Are the Clinical and Pathological Features of Differentiated Thyroid Carcinoma Really Changed over the Last 35 Years? Study on 4187 Patients from a Single Italian Institution to Answer this Question

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Background: In the last decades, a marked increased prevalence of differentiated thyroid cancer (DTC) has been observed worldwide. The aim of this study was to evaluate the changing features of DTC referred to our institution between 1969 and 2004.

Methods: Clinical and pathological features and prognostic factors were analyzed in 4187 DTC patients, subdivided into two groups: group 1 (n = 1215) and group 2 (n = 2972) diagnosed before and after 1990, respectively.

Results: Group 2 showed an increased proportion of micropapillary carcinoma and a concomitant decrease of follicular histotype. Male percentage was greater in group 2, whereas median age at diagnosis was unchanged. DTC of group 2 were more frequently associated with multinodular goiter or autoimmune thyroiditis, but many were unexpected findings. Features of aggressiveness were significantly less frequent in group 2, and the survival rate was greater (98.7 *vs.* 91.4%, $P < 0.0001$). Gender, age, histotype, tumor size, extrathyroidal macroinvasion, and lymph node and/or distant metastases were found to be poor prognostic factors in both groups using univariate analysis, but with multivariate analysis, only advanced age (odds ratio = 22.52 for older patients) and advanced stage (odds ratio = 53.54 for more advanced cases) were independently correlated with a lower survival.

Conclusions: DTC patients diagnosed after 1990 have smaller tumors with less advanced stage and a better prognosis. The question of whether this is related to the finding of tumors with a low clinical penetrance or to the anticipation of diagnosis remains to be clarified. Despite these significant differences, both advanced stage and older age still represent the most important poor prognostic factors for survival. **(*J Clin Endocrinol Metab* 95: 1516–1527, 2010)**

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Abbreviations: DTC, Differentiated thyroid cancer; FNA, fine-needle aspiration; FTC, follicular variant of PTC; IRMA, immunoradiometric assay; mPTC, micro-PTC; OR, odds ratio; PTC, papillary thyroid carcinoma; Tg, thyroglobulin; TgAb, anti-Tg antibodies; TNM, tumor-node-metastases; WBS, whole-body scan.

In the last few years, a relevant increased incidence of thyroid cancer has been documented worldwide (1–10). Epidemiological studies in the United States demonstrated that, in the last 5 yr, the thyroid cancer rate of incidence has been the highest among all tumors (11). The increased number of cases is mainly due to small papillary thyroid carcinoma (PTC) (1–3, 6, 10), which is typically well differentiated and curable (12–15).

The Department of Endocrinology at the University Hospital of Pisa is a national referral center for the diagnosis and treatment of thyroid cancer. From 1969 to date, almost 8000 thyroid cancer (all histotypes) have been registered at this institution. In the present study, we focused our attention on a group of 4187 consecutive differentiated thyroid cancer (DTC) patients having at least a 3-yr follow-up. To our knowledge, this is the largest series of DTC patients followed at one single institution. The series is characterized by a considerable homogeneity in treatment and follow-up strategies, a very low percentage of patients lost at follow-up (<6%), and an almost unique long-term follow-up, with more than 500 patients followed for more than 20 yr.

Clinical and pathological features of DTC in patients diagnosed before and after the year 1990 were separately analyzed and compared. The rationale to distinguish the two groups before and after 1990 derived from the observation that around this time period, a sharp and progressive increase in the number of patients referred to our institution occurred (Fig. 1) in conjunction with the widespread use of neck ultrasound for diagnostic procedures in our country (16). The study hypothesis was that the two groups should be characterized by a different biological and clinical behavior. The results of this analysis have been discussed in the present report.

