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"The potential role of CXCR4 and SDF-1 as indicators of tumor aggressiviness in patients with conventional papillary thyroid carcinoma"

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- **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. Review.

- Biondi B, Pulcrano M, **Pagano L,** Lombardi G. Adjuvant treatment with thyrotropin alpha for remnant ablation in thyroid cancer. Biologics: Targets & Therapy 2008:3(1) 1–5 *in press*

Charter in a book:

- **Pagano L,** Pulcrano M, Ippolito S, Klain M, Lombardi G, Salvatore M, Biondi B. Ruolo della PET con FDG nel carcinoma differenziato della tiroide. Carcinoma Tiroideo. Teoria e Gestione Pratica. Autore: Daniele Barbaro. Società Editrice Universo, I edizione 2008.

Abstract

Background: Functional chemokine receptors are expressed in many malignant tumors, including papillary thyroid carcinoma (PTC). These receptors promote tumor growth and metastasis in response to endogenous chemokines.

The purpose of this study was to examine the expression of SDF-1 and its chemokine receptors, CXCR4, in a series of PTCs, considered as low risk for tumor size and hystotypes.

In this study, we correlated CXCR4 and SDF-1 with indicators of clinical and tumor aggressiveness, including age, gender, tumor size, extrathyroidal extension, multifocality and lymph node metastasis.

Methods: CXCR4 as well as its specific chemokine ligand, SDF-1 , were assessed in 48 PTCs using a semiquantitative measure of immunohistochemical (IHC) staining intensity for each molecule. Staining intensity was compared with clinicopathological features. Expression in CXCR4 and SDF-1 mRNA levels were sought in a subset of tumors, using gene microarrays and quantitative RTPCR.

Results: In 29 cases, the PTC was associated with chronic thyroiditis. High-intensity IHC staining for CXCR4 correleted with T1 ($p=0.003$), classical variant ($p=0.02$) and lymph node metastasis at initial diagnosis ($p=0.01$). In contrast SDF 1 correlated with female gender ($p =0.05$), but this association was not shown following multivariate analyses.

Conclusion: In our study, the expression of CXCR4 and its ligand are more frequent in tumors that have a smaller dimension and a classical variant of PTC. Therefore, CXCR4 and its ligand are associated with lymph node metastasis involvement at the initial diagnosis, which is an indicator of negative prognosis. Finally, the chemokine pathways may be expressed early in PTC tumorigenesis. Although these markers are found in a less aggressive PTC variant, they may be responsible for early and preferential lymphnode neck metastases. Further studies are necessary to define the mechanisms underlying this association and to determine its potential prognostic and therapeutic implications.

1.Introduction

Thyroid carcinoma accounts for roughly 1% of all new malignant diseases; about 0.5% of cancers in men and 1.5% in women. Of these, about 94% are differentiated thyroid tumors that derive from the follicular epithelial cells, such as papillary and follicular thyroid carcinomas. Another 5% are medullary thyroid carcinoma, a neuroendocine tumor. The remaining 1% are anaplastic carcinoma that generally derive from dedifferentiation of the differentiated type. (Schlumberger 1998; Sherman, 2003)

The incidence of thyroid cancer has been increasing in many countries over the last 30 years (from 3.6/100 000 people in 1973 to 8.7/100 000 people in 2002) while mortality has been slowly decreasing. The increase is attributable to better detection of small papillary carcinomas (PTC) as a result of improved diagnostic accuracy (neck ultrasound and fine needle aspiration cytology). In fact, nearly 60–80% of thyroid carcinomas detected nowadays are micropapillary thyroid carcinomas (<1 cm in size), carrying an excellent long-term prognosis. (Davies L et al, 2006, Pacini F et al 2008,). Despite the generally favorable prognosis of these tumors with a 10-year survival rate exceeding 90%, the rates of locoregional and distant recurrences are as high as 5.9% and 1.5%, respectevely, and cancer related mortality may be 1- 2%. Consequently, it is necessary to identify patients who have a risk of progressive disease at the time of diagnosis. The importance of recognizing prognostic variables is also relevant for the best management such as, the extent of thyroid surgery and the indications for post operative radioiodine therapy (Sugitani I et al, 1999, Mazzaferri EL 2007). A careful prognostic classification is also critical for the comparison of treatment results. Therefore, a reliable new prognostic classification is needed in PTC patients to modify initial therapy and follow up schemes to the risks of persistent or recurrent disease.

Conventionally, patient age at diagnosis, gender, tumor size, extrathyroidal invasiveness, multifocality, lymph node involvement at the initial diagnosis and certain histological markers are known to be associated with recurrence and poor prognosis (DeGroot LJ et al, 1990; Mazzaferri EL et al 1981).

Moreover, lymphocytic infiltration is frequently observed in PTC, although there are few and conflicting reports concerning the association between the prognosis of PTC and the degree of lymphocytic infiltration surrounding and /or inside the tumor (Hirabayashi RN, et al 1965; Clark OH et al 1980; Aguayo J et al 1989; Matsubayashi et al 1995).

Consequently, three distinguishing features of PTC should be considered as the basis to formulate new criteria for the prognosis. The first feature regards the activation of downstream signal transduction pathway and modulation of gene expression induced by RET/PTC. The second is a prominent peritumoral inflammatory reaction, which suggests cross-talk between tumor cells and the inflammatory system. Third, PTC is often characterized by early metastases

spread to regional lymph nodes and by multifocal involvement of the gland, which suggest highly invasive behavior.

1.1 Papillary Thyroid Cancer (PTC): hystological variant and molecular genetics

Numerous variants of PTC are already known. The classical variant (45%) and the follicular variant (18%) are the most common and frequently diagnosed tumors. The follicular variant tumors may be encapsulated or non-encapsulated and are composed almost exclusively of follicles having the characteristic nuclear features of PTC. Inter observation variation in the diagnosis of these tumors, particularly the encapsulated type, is high since the nuclear features may be focal or poorly developed. Lymph node metastases are less common in the follicular variant than in classical PTCs. Microfollicular, oncocytic, Warthin-like, and clear cell variants have a prognosis that is similar to conventional PTCs. The solid variant comprises approximately 8% of sporadic PTCs and is relatively common in children following radiation exposure. This variant is associated with a slightly higher frequency of distant metastases and a somewhat less favorable prognosis than conventional PTC. The diffuse sclerosing variant is more of pulmonary metastases than conventional PTCs although overall survivals do not differs have a worse prognosis than conventional PTCs; however, stage and grade may be more important than histological subtypes. The prognosis of PTCs with poorly differentiated, undifferentiated, or squamous carcinoma components depends on the proportion of the non-PTC component. The term papillary ‘‘microcarcinoma’’ is reserved for those tumors measuring less than 1 cm in diameter. Although most of these tumors have follicular or papillary architectural features, any of the variants may measure less than 1 cm. Papillary microcarcinomas are extremely common, occurring in up to 30% of autopsies and in up to 24% of surgical thyroidectomies performed for disorders unrelated to PTC (Asklen LA et al 2000; Nikiforov YE et al 2001; Lloyd et al 2004; Delellis R, 2006).

Recent years have been marked by dramatic expansion in the understanding of the molecular basis of thyroid carcinogenesis. It has become apparent that thyroid tumors, especially those of the papillary type, frequently go through genetic alterations leading to the activation of the mitogenactivate protein kinase (MAPK) signaling pathway. This crucial intracellular cascade regulates cell growth, differentiation and survival in response to growth factors, hormones and cytokines that interact with receptor tyrosine kinases present on the cell surface (Fusco A et al 1987; Grieco M et al 1990; Ciampi R, 2006; Santoro M et al 2006) (Fig. 1).

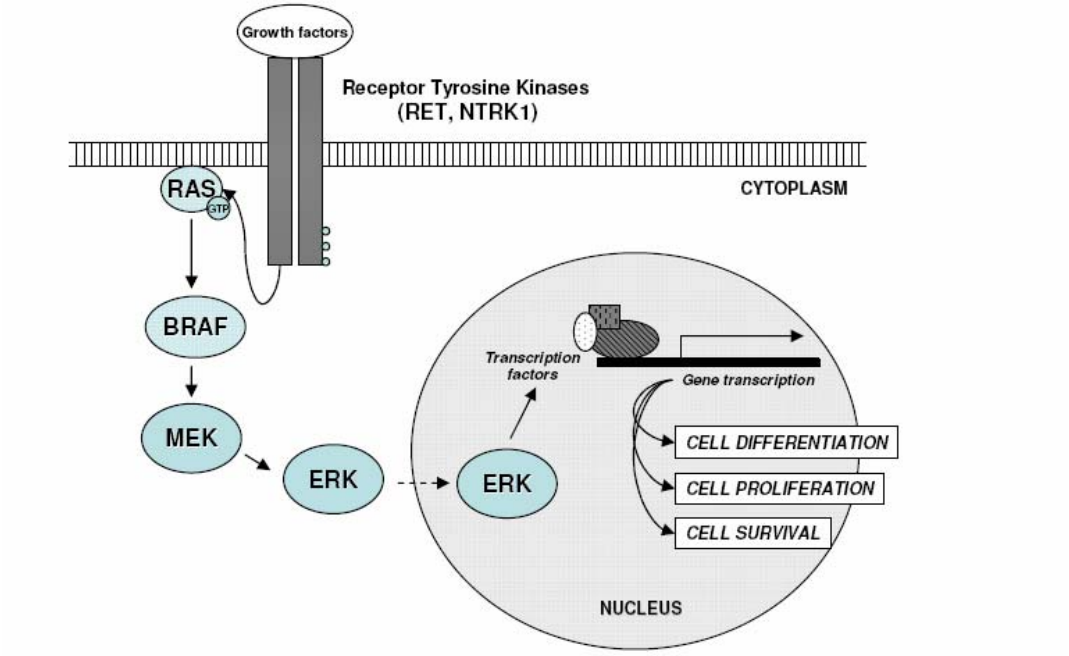

Fig. 1. Schematic representation of the mitogen-activated protein kinase (MAPK) signaling pathway. Physiologically, binding of growth factors to receptor tyrosine kinases, such RET and NTRK1, results in receptor dimerization and activation via autophosphorylation of tyrosine residues in the intracellular domain. The activated receptor, through a series of adaptor proteins, leads to activation of RAS located at the inner face of the plasma membrane by substitution of GDP with GTP. The GTP-bound form of RAS binds to and recruits RAF proteins, mainly BRAF in thyroid follicular cells, to the plasma membrane. Activated BRAF is now able to phosphorylate and activate the mitogen-activated protein kinase/ERK kinase (MEK), which in turn phosphorylates and activates the extracellular-signal-regulated kinase (ERK). Once activated, ERK phosphorylates cytoplasmic proteins and translocates into the nucleus, where it regulates transcription of the genes involved in cell differentiation, proliferation and survival. Alterations of this pathway in thyroid cancer can occur at different levels as a result of point mutation or rearrangement involving the RET, RAS, and BRAF genes. (Ciampi R and Nikiforov YE, Endocrinology 2006)

Molecular alterations found in papillary carcinomas involve genes coding for the receptor tyrosine kinases RET and NTRK1, and for two intracellular effectors of the MAPK pathway, a GTP-binding protein RAS and a serine-threonine kinase BRAF. Mutation of one of these genes can be found in more than 70% of papillary carcinomas and they rarely overlap in the same tumor, suggesting that activation of this signalling pathway is essential for tumor initiation and alteration of a single effector of the pathway is sufficient for cell transformation.

