expression of various markers of cell proliferation and differentiation (cyclin A, cyclin B1, cyclin D1, cyclin E, Ki67, thyroperoxidase, galectin 3, dual oxidase [Duox], vascular endothelial growth factor, epidermal growth factor receptor, and p53). *Main outcome*: At 5 years, the overall survival rate was 63% and the metastasis-free survival rate was 57%. An older age at the time of diagnosis and a larger tumor size were associated with an increased risk of distant metastases and of cancer-related death. Polymorph architecture was associated with a reduced risk of metastases, whereas a high expression of Duox was associated with a reduced risk of death. In these patients with PDFC, no other histological features or expression of any other marker had a prognostic significance. *Conclusion*: PDFC has a more aggressive behavior than well-differentiated carcinomas; prognosis is related to indicators that are also relevant in patients with well-differentiated carcinomas.

Introduction

Follicular thyroid carcinoma accounts for approximately 10% of all thyroid malignancies (1) and constitutes a heterogeneous group of tumors that includes tumors with favorable prognosis and tumors with an aggressive behavior.

The World Health Organization classification individualizes the poorly differentiated carcinoma (PDC), which is defined as "a tumor of follicular cell origin with morphological and biological attributes intermediate between differentiated and anaplastic carcinomas of the thyroid" (2–4). Its clinical course is more aggressive than that of well-differentiated carcinomas (5–11). However, the histological definition of PDC is still controversial and various diagnostic criteria have been proposed (5–9). The most distinctive histological feature is the presence of trabecular, insular, microfollicular, and/or solid growth pattern. Furthermore, foci of necrosis, high mitotic rates, extra-thyroid extension, and blood vessel invasion are common (5–9).

Moreover, prognostic indicators are not clearly identified in PDC. Indeed, the pathological tumor-node-metastasis (pTNM) classification was established in patients with well-differentiated thyroid carcinoma, and has not yet been validated for PDC (12,13).

Recent studies in various human malignancies indicate that the expression of cell cycle regulators has a prognostic utility (14,15). Ki67, a nuclear protein expressed throughout the cell cycle, but not in the G0 or early G1 phase, is an important prognostic indicator in various types of tumors. In papillary thyroid carcinoma, low nuclear expression of cyclins D1 and E was observed in stage IV (16). In anaplastic thyroid carcinoma, cyclin A is overexpressed in most tumors

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and cyclin B is overexpressed in only 19% of these tumors (17).

Also, in well-differentiated thyroid carcinomas, the expression of thyroid functional genes, including thyroperoxidase (TPO), is decreased and that of Galectin 3 is increased, but their expression has not been carefully studied in PDC (18–20). Dual oxidase (Duox) is a glycoflavoprotein involved in thyroid hormone biosynthesis as the hydrogen peroxide generator in thyroid. Duox expression is found in well-differentiated thyroid carcinomas that are able to pick up radioiodine (^{131}I), and with detectable expression of TPO, pendrin, and sodium/iodide symporter (21). Finally, an increased epidermal growth factor receptor (EGFR) expression and frequent inactivating p53 mutations were found in PDC and seemed to correlate with tumor dedifferentiation (22).

In the current study, 40 patients affected by poorly differentiated follicular thyroid carcinoma (PDFC) were identified on the basis of a trabecular, insular, or solid (TIS) growth pattern. We studied their clinical outcome according to histological architecture, cytological characteristics, and expression of various markers of cell proliferation and differentiation with the intent of defining prognostic indicators.

Materials and Methods

Patients

A list of patients initially treated at the Institut Gustave Roussy (IGR), Villejuif, France, between 1975 and 2000 was obtained from the central computer system of IGR. This search was conducted using the terms "thyroid," "carcinoma," and "follicular carcinomas." Patients affected by papillary, medullary, well-differentiated follicular, or undifferentiated thyroid carcinomas were not included in the study. Clinical charts of 200 patients were retrieved from the archives, and histological hematoxylin and eosin–stained slides were reviewed by a pathologist (B. Caillou), who was unaware of clinical data. This permitted the selection of 40 patients with PDFC, based on the presence of focal, extensive, or predominant TIS growth patterns. Poorly differentiated tumors along with oxyphilic (Hürthle cell) tumors, which differed from classical follicular carcinomas with respect to their mitochondrion-rich cytoplasm, were included in the study. The follicular cell origin of the tumors was confirmed by a positive thyroglobulin (Tg) staining and a negative staining for calcitonin.

Information regarding gender, age at diagnosis, TNM stage (23), therapy, and outcome was collected from all 40 patients. Date and site of recurrence and date of death were recorded. The end point was June 2005, the date of death or the date of loss to follow-up.

Histological study

The following pathological parameters were evaluated on hematoxylin and eosin–stained slides:

1. Tumoral architecture, defined as the presence of a TIS and/or microfollicular pattern—some tumors showed a polymorph pattern associating two or more of these features in separated areas of the tumor.
2. Hürthle cell carcinoma, defined as the presence of a majority (>75%) of tumor cells with eosinophilic and granular cytoplasm.
3. Presence of atypical nuclear pattern—we used a score between 1 and 3 (1: <1% atypical cells, 2: between 1% and 5% atypical cells, 3: between 6% and 10% atypical cells).