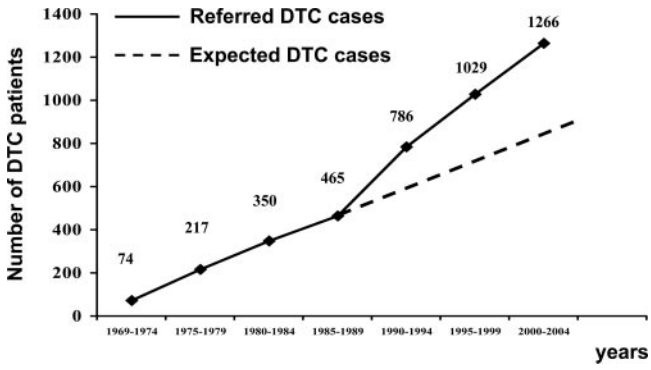

FIG. 1. Number of patients with DTC observed over the years 1969–2004 at the Thyroid Cancer Center of the Department of Endocrinology, University Hospital of Pisa, after it was established in 1969. The number of referred patients linearly increased over the years, whereas a sharp unexpected increase occurred between the late 1980s and early 1990s.

Patients and Methods

Patients

The study group consisted of 4187 patients (3166 females and 1021 males, 3:1 ratio) ranging in age from 5–88 yr (mean 42.4 yr ± 14.4 yr, median 42 yr). A total of 3684 (88%) patients had PTC, and 503 (12%) had a follicular thyroid cancer (FTC), including Huerthle cell variant. Poorly differentiated, anaplastic, and medullary thyroid carcinomas were not included in the study.

The mean follow-up was 9 ± 7.6 yr, ranging from 0.5–34.5 yr (median, 7 yr). A scheduled annual visit for clinical and biochemical control generally guarantees keeping the majority of patients under active follow-up. When such controls were not performed, patients were contacted directly or through their family doctor. As a result, at the time of this analysis, the clinical outcome of 3952 of 4187 patients (94.4%) was known with only 235 of 4187 lost patients (5.6%).

As common practice, patients have been requested to sign an informed consent to the collection of clinical and pathological data. The present study has been approved by the Internal Reviewing Board of the Department of Endocrinology and Metabolism of the University of Pisa.

Management of DTC patients

Since 1969, most of the same endocrinologists, endocrine surgeons, nuclear medical physicians, and pathologists has been involved in the management of DTC patients. Strict collaboration and shared protocols helped to maintain uniform treatment and follow-up strategies.

Initial treatment included a near-total thyroidectomy followed by radioiodine (^{131}I) ablation (100 mCi before 1980, 30 mCi thereafter) of postsurgical thyroid remnant. Patients referred after initial surgery were submitted to completion thyroidectomy if the first operation had been less than a near-total thyroidectomy.

Patients with metastatic disease underwent additional treatments consisting of ^{131}I therapeutic doses (100–200 mCi) until the evidence of a negative posttherapeutic whole-body scan (WBS). Whenever necessary, the ^{131}I treatments were repeated at interval times not shorter than 6 months. Neck recurrences and single lung or bone metastases were treated by surgery whenever technically possible. Neck external radiotherapy was indicated after an incomplete surgical treatment due to local infiltration or repeated relapses in the neck. Bone radiotherapy was used for pain control. Chemotherapy was administered in patients with systemic metastatic disease who did not respond to radioiodine any longer.

All patients were submitted to L-T_4 therapy. Tailored TSH-suppressive doses were administered until evidence of a disease-free status. Partially suppressive or substitutive doses were given thereafter.

Follow-up strategy

The first postthyroid remnant ablation control was performed after 3–6 months. The follow-up controls were scheduled every 12 months and consisted of clinical and laboratory evaluation on L-T_4 therapy, including free thyroid hormones, TSH, thyroglobulin (Tg) (from 1976) and anti-Tg antibodies (TgAb) measurements. In 1989, neck ultrasound was routinely introduced in the follow-up of DTC patients. When a suspicious lymph node was found, fine-needle aspiration (FNA) cytology

and Tg measurement in the needle washout were performed (17). Other imaging techniques were carried out when needed.

In 2001, the recombinant human TSH-Tg stimulation test was introduced in our department to ascertain the persistence of the disease (18, 19). A diagnostic WBS was performed only when high titers of TgAb were detected.