Notably, in about 40-50% of papillary thyroid carcinomas the kinase domain of the tyrosine kinase receptor for the GDNF, c-Ret (REarranged during

Transfection), is fused with the N-terminal region of constitutively expressed, heterologous genes, such as H4 (in RET/PTC1) or RFG (in RET/PTC3). In RET/PTC rearrangements, fusion with protein partners, possessing protein-protein interaction domains, provides RET/PTC proteins with coiled-coil domains, thereby resulting in ligand-independent activation of c-Ret tyrosine kinase activity (Santoro et al. 1995). Similar rearrangements of the high affinity receptor for NGF (Nerve Growth Factor), TRKA, can be also found, at a low prevalence (in about 10% of the tumors), in human PTC. RET activates many intracellular signaling pathways. Upon binding to ligand, it dimerizes and autophosphorylates various cytoplasmic tyrosines. The phosphorylated tyrosines thus become binding sites for intracellular molecules containing phosphotyrosine-binding motifs, thereby initiating a diverse array of signaling pathways (Santoro et al. 2004). In RET/PTC rearrangements, fusion with protein partners possessing coiled-coil domains provides RET/PTC kinases with dimerizing interfaces, thereby resulting in ligand-independent autophosphorylation. The most common rearranged forms of RET are RET/PTC1 and RET/PTC3, resulting from paracentric inversions of the long arm of chromosome 10, and RET/PTC2, resulting from a 10; 17 reciprocal translocation involving R1a on17q23. More than 11 additional types of RET rearrangements, occurring primarily in radiation-induced tumors, have been described but their frequencies are low. The role of RET/PTC in the development of PTC has been demonstrated convincingly in transgenic mice with targeted overexpression of RET/PTC1 and RET/PTC3. The RET/PTC1 rearrangement is more common in classic PTCs, papillary microcarcinomas, and the diffuse sclerosing variant than in other subtypes. RET/PTC3, on the other hand, has been associated with the solid variant.

The RET intracellular domain contains at least 12 autophosphorylation sites, 11 of which are maintained in RET/PTC proteins (Kawamoto et al. 2004). Tyr 905 is located in the activation loop and its phosphorylation stabilizes the enzyme in an active conformation. Moreover, when phosphorylated, Tyr 905 binds some SH2- containing proteins, such as Grb7 and Grb10 (Pandey et al. 1996). Phosphorylated Tyr 1015 and Tyr 1096 are responsible for binding of PLCγand GRB2, respectively (Jhiang S.M. 2000; van Weering et al. 1998). Several protein adaptors, Shc, FRS2, IRS1/2, DOK1/4/5, RAI/NShc and Enigma recognize phosphorylated Tyr 1062 (Lorenzo et al. 1997, Pelicci et al. 2002, Melillo et al 2001).

The multi-docking site Tyr1062, upon phosphorylation, is responsible for the activation of the phosphatidylinositol 3- kinase (PI3K)/AKT and the mitogen-activated protein kinase (MAPK) pathways, the latter involving the extracellular-regulated kinase (ERKs), c-Jun amino-terminal protein kinase (JNKs) and the p38 MAPK (Jhiang S.M. 2000; van Weering et al. 1998). Moreover, RET/PTC induced the expression of a pro-inflammatory transcriptional program. Such a program included the up-regulation of various cytokines (OPN/SPP1, GM-CSF, M-CSF, G-CSF, IL1A, IL1B, IL6, and IL24), chemokines (CCL2, CCL20, CXCL8, CXCL1, CXCL10, and

CXCL12) chemokine receptors (CXCR4) pro-inflammatory enzymes (cyclooxygenase-2, and microsomal prostaglandin E2 synthase), and interferon-dependent genes. (fig 2)

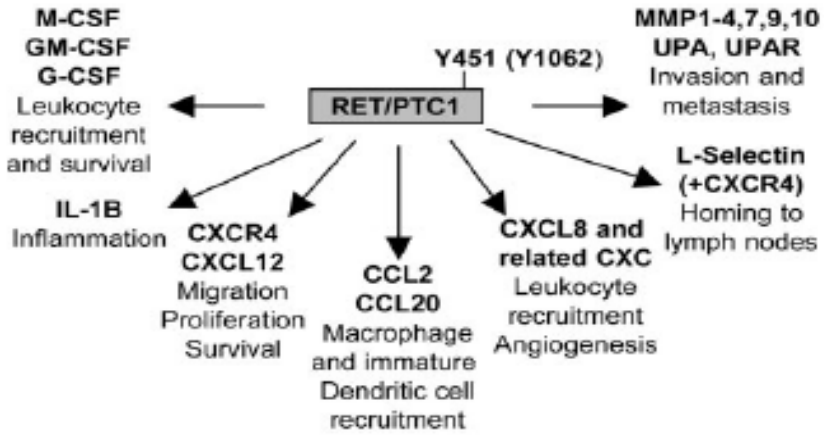

Fig 2. Induction of an inflammatory-type reaction might be part of the oncogenic effect of RET/ PTC on thyroid cancer and may explain the chronic inflammatory reaction that is characteristic of this tumor type. (Borrello et al . PNAS 2005)

By activating point mutations in RAS small GTPase are found roughly in 10% of PTC, mainly in those belonging to the follicular variant (PTC-FV) (Zhu et al. 2003). Point mutations in BRAF are the most common genetic lesion found in PTC (up to 50% of the cases) (Kimura et al. 2003; Xu et al. 2003). BRAF is a member of the RAF family of serine/threonine kinases and it is a component of the RAF-MEK-ERK signaling module. Activation of the RAF proteins is mediated through binding of RAS in its GTP-bound state. Once activated, RAF kinases phosphorylate MEK which in turn phosphorylates and activates ERK (Malumbres et al. 2003). A Glutamine for Valine substitution at residue 600 (V600E) in the activation segment accounts for more than 90% mutations of BRAF in PTC. This mutation enhances BRAF activity through the disruption of the autoinhibited state of the kinase. In human PTCs the genetic alterations of RET/PTC, RAS and BRAF are mutually exclusive, suggesting the existence of a common signaling cascade; moreover, mutations at more than one of these sites are unlikely to provide an additional biological advantage (Kimura et al. 2003, Cohen et al. 2003, Soares et al. 2003).

1.2 The Correlation between Papillary Thyroid Carcinoma and Inflammatory reaction

.

PTC is associated with a striking chronic inflammatory reaction in about 30% of cases, (Ott et al., 1987; Wirtschafter.et al., 1997; Mechler et al., 2001; Di Pasquale, 2001; Pisanu et al., 2003) in particular several observations suggest that the immune response might be important in preventing metastases and recurrence of thyroid cancer, 65% of PTC in children and young adults contained lymphocytes in the immediate vicinity of thyroid cancer, but only 18% of patients also had a diagnosis of autoimmune thyroiditis . In these cases, the role in neoplastic transformation of the infiltrates of lymphocytes present in both autoimmune thyroid disease and PTC is not yet clear; patients with Hashimoto thyroiditis and PTC have generally improved disease-free survival, while Graves disease is associated with worse prognosis.

In particolar, Hashimoto thyroiditis is an autoimmune disorder in which the immune system reacts against a variety of thyroid antigens. The overriding feature of Hashimoto thyroiditis is the progressive depletion of thyroid epithelial cells (thyrocytes), which are gradually replaced by mononuclear cell infiltration and fibrosis. Multiple immunologic mechanisms may contribute to the death of thyrocytes. Sensitization of autoreactive CD4+ T-helper cells to thyroid antigens appears to be the initiating event. Hashimoto's thyroiditis is characterized by proliferating nodules as well as cytological alterations and nuclear modifications similar to those of the papillary carcinomas (Sclafani et al., 1993;).

Originally, the rearrangement RET/PTC was considered to be specific for PTC but recent data was found that this rearrangement is expressed even in some non neoplastic conditions such as Hashimoto's thyroiditis. Several other evidences suggest a role for RET/PTC in the association between thyroiditis and cancer. In fact, patients exposed to radiation from the Chernobyl nuclear power plant disaster often develop not only RET/PTC-induced papillary tumors but also an associated autoimmune thyroiditis (Williams et al 2002). Accordingly, transgenic mice engineered to express RET/PTC develop papillary carcinomas and chronic thyroiditis (Powell et al. 1998). Finally, Wirtschafter and colleagues have detected RET/PTC expression in about the 90% of cases of the Hashimoto's thyroiditis they have analyzed (Wirtschafter et al., 1997). These data are, however, partially in contrast with the report by Rhoden and colleagues. These authors have found only few follicular cells expressing very low levels of the rearranged protein in Hashimoto's thyroiditis, thus suggesting that RET/PTC expression does not necessarily predict the development of a papillary carcinoma in patients with thyroiditis (Rhoden et al., 2006).

Two hypotheses may explain the association between Hashimoto's thyroiditis and RET/PTC: 1) the inflammatory favors the occurrence of the rearrangement; 2) RET/PTC oncoproteins may be directly involved in inducing inflammatory responses in thyroid tissues. According to first hypothesis, free radicals

production, cytokine secretion, cellular proliferation as well as other phenomena correlated with inflammation might predispose to the rearrangement in follicular cells (Gandhi et al 2006) .The second hypotesis is supported by the observation that RET/PTC3 expressing thyrocytes express high levels of proinflammatory cytokines (Melillo et al 2005; Puxeddu et al 2005, Rhoden et al 2006).

Inflammatory cytokines produced by transformed epithelial cells or infiltrating leukocytes are known to positively influence tumor development by affecting angiogenesis, growth, survival, immune suppression, DNA damage, tumor suppression, and metastasis. In contrast, tumor-infiltrating leukocytes can negatively regulate tumor progression by producing cytostatic or cytotoxic molecules and induce the death of targeted cells, but the molecular basis for this underlying cellular interaction is not well understood. (Mantovani et al 1992; Russell et al 2004; Melillo et al., 2005; Puxeddu et al., 2005) In the PTC, multiple signaling pathways are required together to work in concert with RET/PTC to cause cancer. A major factor is the host's contribution to the tumor microenvironment including angiogenesis, lymphangioenesis, stromal and immune reactions. Indeed, despite the overwhelming data to support a role of proinflammatory mediators in causing cancer, very little is known about how they influence the host microenvironment. Cytokine secretion by transformed thyrocytes may play a role in the development and progression of thyroid cancer. For instance, low level cytokine production by thyrocytes can directly stimulate, in a paracrine or autocrine fashion, the growth of thyroid cells via the secretion of Il1, Il6, or IL4 and IL10 (Zeki et al 1993; Basolo et al 2002). Moreover, previous work has demonstrated that RET/PTC3 can directly induce the production of these and other inflammatory factors and, when such cells are transplanted into mice, induce the attraction of activated macrophages. These and other data suggest that RP3-induced thyroid hyperplasia and carcinoma evokes multiple systems in vivo to manifest carcinogenic effects of RET/PTC (Russell et al 2003). The consequence of this oncogene-induced cytokine secretion is to recruit leukocytes that secrete additional cytokines (Scarpino et al 2000). Although much is known about the capability of tumors or tumor cells to produce proinflammatory mediators at late stages of disease, little is known about how these factors may be available early in the process to promote the support and progression of transformed epithelial cells.