Immunohistochemical analysis

Slides were deparaffinized in xylene and rehydrated in a graded series of ethanol. To retrieve the antigenicity, sections were then placed in a preheated 10 mM citrate buffer (pH 6.0) and heated in an 850 W microwave. Sections were then immersed for 5 minutes in methanol containing 0.3% hydrogen peroxidase to block the endogenous peroxidase activity and were incubated for 30 minutes with 2.5–5% blocking horse serum to reduce nonspecific binding. Sections were incubated with a primary antibody according to previously validated conditions. Following several washes with phosphate-buffered saline, the slides were allowed to react for 30 minutes with a universal secondary biotinylated antibody and then with avidin-biotinylated horseradish peroxidase H complex (ABC kit, Vector Laboratories, Burlingame, CA). Diaminobenzidine was used as a chromogen, and commercial hematoxylin was used for counterstaining. For positive controls for each antibody, sections of thyroid previously validated by us to be strongly positive were used; for negative controls, incubation was performed without the primary antibody. Indeed, the expression of these markers in the tumor and in the normal thyroid tissue was compared in the 33 samples for which nontumoral tissue was available.

Specific antibodies used in the study are as follows: anti-cyclin A, anti-cyclin B, anti-cyclin E (1/25 dilution; monoclonal antibody; Novocastra, Newcastle upon Tyne, UK),

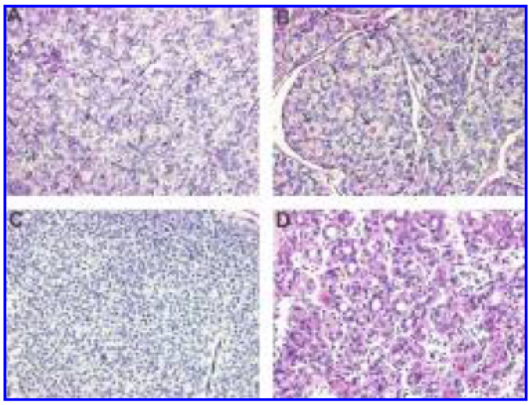

FIG. 1. Histological aspects of poorly differentiated follicular thyroid cancer (hematoxylin and eosin stain; original magnification: ×100). (**A**) Poorly differentiated trabecular carcinoma: dense proliferation of tumor cells without papillary or follicular structures. Note the absence of colloid formation and the presence of a trabecular pattern (arrows). (**B**) Poorly differentiated insular carcinoma: presence of "islands" of tumor cells with well-defined limits and with "holes" filled up with colloid (arrows). (**C**) Poorly differentiated solid carcinoma: diffuse solid pattern of tumor cells without any architecture. (**D**) Poorly differentiated microfollicular carcinoma: multiple microfollicles are seen.

Table 1. Clinical and Histological Factors of Distant Metastasis and of Death in 40 Patients with Poorly Differentiated Follicular Thyroid Carcinoma (Univariate Analysis)

Clinical characteristics	*Metastases*	*RR*	*95% CI*	p	*Death*	*RR*	*95% CI*	p
Age (years)								
≤45	04/18				0/18			
>45	14/22	1.08	1.04–1.12	<0.0001	12/22	1.12	1.06–1.17	<0.0001
Gender								
Male	07/18				4/18			
Female	11/22	1.47	0.57–3.8	0.42	8/22	1.76	0.53–5.87	0.36
T (TNM)[a]								
Stages 0, 1, 2	03/20				1/20			
Stages 3, 4	14/19	3.50	1.84–6.64	0.0001	10/19	4.36	1.80–10.6	0.001
Lymph nodes (TNM)[b]								
Absent	05/20				3/20			
Present	09/16	2.50	0.84–7.55	0.1	6/16	2.28	0.57–9.20	0.29
Histological characteristics								
Microfollicular								
Yes	12/27				8/27			
No	06/13	0.91	0.34–2.45	0.86	4/13	0.86	0.26–2.86	0.8
Trabecular								
Yes	16/31				10/31			
No	02/09	2.50	0.57–10.9	0.22	2/9	1.5	0.33–6.87	0.6
Insular								
Yes	03/08				3/8			
No	15/32	0.72	0.21–2.50	0.6	9/32	1.01	0.27–3.73	0.99
Solid								
Yes	02/03				1/3			
No	16/37	1.69	0.38–7.4	0.48	11/37	1.23	0.16–9.57	0.85
Polymorph								
Yes	03/14				3/14			
No	15/26	0.26	0.07–0.93	0.04	9/26	0.46	0.12–1.72	0.25
Hürthle[c]								
Yes	06/13				4/13			
No	12//26	1.04	0.38–2.84	0.94	8/26	1.02	0.30–3.44	0.98
Nuclear atypia								
Yes	09/24				9/24			
No	09/16	1.0	0.48–2.08	0.99	9/16	1.63	0.68–3.90	0.27

[a]One missing value for tumor size.
[b]One missing value for lymph node metastases.
[c]One missing value for the histological classification into Hurthle cells.
RR: recurrence rate; 95% CI: 95% confidence interval; TNM: tumor node metastasis.

anti-cyclin D1 (1/100 dilution; monoclonal antibody; Neomarkers, Fremont, CA), anti-Ki67 (1/25 dilution; monoclonal antibody; Dako, Glostrup, Denmark), anti-TPO (1/4 dilution; monoclonal antibody; BioCytex, Marseille, France), anti-galectin-3 (1/25 dilution; monoclonal antibody; Novocastra), anti-Duox [1/25 dilution; polyclonal antibody raised at IGR (21)], anti-Tg (1/100 dilution; monoclonal antibody; Dako), anti-vascular endothelial growth factor (VEGF) (1/50 dilution; polyclonal antibody; Santa Cruz, Heidelberg, Germany), anti-p53 (1/25 dilution; monoclonal antibody; Dako), and anti-EGFR (monoclonal antibody; Ventana, Illkirch Cedex, France) antiserum.