Pathology

Because our department is a national referral center, almost half of the DTC patients [2344 of 4187 (52.1%)] were referred after initial surgery. All cases operated on at the University Hospital of Pisa have been analyzed by the same team of pathologists. Starting from 1990, the histological slides of patients referred after surgery were routinely reexamined by our endocrine pathologists. The histological slides of DTC diagnosed before 1990 were revised when available.

Histopathological examination and classification were carried out following the morphological criteria used at the time of observation (20, 21). Whenever requested, immunohistochemistry for Tg or other relevant proteins was performed. Tumor size, foci number, local extrathyroidal extension, and the presence of metastatic lymph nodes were reported and used for tumor-node-metastases (TNM) classification (22).

Over the last 30 yr, three major changes have occurred in the practice of thyroid pathology. 1) The follicular variant of PTC was recognized and introduced in the thyroid tumor classification (23, 24). The patients treated before these changes were reclassified whenever their histological slides could be traced. This was possible for the majority of patients submitted to surgery in Pisa (*i.e.* 41 of 49 FTC) but for only a minority of those referred after surgery (*i.e.* 20 of 78 FTC). 2) The sampling collection method was changed. Only one sample of apparently normal thyroid tissue was prepared up until 1990, whereas several 3- to 4-μm sections were prepared thereafter, and the possibility of recognizing microscopic tumor foci was increased. 3) In 2002, the new sixth edition TNM staging was introduced in the clinical practice (22).

As a staging system, we used the De Groot's classification (25), which is based on the combination of clinical, surgical, and pathological data. Differently from the TNM staging, the De Groot's classification does not take into account the age of the patient at the time of diagnosis.

Biochemical tests

In 1976, serum Tg measurement became the routine test for the follow-up of DTC patients. From 1976–1982, it was measured by a homemade RIA, whereas from 1982–1998 by a commercial immunoradiometric assay (IRMA) (Sorin, Saluggia, Italy). In both cases, the sensitivity was 3 ng/ml. A more sensitive solid-phase chemiluminescent IRMA with a functional sensitivity of 0.9 ng/ml was adopted in 1998 (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA).

To avoid misinterpretation of Tg measurements, patients were routinely screened for TgAb (26, 27). The TgAb measurement was performed by passive hemagglutination up until 1990 (thyroid test kit; Fujizoki, Fujirebio Inc., Tokyo, Japan), by IRMA up until 2000 (ICN Pharmaceutical, High Wycombe, UK) and by an immunoenzymometric assay (AIA PACK TgAb System; Tosoh Bioscence, Tessenderlo, Belgium) thereafter.

TSH measurement was performed using different methods with increasing sensitivity. A RIA (clinical assay γ-coat hTSH; Incstar Corp., Stillwater, MN) was used up until 1988, a more sensitive method (hTSH Ultra; Delfia, Wallace Oy, Finland) was employed up until 1996, and the ultrasensitive immunochemiluminescent assay method (Diagnostic Products) has been used since.

Urinary iodine content was measured with the UV irradiation method (28) employing an automated autoanalyzer (Bran-Luebbe S.P.A., Gallarate, Italy).

^{131}I WBS

Until 1997, the postsurgical thyroid remnant ^{131}I ablation was preceded by a diagnostic 1-mCi WBS. Since 1997, a 50-μCi thyroid bed radioiodine uptake measurement (New Atomlab 950; Biodex Medical System, Urbino, Italy) was substituted for the diagnostic WBS and a 3- to 7-d post-^{131}I-ablation WBS was routinely performed.

After remnant ^{131}I ablation, patients were followed with a yearly control of serum Tg and 4- to 5-mCi diagnostic ^{131}I WBS while hypothyroid (TSH $>$ 30 μU/ml). A therapeutic dose of ^{131}I was administered whenever serum Tg was detectable and/or the diagnostic WBS was positive. After 1998, the diagnostic WBS was no longer performed, and patients directly underwent ^{131}I therapy when basal or TSH-stimulated serum Tg, either by endogenous or exogenous TSH, was detectable. A posttherapeutic WBS was performed 1–5 d after the ^{131}I treatment.