Therefore, the factor that seems to influence the relationship between thryroid cancer and inflammatory infiltration is the "initiating oncogene", rearrangement RET/PTC, that may therefore be critical for promoting tumor progression by the activation of specific signaling pathways that not only include those that regulate autocrine growth factors or their receptors, but also those that influence the immune responses (Russell et al 2006) Furthermore, the RET/PTC- RAS-BRAF axis was shown to trigger an up-regulation of CXC chemokines and CXC chemokines receptors, which in turn stimulates proliferation and invasion (Borrello et al 2005, Puxeddu et al 2005, Melillo et al, 2005) .

Chemokines are small chemotactic cytokines that are subdivided into 4 groups (CXC, CC, C and CX3C chemokines) on the basis of the relative position of

cysteine residues. Chemokines bind to 7-transmembrane G-protein –linked receptors. Most tumors produce CXC and CC chemokines which interact with CXCR and CCR respectively. These receptors mediate several biological activities (chemotaxis, cytoskeletal rearrangements and adhesion to specific cells), triggers a cascade of events that results in activation of AKT and ERK and in cell polaritation, adhesion and migration (fig 3). Chemokines regulate some important features of cancer cells; moreover they are also involved in the regulation of tumor angiogenesis and leukocyte recruitment (Kulbe H et al 2004; Kucia et al 2006)

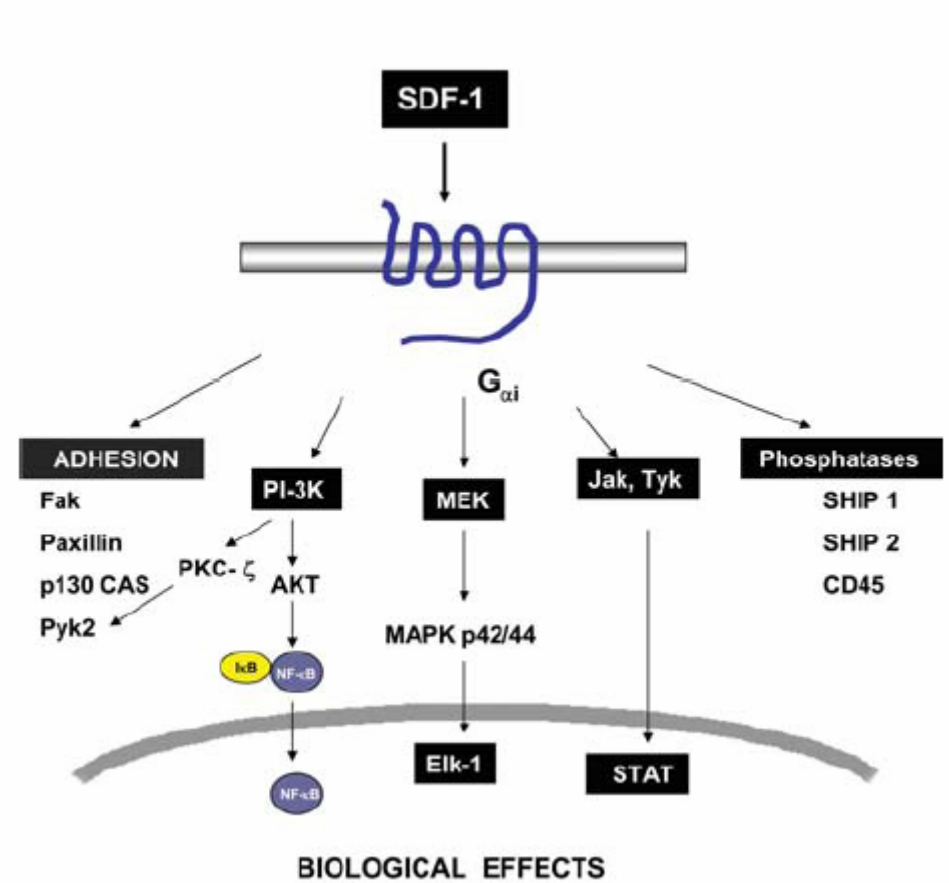

Figure 3. Signal transduction pathways activated by the SDF-1– CXCR4 axis. Interaction of SDF-1 with G-protein–coupled sevenspan transmembrane receptor CXCR4 activates several pathways in cells, with activation varying between cell types. Activation of these pathways in CXCR4+ cells (e.g., normal and malignant stem cells) regulates locomotion, chemotaxis, adhesion, and secretion. (Kucia M et al, Stems Cell 2006)

More than 50 different chemokines and 20 different chemokine receptors have been cloned. Chemokines usually bind to multiple receptors, and the same receptor may bind to more than one chemokine. However, there is one exception to this rule: the chemokine stromal-derived factor (SDF)-1, which binds exclusively to CXCR4 and has CXCR4 as its only receptor. The

directional migration of tumor cells to distant organs via lymphatics and blood resembles chemokine-directed lymphocyte migration. Recent papers suggest (Kulbe et al 2004; Kucia et al 2006) that tumor cells may express restricted and specific patterns of chemokine receptors and that responses to chemokine gradients may contribute to metastatic spread (fig 4) . The composition of the leukocyte infiltrate in many carcinomas is related, in particular, to tumor and stromal cell production of CC chemokines (Liao et al 1989;Rollins et al 1997)

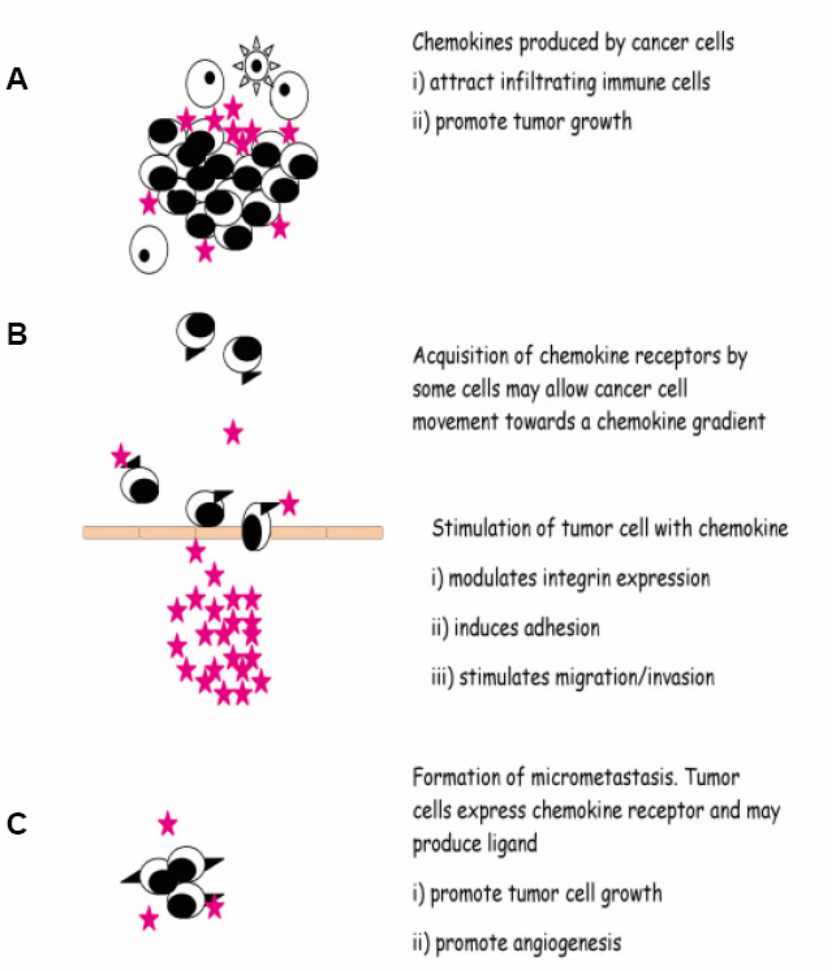

Fig. 4 . Role of chemokines in tumor progression/invasion/outgrowth. (A) Chemokines (stars) produced by tumor cells within a solid tumor, attract a leukocyte infiltrate and/or promote proliferation of cancer cells. **(B)** Cancer cells which acquire expression of chemokine receptors may migrate towards a chemokine gradient. Chemokine stimulation of receptors may also alter the adhesive capacity of tumor cells. **(C)** Within a metastatic deposit, chemokine production may stimulate tumor growth, but also effect the microenvironment by promoting angiogenesis. (Kulbe H et al, Int. J. Dev. Biol., 2004)

Chemokines control the directional migration of leukocytes and it seems that mechanisms utilised for leukocyte trafficking may also be used by tumor cells. It is described how certain cancer cells can have restricted and specific

expression of chemokine receptors, in particular CXCR4 and CCR7 and this may be one factor in the development of site specific metastasis. In particular, the chemokine receptor CXCR4 and its ligand stromal cell-derived factor-1 (SDF-1)/CXCL12 appears to be expressed by a majority of cancer types including cancers of epithelial, mesenchymal and haematopoeitic origin. This fact alone suggests that the SDF-1–CXCR4 axis plays a uniquely important biological role and may be involved in tumor cell proliferation and survival, as well as in the metastatic capacity of tumor cells (Bajetto et al 2006, Kucia et al 2006, Orimo et al 2005) .
Recent investigation has demonstrated a potential role of chemokines and their receptor in PTC and in anaplastic thyroid carcinoma (as CXCL12,CXCL1, CXCL10, and CXCR4, CXCR2 and CXCR3 receptors respectively). These data have showed that the RET/PTC rearrangemnt (RET/PTC 1 and 3 especially) induces the expression and the up-modulation of several inflammatory genes, including chemokines(CXCL12), their receptors (CXCR4), cyokines, thus suggesting a possible autocrine loop (Castellone et al 2004; Borrello etal 2005, Hwang et al 2003; De Falco et al 2007).

1.3 Staging and Risk Assessment

Conventionally, staging of PTC is based on the pathological results combined with the information derived from the post-ablation 131IWBS and from neck ultrasound at the moment of ablation (Mazzaferri et al 1994). Several tumor staging systems have been devised to accurately predict a patient's long-term outcome. The first such staging system identified age, sex, cell type (eg, PTC, follicular thyroid cancer, etc.), tumor size, and number of metastatic sites as the main characteristics in identifying prognosis. Moreover, there are the different staging systems (Cady et al 1988;Hay et al 1989; DeGroot et al 1990; Shaha et al 1996; Sherman et al 1998; Hay et al 2002). Statistical analyses of staging systems show that the proportion of variance explained averages from about 6% to 20% to predict mortality and about 12% to predict the likelihood of a patient being disease free. A perfect staging system would predict the correct outcome in every patient, with a proportion of variance explained of 100% (Byar et al 1979). None of the staging classifications accounts for more than a small proportion of the uncertainty in disease specific mortality or the likelihood of remaining disease-free over time. The accuracy of predicting outcome with these staging systems is thus too low to guide therapy in an individual patient. None is superior to the TNM staging system used by most tumor registries, but it also has a similarly low proportion of variance explained. The American Joint Committee on Cancer/ International Union Against Cancer TNM staging system (Hermanek et al. 1992, American Joint Committee on Cancer 2002 and 2005) based mainly on the rational that it allows risk stratification to plan the

frequency and type of follow up for individual patients. The 2002 TNM classification is to our knowledge one of the few scoring systems that incorporates information regarding the prognostic impact of regional lymph node metastases (Tab 1-2). Infact neck lymph node (LN) metastases are found in up to 70% of cases of PTC. The presence of neck LN metastases and tumor extension beyond the thyroid capsule at diagnosis have been identified as indipendent risk factors of recurrence .

Table 1: The Tumor, Node, Metastases (TNM) scoring system (Hermanek et al. 1992, American Joint Committee on Cancer 2002 and 2005)

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.