For each patient, all spots were analyzed and representative zones were chosen to count 500 cells. For each marker, the three following parameters were evaluated:

1. Localization of staining—nucleus, cytoplasm, and/or membrane.
2. Number of positive cells—we used a score between 1 and 4 (1: <1% positive cells, 2: between 1% and 5% positive cells, 3: between 6% and 10% positive cells, 4: >10% positive cells).
3. Intensity of expression—we used a score between 1 and 3 (1: low, 2: moderate, 3: high).

Statistical analysis

Overall and metastasis-free survival rates were studied according to clinical, histological, and immunohistological parameters by using univariate and multivariate analysis. Multivariate analysis included the variables for which *p*-value was less than 0.1 in the univariate analysis. For all analyses, two-sided tests were employed and the 0.05 level of significance was used. Survival rates and their 95% confidence intervals were estimated by the Kaplan–Meier method (24). The log-rank test (25) was used to compare overall and metastasis-free survival rates (univariate analysis). The relative risks of death and of occurrence of metastases according to each variable were estimated using Cox's proportional

Table 2. Immunohistochemical Factors of Metastases and Death in 40 Patients with a Poorly Differentiated Thyroid Carcinoma (Univariate Analysis)

	RR (for metastases)	*95% CI*	p	*RR (for death)*	*95% CI*	p
Cyclin A						
Cells with nuclear staining per percentage of positive cells	1.37	0.92–2.04	0.1	1.23	0.78–1.95	0.36
Cyclin B1						
Cells with cytoplasmic staining per percentage of positive cells	1.26	0.88–1.79	0.21	1.13	0.74–1.73	0.56
Cyclin D1						
Cells with nuclear staining per percentage of positive cells	0.81	0.37–1.79	0.61	0.81	0.37–1.79	0.61
Cycline E						
Cells with nuclear staining per percentage of positive cells	0.91	0.67–1.22	0.53	0.83	0.58–1.18	0.31
Ki67						
Cells with nuclear staining per percentage of positive cells	1.14	0.84–1.54	0.39	1.10	0.76–1.60	0.61
TPO						
Cells with cytoplasmic staining per percentage of positive cells	0.74	0.57–0.97	0.03	0.79	0.58–1.07	0.13
Galectin 3						
Cells with cytoplasmic staining per percentage of positive cells	1.01	0.78–1.29	0.93	0.96	0.71–1.31	0.80
Galectin 3						
Cells with nuclear staining per percentage of positive cells	0.95	0.74–1.22	0.69	0.95	0.70–1.29	0.76
VEGF						
Cells with cytoplasmic staining per percentage of positive cells	0.91	0.53–1.54	0.71	0.77	0.45–1.31	0.34
EGFR						
Cells with membrane staining per percentage of positive cells	0.86	0.67–1.13	0.28	0.79	0.58–1.07	0.13
Duox						
Per percentage of positive cells	0.71	0.44–1.16	0.18	0.14	0.02–0.97	0.04
P53						
Cells with nuclear staining per percentage of positive cells	0.9	0.71–1.15	0.4	0.86	0.62–1.16	0.32

A score from 1 to 4 was used to quantify the number of positive cells (1: <1% of positive cells; 2: between 1% and 5% of positive cells; 3: between 6% and 10% of positive cells; 4: >10% of positive cells). RR: recurrence rate; 95% CI: 95% confidence interval; TPO: thyroperoxidase; VEGF: vascular endothelial growth factor; EGFR: epidermal growth factor receptor.

hazards regression model (26). The analysis was performed using SAS® software (version 9.1, SAS Institute, Cary, NC).

Results

Patients

Forty patients with PDFC were included in the analysis; there were 22 females and 18 males, with a mean age of 46.7 years (range: 16–81 years) at diagnosis. No patient had a history of thyroid carcinoma in the family or of radiation exposure. The mean tumor size was 3.9 cm (range: 0.5–12 cm). Twenty patients were classified as T0, T1, or T2; 19 as T3 or T4; and 1 patient as Tx. Lymph-node metastases were present in 16 patients. Nine patients had distant metastases at discovery of the disease: in lungs (five patients), in bones (one patient), and in both lungs and bones (three patients).

Median follow-up was 3.9 years (range: 0–19.7 years), during which nine patients developed distant metastases: in lungs (two patients); in bones (two patients); in both lungs and bones (four patients); and in lungs, bones, and brain (one patient).

Radioiodine uptake was present in metastases on radioiodine whole body scan performed 3 to 5 days after the administration of 3.7 GBq in only 6 of the 15 patients with distant metastases who were treated with radioiodine; the other 3 patients did not receive any radioiodine treatment for poor clinical condition and the presence of uptake could not be determined. When present, radioiodine uptake in the metastases was low, and no significant clinical benefits were observed after treatment with radioiodine.

Twelve patients, all with distant metastases, died after a mean follow-up of 2.6 years (range: 0–6.5 years) after initial treatment.