The WBS was performed using a rectilinear scanner up until 1995, a one-head γ camera (Apex SPX 4000, Elscint, Milano, Italy) with a higher energy collimator up until 2000 and a double-head γ camera (Axis/Iris, Marconi Medical System, Cleveland, OH) with high-energy collimators thereafter.

Neck ultrasound

At our center, neck ultrasound was routinely introduced in the work-up of patients with any type of thyroid disease, starting from 1986. In particular, DTC patients were submitted to neck ultrasound at any follow-up control. Central, laterocervical, and supraclavear nodes compartments were evaluated on a routine basis by the endocrinologist who was visiting the patient.

Disease-free, recurrence, and persistence

Patients were considered disease-free when WBS was negative and both endogenous or exogenous TSH-stimulated Tg and TgAb were undetectable (29). The recurrence was defined as the evidence of the disease at least 6 months after the definition of a disease-free status.

Patients with detectable levels of serum Tg or elevated titers of TgAb were not defined as disease-free but with persistence, even if both WBS and other imaging techniques were unable to detect any lesion (*i.e.* biochemical persistence).

Data analysis

Clinical data are presented with descriptive statistical analysis. Statistical analysis was performed using Mann-Whitney *U* and χ^2 tests according to the data to be analyzed. Survival curves were plotted by the Kaplan-Mayer method with the log rank statistic. P values < 0.05 were considered significant. Multivariate analysis was performed using a Cox regression model.

Results

Gender, age, and histotype observed in the study groups are reported in Table 1. The comparison of these features

TABLE 1. Epidemiological and histological data of patients with DTC referred to University Hospital of Pisa from 1969–2004

	Total series	1969–1989, group 1	1990–2004, group 2	*P* (group 1 *vs.* group 2)
No. of patients	4187	1215 (29.0%)	2972 (71.0%)	
Sex				
Female (n)	3166 (75.6%)	944 (77.7%)	2222 (74.8%)	0.04
Male	1021 (24.4%)	271 (22.3%)	750 (25.2%)	
Age at diagnosis (yr)	42.5 ± 14.4 (5–88)	42.2 ± 15.8 (5–84)	42.6 ± 13.9 (7–88)	NS (0.56)
Histotype				
Papillary cancer (PTC)	3684 (88%)	979 (80.5%)	2705 (91.0%)	<0.0001
Follicular cancer (FTC)	503 (12%)	236 (19.5%)	267 (9.0%)	

Data were analyzed in the entire group (total series) and separately in patients diagnosed before 1990 (group 1) and after 1990 (group 2). Median age for total series and both groups was 42 yr. The FTC group includes Huerthle cells carcinoma. NS, Not significant.

showed that, as expected, the vast majority of patients were female in both groups. There was, however, a significant albeit small increase of males, from 22% in group 1 to 25% in group 2. The mean age at diagnosis was 42.5 yr, with no difference between the two groups. A PTC diagnosis accounted for the majority of patients in both groups. However, a significant reduction of FTC, from 19.5% in group 1 to 9.0% in group 2 was observed.

As shown in Table 2, 63.6% of the total series did not show a coexisting thyroid disease, and this percentage decreased from 72.6% in group 1 to 59.8% in group 2. Nodular goiter was the most frequent coexisting disorder. It was found in 26% of the total series and was significantly more frequent in group 2 than group 1 (27.5% *vs.* 22.3%). Autoimmune thyroiditis accounted for 7.5% of coexisting disorders in all subjects, with an interesting increase from 2.0% in group 1 to 9.8% in group 2. Graves' disease and toxic adenoma accounted only for a minority of coexisting diseases with no difference between the two groups. A positive history of neck irradiation was found in 3.2% of the total series with a significant drop from 6.1% in group 1 to 1.9% in group 2.