Table 2: TNM Staging (Hermanek et al. 1992, American Joint Committee on Cancer 2002 and 2005)

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

Recently, in accordance with this system, several attemps to reach consensus about treatment of thyroid carcinoma have resulted in guidelines for diagnosis and disease management. A European Consensus Report (Pacini et al 2006) defined three risk categories for DTC: Very low risk: unifocal T1 (<1 cm) N0 M0, no extending beyond the thyroid capsule, and absence of aggressive histology (e.g. tall cell, insular, columnar, cell carcinoma) Low risk: T1 (>1 cm) or T2 N0 M0 or multifocal T1 N0 M0. High risk: any T3 and T4 or any T, N1, or any M1. In the guidelines stipulated by the American Thyroid Association Guidelines (Cooper et al 2006) patients are stratified as follows: Low risk: T1–2 N0 M0 and absence of aggressive histology or vascular invasion; Intermediate risk: T3 or tumor with aggressive histology or vascular invasion; High risk: T4 or any T, N1 or M1. (Tab.3)

Table 3
Cancer Risk Stratification According to the American Thyroid Association and the European Thyroid Association

Risk	Characterization
ATA guidelines[a]	
Low	Stage I
Intermediate	Stage II
High	Stage III and IV
ETA consensus[b]	
Very low	T1N0M0
Low	All patients not classified in the very low- or high-risk categories
High	Persistent disease or high risk of recurrence

Abbreviations: ATA, American Thyroid Association; ETA, European Thyroid Association.
[a]Based on the sixth edition of the American Joint Committee on Cancer and the International Union Against Cancer TNM staging system.
[b]Based on the fifth edition of the American Joint Committee on Cancer and the International Union Against Cancer TNM staging system.

Nevertheless, tumor staging systems are too inaccurate to guide therapy because they fail to identify patients with adverse outcomes. A recent study (Leboulleux et al 2006) has demonstrated that the pTNM system is not suitable to assess the risk for persistent or recurrent disease. In fact, several important prognostical features of thyroid cancer such as the presence of multifocal cancer, vascular invasion, autoimmune thyroid disease in the remaining thyroid tissue and /or incomplete surgical resection, are not included in the TNM staging system. Moreover, TNM does not take into account the extent of LN involvement. Therefore, the authors proposed that other criteria,such as hystopatological subtypes, should be taken into account for the evaluation of prognostic factors of survival.

2. AIM OF THE STUDY

Although the expression of the chemochine receptor, CXCR4, in thyroid carcinoma has been well documented, in contrast the expression of its ligand, SDF-1, has not been evaluated in thyroid carcinoma. Moreover, the association of these molecules with clinicopathological features has not been systematically addressed.
The purpose of this study was to quantify the expression of these molecules in a series of small PTCs and to determine whether their expression level correlates with patient and tumor characteristics.
This study was of particular interest for several reasons: PTC, including small ones, preferentially metastasize to neck lymph-nodes via lymphatic stream. This preferential localization may also depend on the repertoire of chemokine receptors expressed on the cell surface of cancer cells and, at the end, could be a potential target for therapeutic intervention.

Therefore, the main objectives of my dissertetion were:

- To analyze initial presentation, treatment, outcome and prognostic factors according to the TNM classification in patients with PTC.

- To evaluate and quantify the expression of inflammatory markers as SDF 1- CXCR4 by RT-PCR and immunohistochemistry.

- To determine whether their expression correlates with clinical and tumor characteristics, indicators of tumor aggressiveness, including sex, age, TNM and/or stage, tumor size, focality, extrathyroidal capsule extension and lymph nodes involvement.

3. MATERIALS AND METHODS

3.1. Clinical Study

Patients

Clinical charts were retrieved from the archives, 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 1996 and 2005. Thyroid tissue samples were obtained from the IGR-CRB with informed consent of each patient and the approval of the IGR committee.This search was conducted using the terms "thyroid", " differentiated carcinoma" and "papillary carcinomas". According to WHO, the inclusion criteria was: PTC of 2±1 cm was diagnosed in all patients, histologically , the classic variant and the follicular variant were diagnosed.
The patients affected by folliculary, medullary, or undifferentiated carcinoma were not included in the study.
For the 48 patients selected , histologic hematoxylin and eosin-stained slides were reviewed by a single pathologist who was unaware of clinical data, in order to confirm the diagnosis. The follicular cell origin of the tumors is immunohistochemically confirmed with positive thyroglobulin immunoreactivity and no staining for calcitonin.
For all 48 patients, informations regarding gender, age, tumor size and stage, therapy administered and outcomes were collected. Clinical parameters including patient age and gender were obtained from hospital records. Pathologic parameters , obtained from pathology reports, included histologic subtype of papillary carcinoma, tumor size, extrathyroidal extension, presence of positive lymph nodes, and presence of chronic thyroiditis.
Stage for PTC was assigned using the TNM system of American Joint Committee on Cancer American Joint Committee on Cancer 2002, (Hermanek and Sobin 1992, AJCC 2002 and 2005). 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 august 2008,or loss to follow-up.

3.2 Gene Expression Analysis

Tissue Collection and RNA extraction and Reverse Transcription Polymerase Chain Reaction (RT-PCR).

Thyroid tumor samples were collected at the time of surgery, snap-frozen in liquid nitrogen, and stored at -80°C. RNA was extracted from frozen tissue by homogenization in RLT lysis buffer (Qiagen, Valencia, CA, USA) using the manufacturer's protocol (RNeasy Mini Kit; Qiagen, Valencia, CA, USA). RNA purity was confirmed by spectrophotometry.
Total RNA was isolated by the RNeasy Kit (Qiagen, Crawley, West Sussex, UK) and subjected to on-column DNase digestion with the RNase-free DNase set (Qiagen) according to the manufacturer's instructions. The quality of RNA was verified by electrophoresis through 1% agarose gel and visualized with ethidium bromide. Random-primed first strand cDNA was synthesized in a 50 µl reaction volume starting from 2 µg RNA by using the GeneAmp RNA PCR Core Kit (Applied Biosystems, Warrington, UK). Primers were designed by using software available at http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi and synthesized by the MWG Biotech (Ebersberg, Germany). To exclude DNA contamination, each PCR reaction was also performed on untranscribed RNA.
Quantitative (real-time) reverse transcription polymerase chain reactions (QRT-PCR) were performed by using the SYBR Green PCR Master mix (Applied Biosystems) in the iCycler apparatus (Bio-Rad, Munich, Germany). Amplification reactions (25µl final reaction volume) contained 200 nM of each primer, 3 mM MgCl2, 300 µM dNTPs, 1x SYBR Green PCR buffer, 0.1U/µl. AmpliTaq Gold DNA Polymerase, 0.01U/µl Amp Erase, RNase-free water, and 2 µl cDNA samples. Thermal cycling conditions were optimized for each primer pair and are available upon request. To verify the absence of nonspecific products, 80 cycles of melting curve (55°C for 10 sec) were performed. In all cases, the melting curve confirmed that a single product was generated. Amplification was monitored by measuring the increase in fluorescence caused by the SYBR-Green binding to double-stranded DNA. Fluorescent threshold values were measured in triplicate and fold changes were calculated by the formula: 2-(sample 1 ΔCt - sample 2 ΔCt), where Δ*Ct* is the difference between the amplification fluorescent thresholds of the mRNA of interest and the ß actin mRNA.

3.3 Immunohistochemistry

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 for 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 treated 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.
Immunohistochemical analysis (IHC) was performed using the BondMax Automated Immunohistochemistry & In Situ Hybridization and Bond Polymer Define peroxidase detection systems for CXCR4, SDF-1 , (Leica-Vision Biosystems, Melbourne, Australia) . For CXCR4 the primary antibody was mouse monoclonal IgG2a clone 12G5(R&DSystems, Minneapolis, MN, USA), while SDF-1 was assessed using mouse monoclonal IgG1 clone 79018 (R&D Systems), each diluted 1:200 in Bond Primary Antibody After antigen retrieval, immunostaining was performed according to the manufacturer's protocol.
Positive controls for each antibody and appropriate negative controls lacking primary antibody were performed. Sections were counterstained with hematoxylin, dehydrated, and embedded in Cytoseal XYL (Richard Allan Scientific, Kalamazoo, MI). Each staining reaction was performed in triplicate.

3.4 Immunohistochemical analysis

The 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, a semiquantitative assessment of cytoplasmic staining intensity for each antibody was carried out by two independent observers, who assigned a score to each tumor core as follows:

1. *localization of the marker:* in the nucleus, cytoplasm and membrane
2. *number of positive cells:* we used a score between 1 and 3 (1: <50% positive cells, 2: between 50-94% positive cells, 3: between 95-100% positive cells)
3. *intensity of expression:* we used a score between 1 and 3 (1: absent or low, 2: moderate, 3: high)

Moreover for each marker, we evaluated the expression in associated chronic thyroiditis and in peritumoral stroma. In particular, when standard histological sections of normal tissue, thyroiditis, stroma and tumor specimens were carefully examined, several types of epithelial structures were identified:
1.Normal appearing follicular structures of which the majority of the thyroid tissue is usually composed;
2. Follicles that were more or less damaged by cytotoxic lymphocytes;
3.Tissue-infiltrating lymphocytes;
4.Peculiar regenerative metaplastic ductal structures (MDS) which were clearly present in thyroiditis samples. They were always found at the periphery of organized lymphoid infiltrates to which they are confined ;
5.Polinuclear macrophage-like population, fibroblastic-like population;
6.Papillary differentiated tumoral cells
7. Epidermoid metaplasia.

3.5 Statistical Analysis

Data were analyzed using SAS version 8.2 (SAS Inc, Cary, NC). Quantitative variables were reported as median (range), and categorical variables as absolute numbers and percentages (%).Univariate association of patient clinical characteristics with chemoattractant stromal cell-derived factor 1 (SDF-1) and chemokine receptor 4 (CXCR4) were tested by means of Kruskal-Wallis exact test. Multivariate analyses were performed to evaluate the association of SDF-1 and CXCR4 with 6 main clinical characteristics included *a priori* in the model. Two different approaches were used. Ordinal logistic regression under a proportional odds model was used for categorical dependent variables (DV). If the DV has three different ordered categories this approach simultaneously models 2 cumulative logits corresponding to using binary cut points at 2 and 3, written as $\log\{Pr(DV\geq 2)/Pr(DV<2)\}$ and $\log\{Pr(DV\geq 3)/Pr(DV<3)\}$, respectively. Under this proportional odds model, one coefficient is estimated for each covariate in the model. The coefficient represents the effect of a one-unit increase in the predictor variable on the logit (log odds), which is assumed to be the same for all two logits. A score test was used to verify the proportional odds assumption in the final model. To further verify the proportional odds model, we fit binary logistic regressions using DEP cutoffs of at least 2 and at least 3, and compared the results with those of the ordinal

regressions. Multiple linear regression analysis was used for continuous dependent variables.