Histological characteristics

All patients had clear-cut criteria of malignancy with different amounts of atypical nuclear pattern, mitosis, and necrosis. The characteristic histological architecture (TIS) represented the vast majority of the tumor areas in all tumor samples (Fig. 1) with the presence of trabeculae in 31 patients (77%), closely intermingled with insular architecture in 8

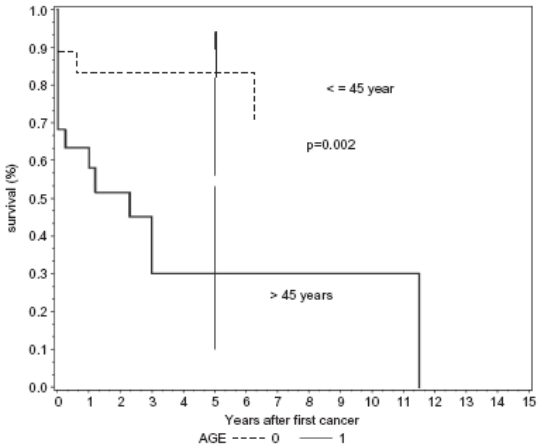

FIG. 2. Metastasis-free survival according to age at the time of diagnosis.

patients (20%), and solid architecture in 3 patients (8%). Microfollicles were found in variable amounts in 27 patients (67%). Polymorph architecture with a clearly separate distribution of various architectures was found in 14 patients (35%), and Hürthle cells were observed in 13 patients (33%).

Among the 18 patients with distant metastases, trabecular architecture was predominant in 16 patients (89%). Insular pattern was found in 3 patients (17%), in association with a trabecular pattern in 1 and with a solid pattern in 2 patients (11%). Microfollicles were observed in 12 patients (67%) and Hürthle cells in 6 patients (33%). Similar features were observed in patients without metastases. The polymorph architecture was found in 3 of the 18 patients with metastases, less frequently than in 11 of the 22 patients without metastases ($p = 0.04$) (Table 1).

Immunohistochemical study

Twenty-nine (72%) PDFCs were positive for cyclin A, and 31 (77%) PDFCs were positive for cyclin B1. A nuclear expression of cyclin E was found in 31 PDFCs (77%), whereas no expression of cyclin D1 was found. Ki67 staining was positive in 28 PDFCs (72%) with a percentage of positive cells of 1–5% in 10 tumors, of 6–10% in 6, and >10% in 12. P53 staining was positive in 20 PDFCs (50%) (Table 2).

Galectin 3 staining was positive in 21 PDFCs (52%). Duox staining was positive in 17 PDFCs (43%) and TPO in 26 (65%), and there was no clear relationship between staining for Duox and for TPO (both stainings being positive in 12 tumors and negative in 9, and either one positive in 19 tumors). In tumors positive for TPO, staining was located in the cytoplasm and not in the membrane. EGFR was positive in 29 PDFCs (72%) and VEGF in 38 (95%).

Prognostic factors for the occurrence of metastases and thyroid cancer mortality

Occurrence of distant metastases. Distant metastases occurred in 4 of the 18 patients aged less than 45 years and in 14 of the 22 patients older than 45 years. Mean age at the first treatment was 58.7 years (range: 32–81 years) in the 18 patients who developed metastases and 36.9 years (range: 16–63 years) in the other 22 patients ($p < 0.0001$) (Table 1). Patients younger than 45 years had metastasis-free survival significantly longer than patients older than 45 years ($p = 0.002$) (Fig. 2). Among the 18 patients with metastases, 14 had a primary tumor larger than 4 cm and/or with an extension beyond the thyroid capsule, 3 had a primary tumor smaller than 4 cm, and 1 was staged as Tx (Table 3). Nine patients with distant metastases had lymph-node metastases at the time of diagnosis. Univariate and multivariate analyses showed that an older age at the time of initial treatment and a larger extent of the primary tumor were significantly associated with an increased risk of metastases (Tables 1–3). Indeed, the recurrence rate (RR) of metastases was 1.08 in patients older than 45 years (95% CI: 1.02–1.14; $p = 0.003$) and was 3.24 (95% CI: 1.56–6.72; $p = 0.001$) in patients classified as T3 and T4, as compared to patients classified as T0, T1, or T2 (Table 3). A polymorph pattern was associated with a lower risk of metastases (RR: 0.26; 95% CI: 0.07–0.93; $p = 0.04$) (Table 3). TPO expression was linked to a lower risk of metastases (RR: 0.74; 95% CI: 0.57–0.97; $p = 0.03$) (Table 4). The expression of the other markers studied was not different between patients with and without distant metastases (Table 2).

Survival. At 5 years, the overall survival rate was 63% and the metastasis-free survival rate was 57% (Fig. 3). Survival of

TABLE 3. CLINICAL AND HISTOLOGICAL FACTORS OF METASTASES AND DEATH IN 40 PATIENTS WITH POORLY DIFFERENTIATED FOLLICULAR THYROID CARCINOMA (MULTIVARIATE ANALYSIS)

Clinical characteristics	*Metastasis*	*RR*	*95% CI*	p	*Death*	*RR*	*95% CI*	p
Age (years)								
≤45	04/18				0/18			
>45	14/22	1.08	1.04–1.12	0.003	12/22	1.08	1.03–1.14	0.002
T (TNM)[a]								
0, 1, 2	03/20				01/20			
3, 4	14/19	3.24	1.56–6.72	0.001	10/19	3.14	1.02–9.7	0.04
Histological characteristics								
Polymorph								
Yes	03/14							
No	15/26	0.26	0.07–0.93	0.04		–	–	–

[a]One missing value for tumor size.
RR: recurrence rate; 95% CI: 95% confidence interval; TNM: tumor node metastasis.