The presence of a thyroid nodule was the most frequently reported presenting symptom (66.8%) with no difference between the two groups (Table 3). A thyroid nodule was listed as an incidental finding if detected during diagnostic procedures, usually neck ultrasound, performed for reasons other than thyroid disease. The incidental finding of a nodule was declared as the reason for referral by 16% of the total cases, with a significant increase from 8% in group 1 to 20% in group 2. The finding of suspicious cervical nodes occurred in nearly 10% of the total series with a sharp decrease from 17% in group 1 to 7% in group 2. Local symptoms such as pressure and/or dysphagia and/or dysphonia, were reported in a minority of patients (3.4%), with a significant decrease from 5% in group 1 to 2.6% in group 2. Hyperthyroidism was the presenting symptom only in approximately 2.5% of subjects with no difference between the two groups. Only few patients arrived at the diagnosis for bone pain or other signs of distant metastases, but nevertheless, a significant reduction from group 1 to group 2 (1.8 *vs.* 0.5%) was observed.

Pathological features are reported in Table 4. Small tumors smaller than 1 cm showed a sharp increase from 7.9% in group 1 to 28.7% in group 2. In contrast, a significant decrease in the percentage of tumors larger than 1 cm was observed in group 2. In particular, tumors of 4 cm or more accounted for 13.3% of the total series, with a significant decrease from 17.4% in group 1 to 11.8% in group 2. Local extrathyroidal extension due to microinvasion of surrounding tissues (*i.e.* T_3 in

TABLE 2. Prevalence of coexisting thyroid diseases in the entire group (total series) and in patients diagnosed before 1990 (group 1) and after 1990 (group 2)

	Total series	1969–1989, group 1	1990–2004, group 2	*P* (group 1 *vs.* group 2)
No. of patients	4187	1215	2972	
Coexisting thyroid diseases (n)				
None	2661 (63.6%)	882 (72.6%)	1779 (59.8%)	<0.0001
Nodular goiter	1089 (26%)	271 (22.3%)	818 (27.5%)	0.0006
Autoimmune thyroiditis	316 (7.5%)	24 (2.0%)	292 (9.9%)	<0.0001
Graves' disease	91 (2.2%)	25 (2.1%)	66 (2.2%)	NS (0.8)
Toxic adenoma	30 (0.7%)	13 (1.1%)	17 (0.6%)	NS (0.1)
Neck irradiation[a]	119/3898 (3.2%)	63/1021 (6.1%)	56/2877 (1.9%)	<0.0001

NS, Not significant.

[a] Information on the radiation exposure was not available in 289 patients: 194 of group 1 and 95 of group 2.

TABLE 3. Prevalence of presenting symptoms in the entire group (total series) and separately in patients diagnosed before 1990 (group 1) and after 1990 (group 2)

	Total series	1969–1989, group 1	1990–2004, group 2	*P* (group 1 *vs.* group 2)
No. of patients with available data	3997	1175	2822	
Presenting symptoms (n)				
Thyroid nodule	2670 (66.8%)	772 (65.7%)	1898 (67.3%)	NS (0.3)
Incidental finding	657 (16.4%)	94 (8.0%)	563 (20.0%)	<0.0001
Cervical nodes	396 (9.9%)	200 (17.0%)	196 (7.0%)	<0.0001
Local symptoms	134 (3.4%)	58 (5.0%)	76 (2.6%)	0.0005
Hyperthyroidism	104 (2.6%)	30 (2.5%)	74 (2.6%)	NS (0.9)
Distant metastases	36 (0.9%)	21 (1.8%)	15 (0.5%)	0.0003

This clinical information was not available in 190 patients: 40 of Group 1 and 150 of Group 2. NS, Not significant.

the new TNM classification) was relatively common and more frequently found in group 2 than in group 1 (19.8 *vs.* 18.3%). Conversely, macroinvasion (*i.e.* T_4 in the new TNM classification) was much less frequent in the total series and particularly in group 2, which did not reach 2.0% as opposed to the almost 7.0% in group 1. In this context, it may be relevant to point out that micro-PTC (mPTC) are often localized close to the thy-