4. Results

4.1 Patient features

Forty-eight PTC patients underwent total thyroidectomy and cervical lymphnode dissection by a single surgeon at a single institution.
There were 34 females and 14 males, with a mean age at diagnosis of 44.0 years [14-80 yrs]. No patient had a family history of thyroid carcinoma or of radiation exposure. Chronic thyroiditis was present in 29 cases but AbTg and AbTPO was positive in only 2 patients. Twenty-five patients were classified as T1, eleven as T2, and nine as T3 and three patients as T4. (Tab 4) The median tumor size was 2± 1 cm. Lymph-node metastases were present in 31/48 patients. One patient had distant metastases at discovery of the disease, in the lung. Twenty-four patients showed extracapsule thyroid extension. (Table 5).
Median follow-up was 6.1 years, during which 9 patients developed metastases : lymph nodes metastases occurred in the central compartment in 7 patients, mediastinal region in one patient and in both neck lymph nodes and lungs in another patient. Lymph-nodes metastases occurred in patients aged less than 45 years while distant metsatses occurred in patients older than 45 years. Among the 9 patients with metastases, 5 had a microcarcinoma at the time of diagnosis and 4 had a primary tumor _> 2cm. Eigth patients (including all patients with distant metastases) showed an extension beyond the thyroid capsule and all of these had lymph nodes metastases at the time of diagnosis. We did not find a correlation between the appearance of metastases and the presence of thyroiditis in any case. The data of recurrent disease was noted but the parameters were excluded from analysis due to their small numbers.
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 all of the 10 patients with recurrent diseases who were treated with radioiodine. In all patients clinical benefits were observed after treatment with radioiodine. In fact, no patient with metastases died after a mean follow-up of 3.1 years after initial treatment.

Table 4: Clinical characteristics in 48 patients with PTC

Clinical characteristics	
Age	
≤ 45	26
> 45	22
Gender	
M	14
F	34
Stage	
Stages 1,2	32
Stages 3,4	16
Thyroiditis	
Absent	19
Present	29

Table 5: Tumor characteristics in 48 patients with PTC

tumor characteristics	
Histotype variant	
Classic	27
Follicular	21
Focality	
Unifocal	34
Multifocal	14
Extracapsule thyroid extension	
Absent	24
Present	24
Dimension	
< 2cm	26
>2 cm	22
*Lymph Nodes (TNM)***	
Absent	17
Present	31
*T (TNM)**	
T1	25
T2	11
T3	9
T4	3

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

4.2 CXCR4 and SDF 1 mRNA expression

Each tumoral tissue sample was analyzed for CXCR4 and SDF 1 gene expression by Real Time -PCR . In all cases, CXCR4 and SDF-1 were found to be up regulated in tumor samples *vs* samples of normal thyroid tissue
The chi-square test showed a highly significant association between CXCR4 and SDF-1 expression ($p<0.05$), indicating a tendency toward co-expression of these markers in a subset of tumors.

4.3 CXCR4 and SDF 1 localization by Immunohistochemistry.

IHC analysis was performed to confirm the data obtained in the RTPCR experiments at the protein level and to determine the cell type that actually expresses CXCR4 and SDF-1 in the tumor site, in peritumoral stroma, in normal tissues and in associated chronic thyroiditis.
In accordance with RT -PCR, all tumoral tissues investigated expressed SDF-1 with high intensity and with a percentage of positive cells of < 50% in 5, of 50-94% in 25, and of 95-100% in 18 tumors. A diffuse citoplasmic expression was found both in papillar tumor cells and in epidermoid metaplasia (figure 5)

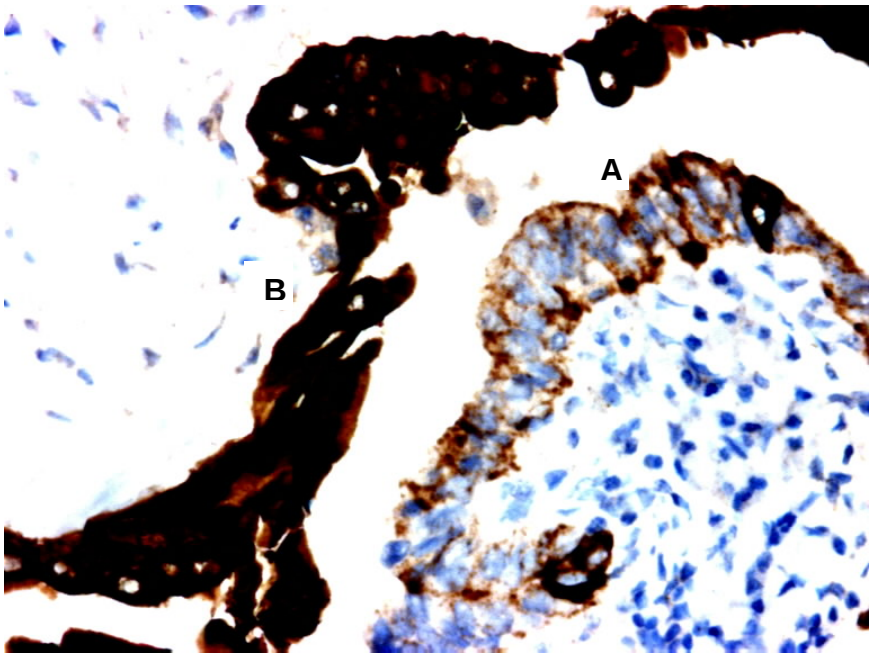

Fig 5 Immunohistochemical analysis for SDF-1 (formalin-fixed, paraffin-embedded thyroid carcinoma) : SDF-1 expression in human papillary thyord cancer: localization of SDF protein was cytoplasmic in papillary differentiated structures (1) and in epidermoid metaplasia (2).

We have also found that SDF-1 is abundantly expressed by peritumoral stromal tissues, but with moderate-low intensity. In particular we observed that the subset of fibroblast-like cells, especially were positive for SDF-1 (as indicated

by figure 6), with a percentage of positive cells of >50% in 42 cases. In contrast, we failed to detect any fibroblastic –like cells positive for SDF-1 in non cancer stroma. Moreover we detected SDF-1 expression to some extent in scartered normal epithelial cells and endothelial cells (figure 7).

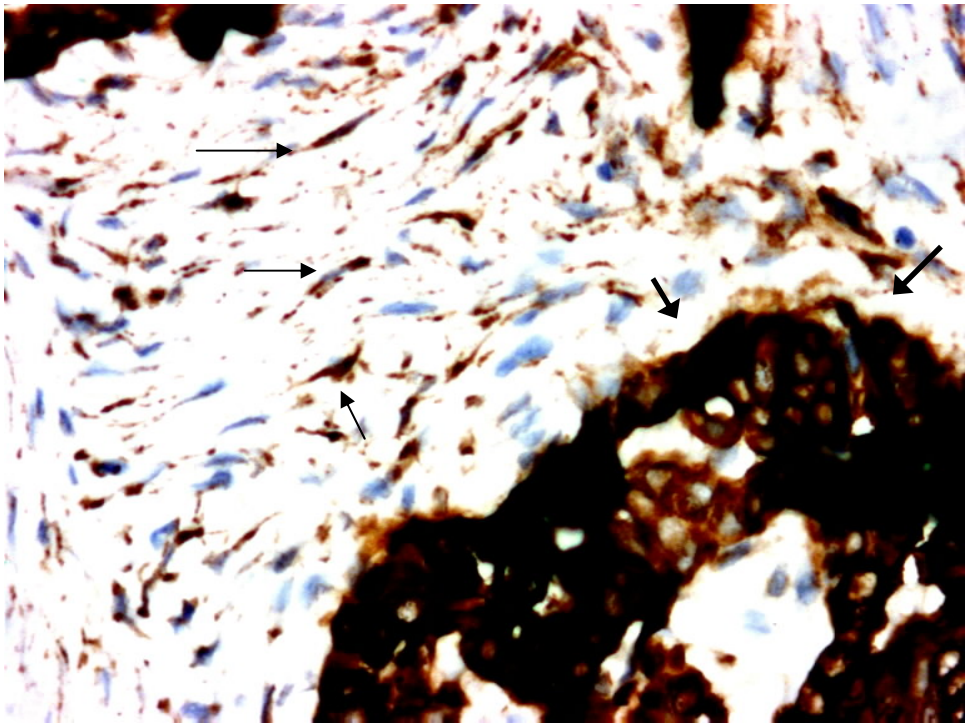

Fig 6 : **Immunohistochemical analysis for SDF-1 (formalin-fixed, paraffin-embedded thyroid carcinoma): Fibroblast-like cells** *(thyn arrows)* **located between the papillary structures moderatly expressed SDF-1, while epidermoid metaplasia was strogly SDF-1 positive** *(thyck arrow)*

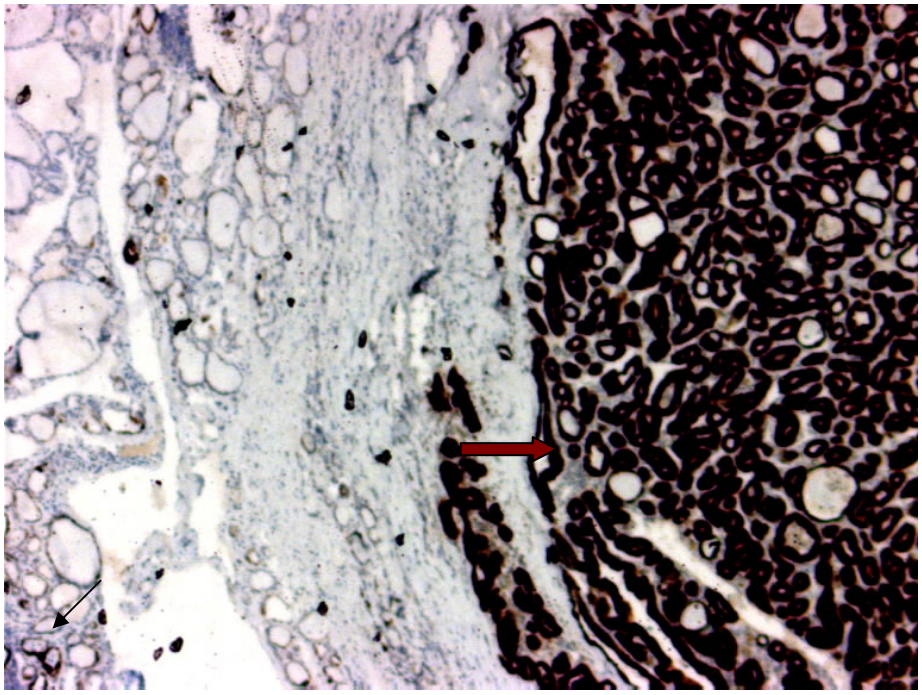

Fig 7 Immunohistochemical analysis for SDF-1 (formalin-fixed, paraffin-embedded thyroid carcinoma): PTC expressed SDF-1 with high intensity *(red arrow)***, whereas normal follicules were faible SDF-1 positive** *(thyn arrows).*

Finally, we have found a characteristic SDF-1 expression in chronic thyroiditis. In particular, we have found a peculiar distribution SDF-1 staining in the metaplastic/hyperplastic ductal structures (MSD) localized at the periphery of organized lymphoid infiltrates, which, on the contrary, appeared widely negative for SDF-1 (figure 8).