TABLE 4. IMMUNOHISTOCHEMICAL FACTORS OF METASTASIS AND DEATH IN 40 PATIENTS WITH POORLY DIFFERENTIATED FOLLICULAR CARCINOMA (MULTIVARIATE ANALYSIS)

	RR	*95% CI*	p
Metastasis			
Thyroperoxidase			
Cells with cytoplasmic expression per percentage of positive cells	0.74	0.57–0.97	0.03
Death			
Duox			
Per percentage of positive cells	0.14	0.02–0.97	0.04

A score from 1 to 4 was used to quantify the number of positive cells (1: <1% of positive cells; 2: between 1% and 5% of positive cells; 3: between 6% and 10% of positive cells; 4: >10% of positive cells). RR: recurrence rate; 95% CI: 95% confidence interval.

patients without metastases was indeed significantly longer than that for patients with metastases ($p < 0.001$). Multivariate analysis showed that an older age at the time of initial treatment and a larger extent of the primary tumor were associated with a higher risk of death. Indeed, the RR of death was 1.08 (95% CI: 1.03–1.14; $p = 0.002$) in patients older than 45 years and 3.14 (95% CI: 1.02–9.7; $p = 0.04$) in patients classified as T3 and T4, as compared to patients classified as T0, T1, or T2 (Table 2). No association was found between histological variables and survival (Table 3). A high expression of Duox was associated with a lower risk of death (RR: 0.14; 95% CI: 0.02–0.97; $p = 0.04$) (Table 4). The expression of the other markers studied was not different between patients who died and those who were still alive at the end of the study (Table 2).

Discussion

PDFCs of the thyroid represent a heterogeneous group of infrequent tumors. We identified 40 patients affected by PDFC defined on the basis of growth pattern, and we evaluated the prognostic significance of various clinical, histological, and immunohistochemical characteristics. The small number of patients is due to the scarcity of PDFC, to the fact that we evaluated only those patients who were initially treated at the IGR, and finally to the exclusion of tumors with a papillary component.

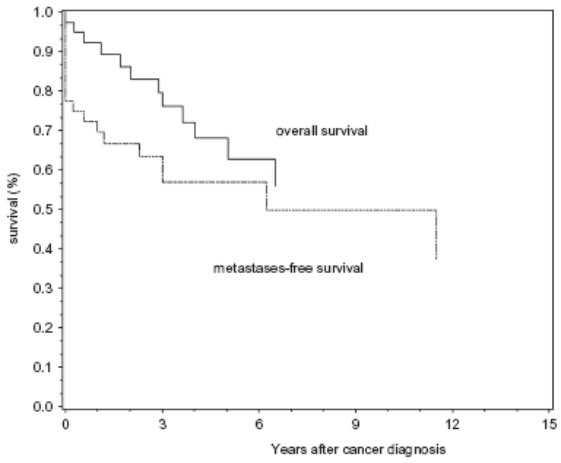

FIG. 3. Overall and metastasis-free survival in the whole series of poorly differentiated follicular thyroid carcinoma (PDFC) patients ($n = 40$).

Our study confirms that PDFC has a clinical course intermediate between the indolent course of well-differentiated thyroid carcinoma and the aggressive course of undifferentiated carcinoma (5,6,9,10). Indeed, at 5 years the overall survival rate was 63% and the metastasis-free survival rate was 57%. In a recent retrospective study of PDC defined by the presence of mitosis and necrosis, the survival rate was similar (9). This similarity is probably due to the aggressive behavior of PDFC, independently of whether they are defined on the basis of growth pattern and/or other criteria. We did not find any relationship between the presence of a particular histological subtype and metastasis-free survival. Only, polymorph architecture was more frequently observed in patients without distant metastases in the univariate analysis, and this may be related to the presence of a more differentiated component in these tumors. As already reported (6), the presence of Hürthle cells was not associated with a more aggressive behavior.

Our study confirms that prognostic factors identified for well-differentiated thyroid cancers also apply to PDFCs. An older age at the time of initial treatment was associated with a poor outcome (6,27–29), and in patients older than 45 years, metastasis-free survival and overall survival rates were lower. A tumor size larger than 4 cm and tumor extension beyond the thyroid capsule were associated with a higher risk of distant metastases and with a poor survival (9,27–29). Finally, survival rate was lower among patients with distant metastases, and 12 deaths were observed in the 18 patients who developed distant metastases.

Concerning the immunohistochemical study, an absence of detectable TPO expression has been reported in differentiated and in undifferentiated thyroid carcinomas (18–20). In the current study, a detectable TPO expression was associated with a lower risk of metastases. In positive cells, TPO was located intracellularly without membrane staining, indicating an abnormal processing of the protein. We found a significant relationship between high Duox expression and reduced thyroid cancer mortality. Similarly, in well-differentiated thyroid carcinomas, Duox expression is related to tumor functional differentiation (20,30). These results suggest that persistent functional differentiation indicates a less aggressive behavior.

Concerning cell cycle markers, expression of cyclins A, B1, and E was found in more than 70% of tumors, in accordance with previous reports. However, no significant differences were observed between patients with and without distant metastases. We do not confirm the low nuclear expression of cyclin E that was reported in papillary and undifferentiated carcinomas (16).

Ki67 is overexpressed in undifferentiated thyroid carcinoma (31). In our series of PDFCs, Ki67 staining was positive in 72% of tumors but was relatively low in most positive tumors, being higher than 10% in only 30% of tumors. It was not different between patients with and without distant metastases, and high Ki67 labeling index was not associated with a shorter survival rate. This is in accordance with the

rapid occurrence of death after the discovery of distant metastases.