TABLE 4. Prevalence of several pathological features in the entire group (total series) and separately in patients diagnosed before 1990 (group 1) and after 1990 (group 2)

	Total series	1969–1989, group 1	1990–2004, group 2	*P* (group 1 *vs.* group 2)
Tumor size: N	3996	1100	2896	
≤1 cm (mPTC)	923 (23.1%)	87 (7.9%)	836 (28.7%)	<0.0001
>1 to <2 cm	1132 (28.3%)	389 (35.4%)	743 (25.8%)	<0.0001
>2 to <4 cm	1409 (35.3%)	432 (39.3%)	977 (33.7%)	0.002
≥4 cm	532 (13.3%)	192 (17.4%)	340 (11.8%)	<0.0001
Local extension: N	3625	824	2774	
No extrathyroid	2967 (81.8%)	673 (81.7%)	2267 (81.7%)	NS (0.4)
Extrathyroid	658 (18.2%)	151 (18.3%)	507 (18.3%)	
T_3 (microinvasion)	545 (15.0%)	93 (11.3%)	452 (16.3%)	0.002
T_4 (macr-invasion)	113 (3.1%)	58 (7.0%)	55 (1.9%)	<0.0001
Lymph nodes metastases				
N	4184	1213	2971	
n	1081 (25.8%)	415 (34.2%)	666 (22.4%)	<0.0001
Distant metastases				
N	4184	1213	2971	
n	127 (3%)	66 (5.4%)	61 (2%)	<0.0001
Clinical classes (De Groot's classification)				
N	3995	1102	2893	
n				
I	2450 (61.3%)	542 (49.2%)	1908 (65.9%)	<0.0001
II	764 (19.1%)	332 (30.1%)	432 (14.9%)	<0.0001
III	655 (16.4%)	163 (14.8%)	492 (17%)	NS (0.1)
IV	126 (3.2%)	65 (5.9%)	61 (2.2%)	<0.0001
Stage (VI TNM classification)				
N	4109	1179	2930	
n				
I	2736 (66.6%)	761 (64.5%)	1975 (67.4%)	NS (0.07)
II	615 (15%)	151 (12.8%)	464 (15.8%).	0.01
III	345 (8.4%)	127 (10.8%)	218 (7.4%)	0.0005
IVA	177 (4.3%)	56 (4.7%)	121 (4.1%)	NS (0.4)
IVB	141 (3.4%)	35 (3.0%)	106 (3.6%)	NS (0.3)
IVC	95 (2.3%)	49 (4.2%)	46 (1.7%)	<0.0001
Multifocality				
N	3726	896	2830	
n	1354 (36.0)	283 (31.5%)	1071 (37.8%)	0.0008
Bilaterality				
N	3662	873	2789	
n	730 (19.9%)	134 (15.3%)	596 (21.4%)	0.0001

N, Number of patients with available data for each pathological feature; n, number of patients with the pathological feature; NS, not significant.

roid capsule and had a limited microscopic extrathyroidal extension (3, 30, 31). Thus, despite being associated with a good prognosis, these tumors fulfilled the definition of a tumor with local extension, thus increasing the percentage of T_3 tumors. Lymph node metastases were present in 25.8% of the total series, whereas only 3.0% of the cases showed distant metastases. Both lymph node and distant metastases were significantly less frequent in group 2 (22.4 and 2.0%, respectively) than in group 1 (34.2 and 5.4%, respectively). To a large extent, similar changes were observed in the class distribution, with a significant increase of class I (49.2 *vs.* 65.9%) and a decrease of classes II (30.1 *vs.* 14.9%) and IV (5.9 *vs.* 2.2%) in group 2. As shown in Table 4, similar results were observed when patients were classified according to the sixth edition TNM classification which, at variance with the De Groot's classification, takes into account both the extension of the disease and the age of patients at diagnosis (22). It is worth noting that the percentage of locally advanced cases without distant metastases, which are included in class III in the De Groot's classification and in stage IVA and IVB in the TNM classification, did not change in the two groups of patients. Both multifocality and bilaterality showed an increase in group 2 (37.8 and 21.4%, respectively) when compared with group 1 (31.5 and 15.3%, respectively).