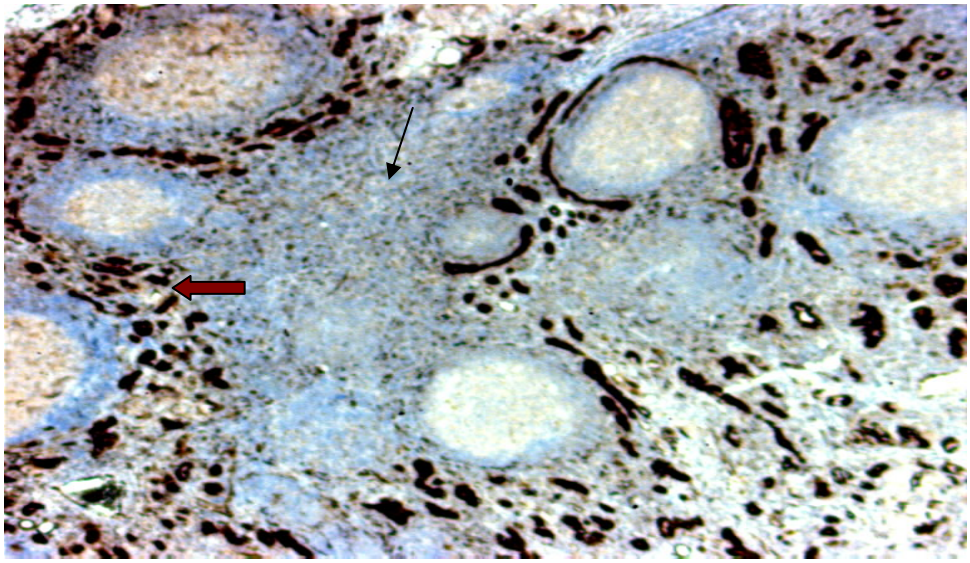

Fig 8 Immunostaining for SDF-1 in chronic thyroiditis shows that MDS *(red arrows)* **are specifically located at the periphery of lymphoid follicles and strongly expressed SDF-1. Lymphocytes were SDF-1 negative** *(thin arrow).*

We then evaluated the expression levels of SDF-1 receptor, CXCR4, in the same PTC tumors. All tumors expressed CXCR4, but with heterogenous intensity (low, moderate and high in 15, 16 and 17 patients respectively) and with a percentage of positive cells of 50-94% in 26 and of 95-100% in 22 cases. CXCR 4 is a membrane receptor.Despite this, in cancer positive cells, CXCR4 staining was located into cytosolic granules. Since this receptor undergoes rapid internalization upon ligand binding (figure 9).This pattern of expression can be explained by receptor down-regulation. Whatever the case, these data indicate that a significant fraction of PTC, similarly to other ephitelial cancers, features high expression levels of the CXCR4.

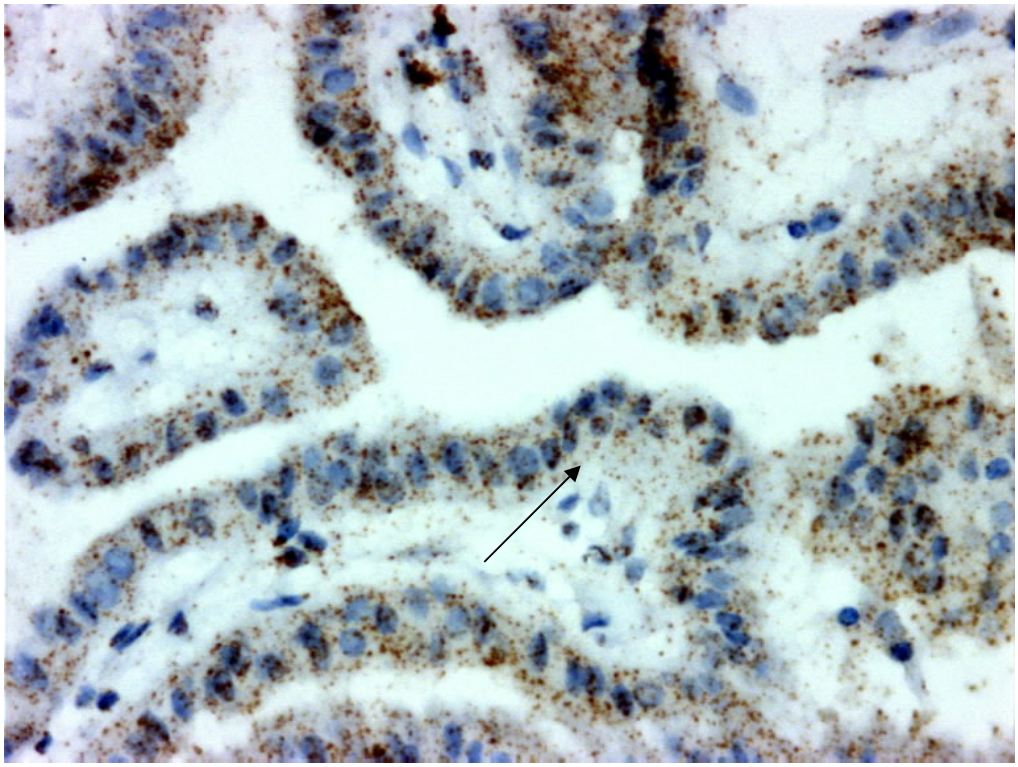

Fig 9 CXCR4 expression in human papillary thyroid carcinoma tissue (hematoxylin-eosin-stained paraffin-embedded tumor sections): immunohistochemical localization of CXCR4 protein was cytoplasmic. Typical granular aspect.

The distribution of CXCR4 in the peritumoral stroma seems completely different from that of its ligand SDF-1. As shown in figures 10 and 11, CXCR4 is expressed only in stromal-derived cells, particularly in macrophage-like cells and lymphocytes cells, with a percentage of positive cells of < 50% in 13 cases, of 50-94% in 23, and of 95-100% in 11 with variabile intensity. In some cases, stromal cells showed positive staining in the nucleus in addition to cytoplasmic staining and an expression in granular form were found in all cases.

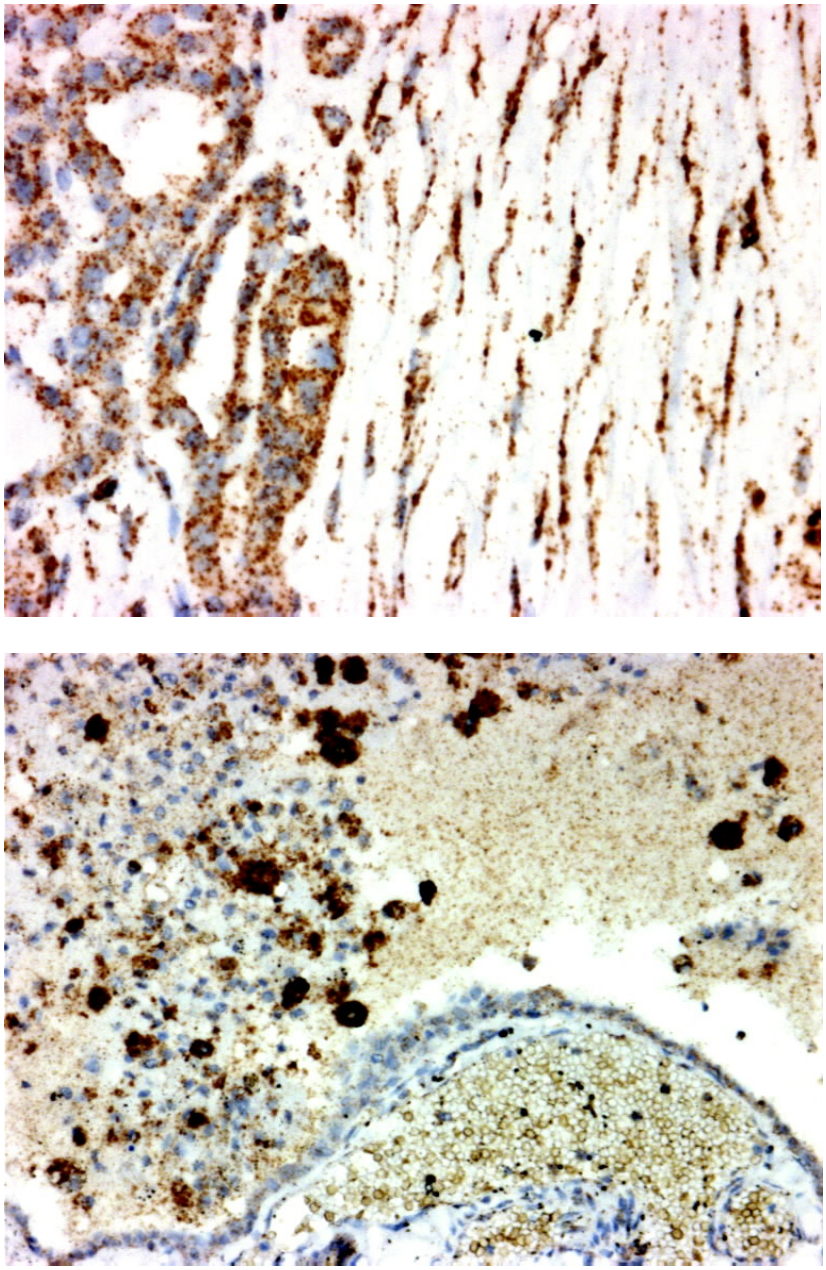

Fig 10 and 11: CXCR4 in peritumoral stroma tissuse: fibroblastic and macrophage-like cells were strongly positive for CXCR4.

In the chronic thyroiditis, in contrast to what observed for SDF-1, CXCR4 was shown to be expressed at significantly higher amounts in lymphocyte populations and not in peripheral MDS (Fig 12)
Finally, peritumoral normal thyroid tissue displayed a lower but detectable expression. This reactivity was localized to follicular thyroid cells, stromal cells or infiltrating lymphocytes. (Fig 13).

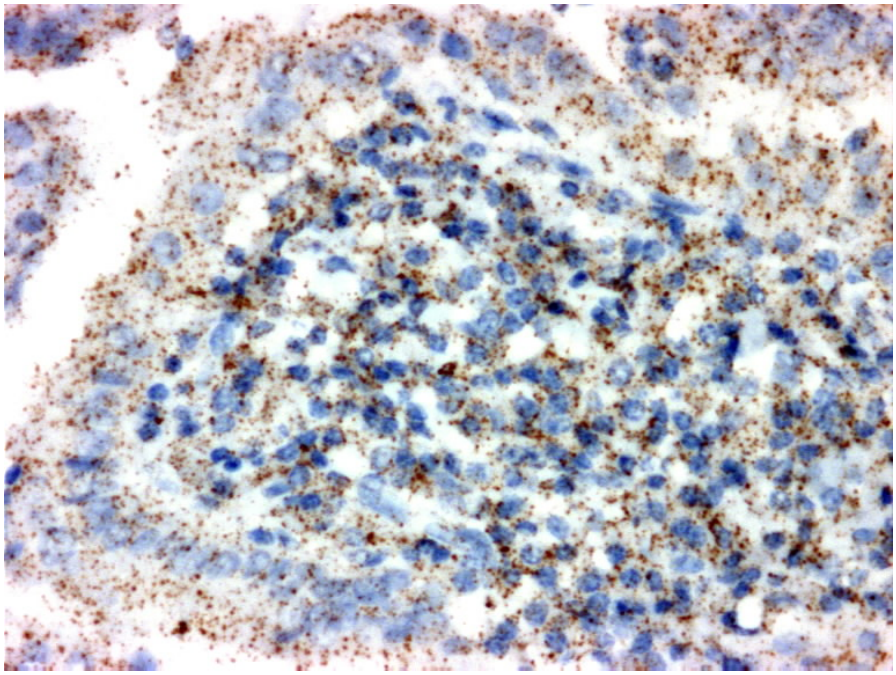

Fig 12. HCI staining for CXCR4 occurred in chronic thyroiditis: intense expression of CXCR4 in the lymphocytes cells