EGFR and VEGF expressions were frequently strongly expressed, but their expression had no prognostic significance. VEGF is one of the major proangiogenic factors in thyroid tumors, and inhibitors of VEGF or of VEGFR reduce the growth of follicular thyroid carcinoma xenografts (32,33). EGFR is a transmembrane cell-surface glycoprotein that stimulates follicular cell proliferation and enhances thyroid cancer invasiveness (34–36). An increased expression of EGFR has been found in poorly and undifferentiated thyroid carcinomas (37–42), and EGFR inhibitors inhibit the growth of anaplastic carcinomas (39).

The absence of prognostic significance of these immunohistochemical parameters may be related to the limited number of cases under study, and to the fact that our selection criteria (the presence of TIS growth pattern) permitted the selection of PDFC patients with a rather homogeneous phenotype.

In conclusion, the current study confirmed that PDFC has a more aggressive behavior and a worse prognosis than well-differentiated carcinoma. We confirmed that in PDFC patients, an advanced age and a larger tumor size are associated with an aggressive behavior, and that death occurred in patients with distant metastases.

Acknowledgments

Melania Pulcrano had a fellowship from the Italian-French University and from the Diplôme d'Université Européen de Recherche en Cancérologie Clinique (University Paris Sud). Houda Boukheris had a fellowship from the International Agency for Research on Cancer (IARC). This study was supported by a grant from Electricité de France to Martin Schlumberger.

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Adjuvant treatment with thyrotropin alpha for remnant ablation in thyroid cancer

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Abstract: Various studies have demonstrated the safety and efficacy of recombinant human thyroid-stimulating hormone (rhTSH) for radioiodine remnant ablation. On this basis, rhTSH was approved in Europe for the radioiodine ablation of low-risk differentiated thyroid cancer (DTC) during thyroid hormone therapy with L-thyroxine (L-T4). Moreover, in December 2007, the US Federal Drug Administration approved the use of rhTSH for adjuvant treatment with radioiodine in patients with DTC without evidence of metastatic thyroid cancer. Quality of life was found to be better with rhTSH preparation than with L-thyroxine withdrawal, thereby resulting in benefits for society as a whole. Furthermore, rhTSH for radioiodine remnant ablation results in a longer effective radioiodine half-life within remnant thyroid tissue and a lower specific absorbed dose in the blood and exposure of bone marrow to X-rays. More studies are required to establish the amount of radioiodine to be administered especially in high-risk patients.

Keywords: thyroid cancer, thyrotropin, radioiodine (^{131}I) remnant ablation (RRA), quality of life, ray exposure

There is general agreement that total thyroidectomy is the initial treatment-of-choice for patients with differentiated thyroid cancer (DTC).[1,2] Radioiodine (^{131}I) remnant ablation is recommended after thyroidectomy to destroy post-surgical residual thyroid tissue especially in patients at high-risk of recurrence and mortality.[1,2] ^{131}I ablation has two advantages: 1) it destroys any remaining microscopic tumoral foci; and 2) it eliminates all normal thyroid cells that would continue to produce thyroglobulin and confound interpretation of measurement of serum thyroglobulin (Tg), which is a specific marker of recurrent or persistent disease. Consequently, this procedure improves the follow-up and treatment of patients with DTC by increasing the specificity and sensitivity of Tg monitoring and ^{131}I treatment. Moreover, ^{131}I administration decreases the frequency of recurrences and mortality.[3–6]

Elevated serum thyroid-stimulating hormone (TSH) levels (above 30 mU/L) are necessary to ensure sufficient trapping and retention of ^{131}I by functioning thyroid tissue.[1] Traditionally, the endogenous increase of TSH was achieved by withdrawal of thyroid hormone therapy (L-thyroxine; LT4) for 4 to 5 weeks, which induces clinical hypothyroidism. However, this short-term hypothyroid condition is associated with cognitive and physical impairment and alteration of quality of life in young and middle-aged patients.[7,8] Moreover, withdrawal of LT4 can impair cardiac, cognitive and neurological function with consequent health risks especially for elderly people.[7,8] Lastly, it may not increase TSH levels in cases of persistent thyroid hormone production by large thyroid remnants or functional metastases, in elderly patients, and in the presence of hypothalamic or pituitary disease or long-term steroid therapy.[9–12]

Recombinant human TSH (rhTSH) is a heterodimeric glycoprotein produced by recombinant DNA technology for the purpose of producing increased TSH levels without

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Biologics: Targets & Therapy 2009:3 000–000

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LT4 withdrawal and the consequent hypothyroidism. The use of rhTSH was initially limited to the field of DTC follow-up and was approved by the US Food and Drug Administration (FDA) in December 1998 for diagnostic use. Subsequently, rhTSH was found to be effective for ^{131}I remnant ablation,[13,14] and in February 2005, rhTSH was approved in Europe for the ^{131}I ablation of low-risk DTC during thyroid hormone therapy with LT4. In December 2007 the FDA approved the use of rhTSH for adjuvant treatment with ^{131}I in patients with DTC without evidence of metastatic thyroid cancer.

Here we review the studies on rhTSH-aided ablation with the aim of addressing such open questions as the exact protocol of rhTSH administration and the dose of ^{131}I to obtain maximum effectiveness.

RhTSH-aided ablation: literature analysis

Table 1 lists the studies that evaluated the effectiveness of rhTSH in the adjuvant treatment for ^{131}I remnant ablation in DTC patients. The criteria used to define successful thyroid ablation differed among studies from no visible uptake at whole body scan after rhTSH or undetectable basal and rhTSH-stimulated serum thyroglobulin. Despite these differences, there is general agreement that rhTSH for thyroid ablation gives results similar to those found after LT4 withdrawal.

The study by Perros et al was the first report on the use of rhTSH to increase ^{131}I uptake for remnant ablation.[15] Subsequently, the effect of rhTSH-aided ablation was evaluated in a prospective non-randomized trial of 10 patients with papillary cancer.[16] The dose of ^{131}I administrated varied between 30 and 250 mCi. The ablation rate was 100% when judged by the absence of visible uptake in the thyroid bed after diagnostic whole body scan 3 months after ablation.[16] Another randomized study confirmed complete ablation after high doses of ^{131}I (approximately 108 mCi) by using TSH to stimulate ^{131}I uptake for the ablation of remnant thyroid tissue.[17]

A subsequent retrospective study from the Memorial Sloan-Kettering Cancer Center confirmed that a high dose of ^{131}I increased the rate of rhTSH-aided ablation in DTC patients.[13] In this study, the rates of complete ablation did not differ significantly between a group of patients who were prepared by thyroid hormone withdrawal (THW) and a group of patients prepared by rhTSH when treated with 100 mCi (84% in 45 euthyroid patients after rhTSH vs 81% in 42 hypothyroid patients)[13].

However, a prospective study by Pacini et al did not confirm these results.[18] In this prospective randomized study in which 1.1 GBq (30 mCi) was used as standard ablative activity, 162 DTC patients were randomized in three treatment arms: in the first arm, patients (n = 50) were treated by LT4 withdrawal (HYPO); in the second arm, patients

Table 1 Studies evaluating the efficacy of rhTSH for remnant ablation

Authors	Patients (n)		Stage of disease		Dose of ^{131}I (mCi)		Outcome	
	rhTSH	LT4W	rhTSH	LT4W	rhTSH	LT4W	rhTSH	LT4W
Robbins et al 2001 Prospective randomized study	10	n.p.	T1–T4	n.p.	30–250	n.p.	100% dWBS negative, 60% Tg < 1.0	
Pacini et al 2002 Prospective randomized study	70	50	T1–T4 N0–N1	T1–T4 N0–N1	30	30	54% dWBS negative 86,8% Tg < 1.0	84% dWBS negative 83% Tg < 1.0
	42 rhTSH + LT4WI		T1–T4 N0–N1		30		78.5% dWBS negative 84.8% Tg < 1.0	
Robbins et al 2002 Retrospective study	45	42	T1–T4 N0–N1	T1–T4 N0–N1	110.4 ± 65	128.9 ± 74	81% dWBS negative	84% dWBS negative
Barbaro et al 2003 Non-randomized prospective study	16	19	1–2	1–2	30	30	77% dWBS negative 86.5% Tg < 1.0	76% dWBS negative 76 % Tg < 1.0
Pacini et al 2006 Prospective randomized study	33	30	T1–T4 N0–N1	T1–T4 N0–N1	100	100	75% dWBS negative 96% Tg < 2.0	86% dWBS negative 86% Tg < 2.0
Pilli et al 2007 Prospective randomized study	36	n.p.	T1–T4 N0–N1	T1–T4 N0–N1	50	n.p.	88.9 % dWBS negative 78.9% Tg < 1.0	
	36		T1–T4 N0–N1	T1–T4 N0–N1	100	n.p.	88.9% dWBS negative 67% Tg < 1.0	

n.p. = not performed.
Abbreviations: rhTSH, recombinant human thyroid-stimulating hormone; dWBS, diagnostic whole body scanner; LT4W, levo-thyroxine withdrawal; Tg, serum thyroglobulin.

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(n = 42) were treated by LT4 withdrawal combined with rhTSH (HYPO + rhTSH); in the third arm, patients (n = 70) were stimulated with rhTSH in euthyroidism (EU + rhTSH). The follow-up was performed 6 to 10 months post ablation. When the criterion for successful ablation was no uptake on the thyroid bed on diagnostic whole body scan, the rate of successful ablation was similar in the HYPO and HYPO + rhTSH groups (84% and 78.5%, respectively) but significantly lower (54% $p < 0.01$) in the EU + rhTSH group.[18] On the contrary when successful ablation was defined as no visible thyroid bed uptake on diagnostic whole body scan or undetectable serum Tg after rhTSH, the success rates were similar (95% vs 74%). However, the reduced rate of ablation in the EU group may be explained by the protocol of ^{131}I administration used by Pacini et al.[18] Indeed, ablative ^{131}I administration was delayed by 24 h and it was delivered 48 h after the second injection of rhTSH. Therefore, the authors suggested that the dose of ^{131}I be increased or that different protocols of rhTSH administration be used to obtain a satisfactory rate of rhTSH-aided thyroid ablation.

An international randomized controlled trial showed that the efficacy of rhTSH for ablation was similar to that of LT4 withdrawal with 100% ablation after 3.7 GBq (100 mCi).[14] The predefined primary criterion for successful ablation was "no visible uptake in the thyroid bed, or a visible uptake less than 0.1%" on neck scans performed 8 months after therapy, and was satisfied in 100% of patients in both groups. A secondary criterion for ablation, a rhTSH-stimulated serum thyroglobulin concentration less than 2 ng/mL, was fulfilled by 23 of 24 (96%) euthyroid rhTSH patients and 18 of 21 (86%) hypothyroid patients ($p = 0.2341$). In this randomized prospective ablation trial, all rhTSH patients had an iodine excretion below 200 μ/L, indicating the absence of overt iodine excess.