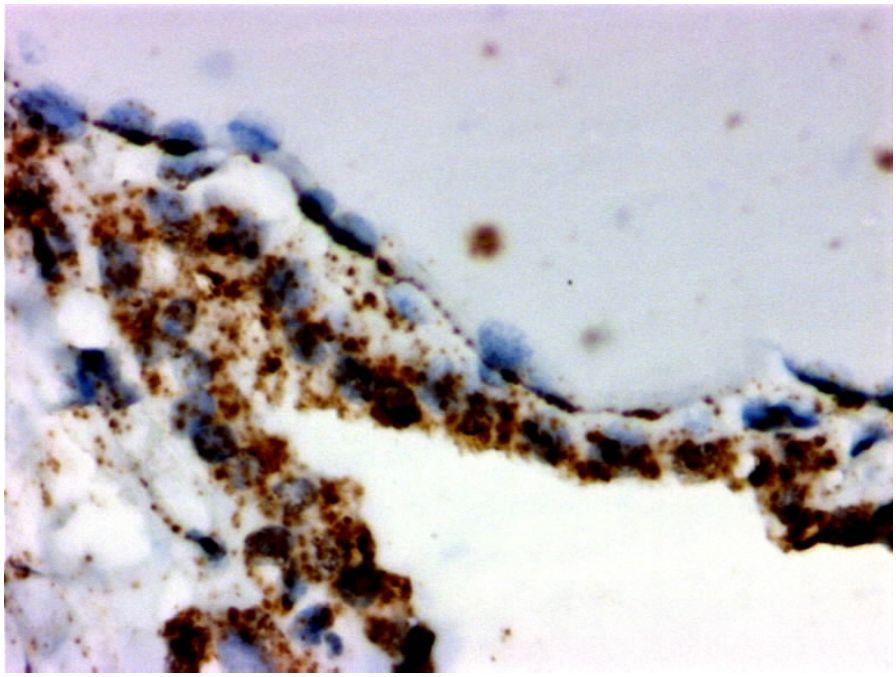

Fig 13: In some cases normal activated stromal cells shoed positive staining for CXCR4 in the cytoplasm

4.4 Correlations between SDF-1/CXCR4expression and clinico and tumor–pathological factors.

In this study, no significant correlation were found between the levels of SDF-1 and CXCR4 mRNA and tumoral and clinical subgroups (Tab.6-7).
For immunohistochemical staining, we have compared the intensity score and the percentage of SDF-1 and CXCR4 positive cells with clinical and tumoral characteristics, indicators of tumor aggressiveness.
We found no significant association of SDF-1 or CXCR4 staining intensity or percentage with any of the patient characteristics evaluated Moreover, we found a significant association between the SDF-1 and female gender($p = 0.05$), (Table 8), this association was not shown following multivariate analyses.
Among tumor features, CXCR4 immunostaining positivity was significantly higher in classical variant relative to follicular variant ($p=0.02$). The intense staining for CXCR4 was also associated with a smaller tumor size ($p= 0.03$),(Tab 9). These association were confirmed also when CXCR4 positivity was expressed through percentage of positive cells ($p=0.0045$) (Tab 10).
Higer CXCR4 expression (Tab. 9) was found among tumors showing lymph-node involvement (N1) at the initial diagnosis ($p=0.01$). No significant association with multifocality, or extrathyroidal extension was observed.
Moreover, tumor features were not significantly associated with SDF-1 expression.
Finally, a multivariate analysis, corrected for known predictors of recurrence, confirmed the CXCR4 univariate results. (table 11-12-13).

Table 6: Correlation between real time RTPCRCXCR4 expression and clinical/tumoral factors – univariate analisys

	RTPCRCXCR4				
	N	Median	Minimum	Maximum	p
Age					
≤ 45	26	4.55	0.38	39.4	NS
> 45	22	4.92	1.21	34.3	
Gender					
F	34	4.38	0.38	39.4	NS
M	14	4.92	1.21	22.16	
Stage					
I	31	3.73	0.38	39.4	NS
II	1	3.03	3.03	3.03	
III	8	5.29	1.21	24.25	
IV A	8	8.57	4.92	34.3	
Thyroiditis					
Absent	19	4.92	0.38	39.4	NS
Present	29	5.66	1.23	34.3	
Histotipe					
Classic	27	4.92	0.38	39.4	NS
Follicular	21	4.6	1.21	34.3	
Focality					
Unifocal	34	4.92	0.38	39.4	NS
Multifocal	14	4.97	1.52	52.99	
Extrathyroid extension					
Absent	24	3.45	1.23	39.4	NS
Present	24	5.66	0.38	34.3	
Dimension					
< 2 cm	26	4.92	1.39	39.4	NS
> 2cm	22	4.92	0.38	34.3	
Lymph Nodes TNM					
Absent	17	3.41	1.39	34.3	NS
Present	31	5.66	0.38	39.4	
T (TNM)					
T1	25	4.87	1.39	39.4	NS
T2	11	3.25	1.21	22.11	
T3	9	6.96	0.38	22.16	
T4	3	14.62	3.03	34.3	

Table 7: Correlation between real time RTPCRSDF-1 expression and clinical/tumoral factors – univariate analisys

	RTPCRSDF-1				
	N	Median	Minimum	Maximum	p
Age					
≤ 45	26	5.29	1.6	22.63	NS
> 45	22	5.39	0.2	21.1	
Gender					
F	34	4.01	0.2	22.63	NS
M	14	7.25	1.9	16	
Stage					
I	31	5.68	1.6	22.63	NS
II	1	2	2	2	
III	8	6.5	0.2	12.13	
IV A	8	3.62	1.9	21.1	
Thyroiditis					
Absent	19	4.92	1.86	19.69	NS
Present	29	5.66	0.2	22.63	
Histotipe					
Classic	27	4.93	1.6	22.63	NS
Follicular	21	5.65	0.2	21.1	
Focality					
Unifocal	34	4.6	1.6	22.63	NS
Multifocal	14	6.07	0.2	16	
Extrathyroid extension					
Absent	24	5.68	1.6	22.63	NS
Present	24	3.62	0.2	22.1	
Dimension					
< 2 cm	26	5.29	0.2	22.63	NS
> 2cm	22	5.11	1.6	13.9	
Lymph Nodes TNM					
Absent	17	11.46	0.2	21.11	NS
Present	31	3.89	1.6	22.63	
T (TNM)					
T1	25	5.65	1.62	22.63	NS
T2	11	6.5	1.6	16	
T3	9	3.2	0.2	13.9	
T4	3	2.87	2	3.73	

Table 8: Correlation between immunoistochemical staining of SDF-1 (number of positive cells) expression and clinical/tumoral factors - univariate analisys

	SDF-1 Numbers of positive cells			
		2	3	
	N	Mean intensity z score (%)		Univariate p value
Age				
≤ 45	26	12(48%)	9 (50%)	NS
> 45	22	13(50%)	9 (50%)	
Gender				
F	34	15(60%)	16(89%)	**0.05**
M	14	10(40%)	2 (11%)	
Stage				
I	31	15(60%)	12 67%)	NS
II	1	0 (0%)	0 (0%)	
III	8	4 (16%)	4 (22%)	
IV A	8	6 (24%)	3 (11%)	
Thyroiditis				
Absent	19	10(40%)	6 (33%)	NS
Present	29	15(60%)	12 67%)	
Histotipe				
Classic	27	16(64%)	9 (50%)	NS
Follicular	21	9 (36%)	9 (50%)	
Focality				
Unifocal	34	20(80%)	11(61%)	NS
Multifocal	14	5 (20%)	7 (39%)	
Extrathyroid extension				
Absent	24	14(56%)	8 (44%)	NS
Present	24	11 44%)	10(56%)	
Dimension				
< 2 cm	26	10(40%)	12(67%)	NS
> 2cm	22	15(60%)	6 (33%)	
Lymph Nodes (TNM)				
Absent	17	9 (36%)	7 (39%)	NS
Present	31	16(64%)	11(61%)	
T (TNM)				
T1	25	12(48%)	10 55%)	NS
T2	11	10(40%)	1 (6%)	
T3	9	2 (80%)	6 (33%)	
T4	3	1 (4%)	1 (6%)	

Table 9: Correlation between immunoistochemical staining of CXCR4 (intensity) expression and clinical/tumoral factors – univariate analisys

CXCR4					
		Low intensity	Medium intensity	High intensity	
	N	Mean intensity z score (%)			Univariate p value
Age					
≤ 45	26	7 (47%)	12 (75%)	7 (41%)	NS
> 45	22	8 (53%)	4 (25%)	10 (59%)	
Gender					
F	34	11 (73%)	10 (62%)	13 (76%)	NS
M	14	4 (27%)	6 (38%)	4 (24%)	
Stage					
I	31	10 (67%)	12 (75%)	9 (52%)	NS
II	1	1 (7%)	0 (0%)	0 (0%)	
III	8	2 (13%)	2 (12.5%)	4 (23.5)	
IV A	8	2 (13%)	2 (12.5%)	4 (23.5)	
Thyroiditis					
Absent	19	4 (27%)	7 (44%)	8 (47%)	NS
Present	29	11 (73%)	9 (56%)	9 (53%)	
Histotipe					
Classic	27	4 (27%)	11 (69%)	12 (70%)	**0.02**
Follicular	21	11 (73%)	5 (31%)	5 (30%)	
Focality					
Unifocal	34	10 (67%)	12 (75%)	12 (71%)	NS
Multifocal	14	5 (33%)	4 (25%)	5 (29%)	
Extrathyroid extension					
Absent	24	8 (53%)	10 (63%)	6 (35%)	NS
Present	24	7 (47%)	6 (37%)	11 (65%)	
Dimension					
< 2 cm	26	10 (67%)	9 (56%)	7 (41%)	NS
> 2cm	22	5 (33%)	7 (44%)	10 (59%)	
Lymph Nodes TNM					
Absent	17	8 (53%)	7 (44%)	2 (12%)	**0.01**
Present	31	7 (47%)	9 (56%)	15 (88%)	
T (TNM)					
T1	25	11 (74%)	6 (37.5%)	8 (47%)	**0.03**
T2	11	2 (13%)	6 (37.5%)	3 (18%)	
T3	9	0 (0%)	3 (19%)	6 (35%)	
T4	3	2 (13%)	1 (6%)	0 (0%)	

Table 10: Correlation between immunoistochemical staining of CXCR4 (Numbers of tumor cells) expression and clinical/tumoral factors – univariate analisys

	CXCR4 Numbers of tumor cells		
	2	3	
	N	Mean intensity z score (%)	Univariate p value
Age			
≤ 45	26	13 (50%)	13 (59%)	NS
> 45	22	13 (50%)	9 (41%)	
Gender			
F	34	21 (81%)	13 (60%)	NS
M	14	5 (19%)	9 (40%)	
Stage			
I	31	16 (62%)	15 (68%)	NS
II	1	1 (4%)	0 (0%)	
III	8	4 (15%)	4 (18%)	
IV A	8	5 (19%)	3 (14%)	
Thyroiditis			
Absent	19	9 (35%)	10 (45%)	NS
Present	29	17 (65%)	12 (55%)	
Histotipe			
Classic	27	14 (54%)	13 (59%)	**0.02**
Follicular	21	12 (46%)	9 (41%)	
Focality			
Unifocal	24	14 (54%)	10 (45%)	NS
Multifocal	24	12 (46%)	12 (55%)	
Extrathyroid extension			
Absent	24	10 (63%)	6 (35%)	NS
Present	24	6 (37%)	11 (65%)	
Dimension			
< 2 cm	26	17 (65%)	9 (41%)	NS
> 2cm	22	9 (35%)	13 (59%)	
Lymph Nodes (TNM)			
Absent	17	9 (34%)	8 (37%)	NS
Present	31	17 (66%)	14 (63%)	
T (TNM)			
T1	25	19 (73%)	6 (27%)	**0.0045**
T2	11	3 (11%)	8 (36%)	
T3	9	2 (8%)	7 (32%)	
T4	3	2 (8%)	1 (5%)	

Multivariate proportional odds regression model

Table11

CXCR4 intensity	OR	95% CI		p-value
Sex, M vs F	0.58	0.15	2.19	0.417
age, >=45 vs <45	1.88	0.54	6.61	0.325
T, T3-T4 vs T1	2.68	0.56	12.77	0.441
T, T2 vs T1	1.10	0.25	4.86	
N, N1 vs N0	3.75	1.00	13.96	**0.049**
Histology, yes vs no	0.13	0.04	0.49	**0.003**
Thyroiditis, yes vs no	0.49	0.14	1.69	0.258

Table 12

CXCR4 Numbers of tumor cells	OR	95% CI		p-value
Sex, M vs F	3.66	0.70	19.25	0.126
age, >=45 vs <45	0.45	0.10	2.16	0.321
T, T3-T4 vs T1	16.17	2.19	119.17	**0.009**
T, T2 vs T1	14.98	1.92	117.15	
N, N1 vs N0	0.22	0.04	1.31	0.096
Histology, yes vs no	0.78	0.18	3.50	0.749
Thyroiditis, yes vs no	0.85	0.19	3.90	0.838

Table 13

SDF-1 Numbers of tumor cells	OR	95% CI		p-value
Sex, M vs F	0.26	0.07	1.02	0.053
age, >=45 vs <45	2.29	0.67	7.86	0.188
T, T3-T4 vs T1	2.09	0.44	9.91	0.546
T, T2 vs T1	0.83	0.18	3.77	
N, N1 vs N0	0.71	0.19	2.62	0.611
Histology, yes vs no	0.78	0.23	2.59	0.683
Thyroiditis, yes vs no	1.60	0.47	5.48	0.454

5. Discussion

Chemokine receptors are transmembrane proteins that interact with specific chemokine ligands, resulting in G-protein-coupled signal transduction, which lead to chemotaxis (a directional movement along a chemical gradient). Chemokine receptors play an important role in many physiological processes, including normal migration of precursor cells during the development and targeting leukocytes to sites of inflammation. Functional chemokine receptors have been shown to be expressed by a large number of human malignancies, leading to the hypothesis that chemokines may stimulate proliferation, chemotaxis, and site-directed metastasis of tumor cells (Muller et al 2001;Burger et al 2003; Hwang et al 2003; Kucia et al 2006). Recently, the literature data have reported that the chemokine receptor is expressed in papillary and anaplastic thyroid carcinomas (Castellone et al 2004; Hwang et al 2003; Melillo et al 2005;De Falco et al 2007). In particular, the majority of research has been focused on the CXCR4 receptor. The CXCR4 receptor interacts specifically with its chemokine ligand, SDF-1, to exert proliferative anti-apoptotic and chemotactic effects in CXCR4-expressing cancer cells (Kucia et al 2004). In several human tumors, including carcinomas of the lung, breast, prostate, and colon, an association between CXCR4 and the ability of metastasis and survival has been found (Salvucci et al 2006;Spano et al 2004; Su et al 2005). Recently, CXCR4, which is not expressed at valuable levels in normal thyrocytes, has been detected in papillary and anaplastic thyroid carcinoma cell lines that undergo chemotaxis in response to the chemokine SDF-1 (Castellone et al 2004;Hwang et al 2003). Moreover, CXCR4 is upregulated in normal rat and human thyrocytes transformed with the RET/PTC oncogene, resulting in increased proliferation, survival, and migration in vitro in response to SDF-1 (Basolo et al 2002;Borrello et al 2005 ; Castellone et al 2004) .

In the present study, we analyzed the expression of CXCR4 and SDF-1 in 48 PTC specimens and their use as molecular markers that predict tumor outcome in low risk patients. These tumors are considered low risk on the basis of tumor size (2±1cm) and histological types (classical or follicular variant) of PTC. Moreover, we found that the chronic lymphocytic thyroiditis was present in 60% of cases, but we found no association between the presence of inflammation and the degree of chemokine or chemokine receptor expression by malignant epithelial cells.

We confirmed that CXCR4 is significantly expressed in PTC, but with a particular and different distribution in tumoral and peritumoral tissues.

Although CXCR4 is a membrane receptor, we found it expressed in cytosolic granules in our tumor cells. This result could be explained by receptor down regulation after rapid internalization with its ligand.

In peritumoral stroma, we observed higher CXCR4 positivity, especially in macrophage-like cells and in lymphocytes. We also found significantly higher amounts of CXCR4 in lymphocyte populations in associated tumoral chronic

thyroiditis. Therefore, this finding leads us to believe that CXCR4 could be involved in the maintenance of inflammatory infiltrate. Moreover, this data suggest that this receptor may mediate the attraction of leukocytes that interact with nascent cancer and form the tumor stroma. The notion that early stages of cancer are dependent on the host (via interaction with leukocytes) has been previously established and more recently shown through animal models. Likewise, in studies of methylcholanthrene-induced carcinomas, the role of host leukocytes, which cause a paradoxical supporting rather than inhibiting, was referred to as a "lymphodependent" stage of cancer (Balkwill et al 2001; Balkwill et al 2004, Russell et al 2004) .

Little is known about the role and expression of SDF-1 in PTC. Several authors have demonstrated that the fibroblast within invasive carcinomas contribute to tumor promotion through the secretion of SDF-1 in solid tumors(Orimo et al 2005; Bajetto et al 2006). Our results are the first to demonstrate by real time RTPCR and IHC that SDF-1 is abundantly expressed in papillary carcinoma cells other than in fibroblasts. Therefore, contrary to previous findings, this evidence suggests that not only thyroid tumoral stroma is a source of chemokines in PTC, but more importantly, cancer cells are the main sourse of SDF-1. This supports the concept that SDF 1 is pivotal in sustaining local pro-tumorigenesis events, such as growth and survival of cancer cells. (Orimo et al 2005). Furthermore, the expression of SDF-1 by stromal cancer cells can recruit endothelial progenitors for tumor angiogenesis. (Burger et al 2006).

In the presence of chronic thyroiditis, we found a different SDF-1distribution compared to CXCR4. Using immunohistochemical analysis, we demonstrated that SDF-1 is clearly overexpressed in MDS. Caillou has demonstrated that lymphocytic autoimmune thyroiditis displays ductal- like structures defined as ductal metaplasia, responsible for chronic inflammatory injury. It has been described that c-Kit, BcL2, HBME, EGFR, vimentin, galectin 3 are positively expressd in this peripheral thyroiditis region. These proteins are often negative in normal thyrocytes but they are usually present in PTCs, correlating with survival, migration and antiapoptotic events

Moreover, the expression of RET/PTC oncogenes in autoimmune diseases provides a close link between the inflammation and the pathophysiology of the cancer (Rodhen et al 2006). Together, these data suggest that the microenvironment of thyroid cancer may be modeled as a complex mixture of resident thyroid epithelial cells, producing chemokines that induce the infiltration of inflammatory cells, (including activated macrophages) that respond to these mediators and secrete additional factors (Melillo et al 2006). The interplay between the inflammatory mediators produced by the organ and those produced by the responding inflammatory cells may accelerate tumor progression by promoting angiogenesis and encouraging tumor cell growth through induction of antiapoptotic proteins or soluble growth factors .

Since we observed the co-expression of both SDF-1 and its receptor in the early PTC stage, the occurrence of an autocrine /paracrine loop can be

hypothesized as an early event in PTC, providing a proliferative advantage for SDF-1 sensitive cells.

We hypothesize that the expression of CXCR4 and SDF-1 in low risk papillary thyroid carcinoma would correlate with clinicopathological indicators of tumor progression. We found that CXCR4 expression is significantly associated with a smaller tumor size (T1 vs T2,T3 and T4). This result is the first to show this association. This could indicate that the chemokines can be expressed early in PTC tumorigenesis. The production of both CXCR4 and SDF-1 provides an autocrine stimulus for the proliferation and metastasis, thus giving an aggressive subset of microPTCs. Interestingly, we observed that classical variant PTC cells express more CXCR4 than follicular subtypes of PTC. We can speculate that the observed differences in CXCR4 expression may reflect underlying molecular alterations of follicular variant PTC, such as a higher frequency of RAS mutations (Fagin et al 1993) and a lower prevalence of RET/PTC and BRAF mutations (Leboeuf et al 2008; Frattini et al 2004) relative to classical PTC (in our cases we found V600E point mutation BRAF in 50% of cases, data not shown). Moreover, we found a correlation between CXCR4 expression and lymphnode involvement at the initial diagnosis. Considering the generally accepted concept that lymph node metastases are independent negative prognostic factors (Leboulleux et al 2006), it appears logical that this correlation was found to be associated with a poor prognosis.

Finally, we found an association ($p = 0.059$) between the SDF-1 and female gender, but other studies are necessary to confirm a correlation between genotype and phenotype in PTC.

The present study did not find any differences in tumor multifocality and extracapsule thyroid extension with CXCR4 or SDF-1 expression in tumor tissue. The relatively small number of tumors studied may have reduced our power to detect differences among tumor subgroups. Therefore, no correlation was found between CXCR4 and SDF-1 mRNA expression and , it is possible that post-transcriptional regulation may be responsible for the observed differences in chemokine receptor protein levels. In addition, the presence of inflammatory and stromal cells in thyroid tissue could confound gene expression analysis, since these cells are likely to contain mRNA for chemokines and chemokine receptors, but should not confound IHC analysis in tumor cells which were evaluated for staining intensity and positive cell percentage.

Conclusion:

In the past years, the increasing incidence of small PTCs are correlated to early diagnosis. However, not all small PTCs are entirely innocuous; therefore, it is crucial to detect markers for aggressive PTC subtypes at the initial diagnosis. The major findings of our study show that:

- CXCR4 is significantly expressed in PTC, confirming previous literature. However, there is a particular and different distribution in tumoral and peritumoral tissues.
- SDF-1 is abundantly expressed in papillary carcinoma cells other than in fibroblasts. This data is a first to demonstrate that the thyroid cancer cells are the main sourse of SDF-1. This supports the concept that SDF- 1 is pivotal in sustaining local pro-tumorigenesis events, such as growth and survival of cancer cells.

Moreover, in this study we found a significant co-expression of both SDF-1 and its receptor CXCR4, in the early PTC stage. The occurrence of this autocrine /paracrine loop can provide a proliferative advantage for SDF-1 sensitive cells. In fact CXCR4 is significantly associated with initial lymphnode involvement , thus giving an aggressive subset of small PTCs .

Future studies in larger sets of patients will be necessary to determine the utility of these molecules as biomarkers of aggressive PTC. Novel therapeutic strategies designed to manipulate the activity of chemokines and their receptors could have efficacy in PTC and other common epithelial malignancies that exploit CXCR4 and SDF-1 during the processes of tumor progression and metastasis.

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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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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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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.

(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

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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