Only two studies have evaluated the efficacy of rhTSH for remnant ablation with lower ^{131}I doses.[19,20] A recent study by Pilli et al showed that 1850 MBq (50 mCi) ^{131}I had a similar success rate to 3700 MBq (100 mCi) in 72 patients prepared with rhTSH for thyroid ablation.[19] This prospective, randomized study showed that 3700 MBq ^{131}I is associated with high rates of successful thyroid ablation after rhTSH preparation and that similar ablation rates (88.9%) were obtained with lower ^{131}I activity (1850 MBq). These results were obtained when the criterion of successful ablation was defined as no visible uptake at the 6- to 8-month control diagnostic ^{131}I whole body scan after rhTSH stimulation, and also when the criterion of successful ablation was undetectable (1 ng/mL) rhTSH-stimulated serum Tg. Furthermore, successful ablation was also obtained in patients with nodal metastases. Lastly, the dosimetric study showed that thyroid uptake was similar in patients treated with 1850 or 3700 MBq.

Since thyroid hormones are an important source of iodine and may interfere with ^{131}I uptake during thyroid ablation, Barbaro et al suggested LT4 therapy be discontinued before rhTSH injection.[20] They compared ablation obtained with doses of 30 mCi in 2 groups of DTC patients: one group was prepared by hypothyroidism and the other group was prepared by rhTSH stimulation. In the rhTSH group, LT4 therapy was interrupted for 4 days starting the day before the first injection. In the rhTSH group, urinary iodine excretion was significantly lower than in a control group of euthyroid subjects who rececived rhTSH stimulation. One year later, patients underwent a whole body scan with a tracer dose of ^{131}I and serum Tg was measured using rhTSH with the same protocol in both groups. The percentage of ablation (undetectable Tg and a negative whole body scan) was 81.2% in patients treated with rhTSH and 76% in patients treated by L-T4 withdrawal.

Similarly, Pitoia et al suggested replacing LT4 with LT3 therapy to maintain the euthyroid state and to minimize the iodine pool during rhTSH preparation.[21] Indeed, LT3 has an iodine content 5-fold less than LT4.[21]

RhTSH-aided ablation: ^{131}I dosimetry, safety and cost

Because ^{131}I activity is associated with such important risks as bone marrow depression and pulmonary fibrosis, several dosimetric studies have been performed to evaluate the absorbed dose in the blood (a surrogate for bone marrow) and ^{131}I activity in the lung to determine the minimum effective dose to reduce the these risks. It has been reported that a dose of 2 Gy of radiation in the blood is dose-limiting,[22] whereas 3 GBq in the lung in 24 h is the safety limit to avoid pulmonary fibrosis.[23]

An international, prospective, randomized study compared the iodine biokinetics, dosimetry and the effectiveness of ablation therapy with 100 mCi in DTC after rhTSH stimulation or LT4 withdrawal.[24] Iodine biokinetics differed between the two groups of patients.[24] In fact, in the euthyroid state, renal clearance of iodine was 50% faster than in hypothyroidism.[31] Indeed, fractional ^{131}I uptake into thyroid remnants was lower after rhTSH stimulation than after LT4 withdrawal.[24] However, this reduction was partially compensated for by an increased half-life of ^{131}I in thyroid cells after rhTSH stimulation.[24] rhTSH-treated patients showed a longer effective ^{131}I half-life within remnant thyroid tissue, and the residence times of the radioisotope were comparable in the two groups.[24] Moreover, the specific absorbed dose in the blood was significantly lower (one-third) after rhTSH preparation, suggesting that

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higher ^{131}I activities might be safely administered after rhTSH stimulation.[24] Finally, another study confirmed that the bone marrow absorbed dose remained under 2 Gy after rhTSH-aided administration of high activities of ^{131}I.[25] Moreover, patients prepared with rhTSH had a better quality of life than hypothyroid patients.[14,29,30] RhTSH-aided ablation was well tolerated with no important side effects, and it can be useful in elderly patients and in patients with associated co-morbidities without increasing the risk of cardiac, cerebrovascular, pulmonary or neurological complications.[8,26,27]

Finally, a recent study compared the cost-effectiveness of ablation after rhTSH stimulation or LT4 withdrawal. The additional cost of rhTSH procurement and administration was considered justified in relation to the clinical benefits and cost offsets such as avoidance of hypothyroidism, increased work productivity and quality life, reduced discharge from radioprotection and period of sick leave.[28] These observations were recently confirmed by Borget et al[30] who found that rhTSH can decrease the duration of sick leave, and that its high cost is compensated for by benefits to patients and society with a modest net cost.[30]

RhTSH-aided ablation: advantages and limits

There is general agreement that rhTSH-aided ablation is effective and safe. Various studies have confirmed the efficacy of rhTSH in aiding ablation and show that rhTSH preparation is more beneficial than LT4 withdrawal[32] in terms of quality of life[1,4,29,30] and well-being and avoids the important side effects of short-term hypothyroidism. Moreover, rhTSH for remnant ablation decreases exposure of bone marrow to X-rays.

Several questions are still open, namely, the amount of ^{131}I to be administered and the effect of iodine intake. More studies are required to evaluate whether rhTSH can be used effectively for remnant ablation in high risk patients with outcomes at least comparable to those seen with ablation after thyroxine withdrawal.

Disclosures

The authors have no conflicts of interest to disclose.

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