The mean follow-up of the total series was 9 ± 7.6 yr (range 0.5–34.5 yr, median 7 yr). A total of 2576 patients had a follow-up of at least 5 yr, 1547 at least 10 yr, and 507 at least 20 yr. There was a marked difference in the follow-up period between group 1 (range 1–34.5; median 19.5 yr) and group 2 (range 1–14.9; median 5.5 yr).

The survival rate of DTC patients is shown in Fig. 2. At the end of the follow-up, the percentage of survivors in the total series was 96.4%, and there was a significant difference between group 1 and group 2 (91.4 *vs.* 98.7%) ($P <$ 0.0001). This was not related to the longer follow-up time in group 1 because the difference remained significant even when the survival data were analyzed by comparing populations within the two groups with equal follow-up periods.

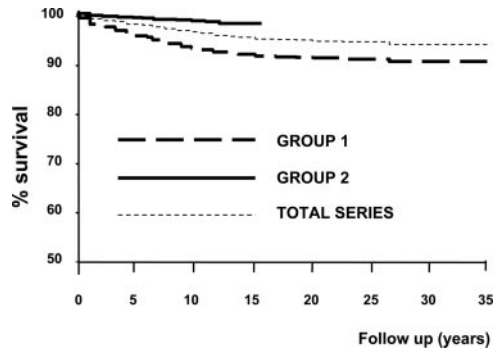

FIG. 2. Survival curves of patients with DTC. The overall survival of the total series was 96.4% after a 35-yr follow-up. A significant difference was observed in the survival rate of group 1 and group 2 patients ($P <$ 0.0001). This was not related to the considerably longer follow-up of group 1 with respect to group 2, because the difference remained significant even when the survival data were analyzed by comparing subsets of the two groups with equal follow-up periods.

Survival curves obtained by univariate analysis of several prognostic factors in the total series are reported in Fig. 3. Prognosis was influenced by gender as shown by the significant, albeit small, reduction of survival observed in males compared with females (Fig. 3A). As shown in Fig. 3B, the influence of age was analyzed by subdividing the patients into three age ranges. Almost all patients younger than 41 yr at diagnosis survived until the end of follow-up. There was a significant decrease of survival in patients 41–60 yr old [odds ratio (OR) = 11.26] and a much greater decrease in those older than 60 yr (OR = 49.2). Patients with FTC had a significantly less favorable outcome than those with PTC (Fig. 3C). A tumor size greater than 4 cm appeared to be an unfavorable prognostic factor for survival, whereas no differences were observed in the survival of patients with smaller tumors (Fig. 3D). Local extrathyroidal extension had a significant negative influence on survival, but only when T_4 (macroinvasion) was considered separately from T_3 (microinvasion) (Fig. 3E). The presence of lymph node metastases was found to have a small but significant negative influence (Fig. 3F). The presence of distant metastases was the most impressive poor prognostic factor for survival, with only 50% of metastatic patients surviving after 10 yr (Fig. 3G). When the clinical stage was considered, a progressive decrease in survival was observed with the increasing number of the class, and class IV was the most unfavorable one (Fig. 3H). Neither multifocality nor bilaterality had any significant influence on survival (data not shown).

Survival curves in relation to prognostic factors were also studied separately in the two groups by univariate analysis (Fig. 4). Essentially the same pattern of the total series was observed in both groups. To a variable extent, however, the survival rate was more affected in group 1 for all prognostic factors except for the presence of metastatic lymph node, which showed a small but significant influence on survival in group 2 but not in group 1 (Fig. 4F).

The prognostic factors found to be significant in the univariate analysis were submitted to the multivariate analysis (Table 5). Gender and tumor size were found to have no independent influence on survival in either the total series or in the two groups. Conversely, age at diagnosis resulted to be a significant independent poor prognostic factor both for the total series and for the two groups with high OR especially for patients over 60 yr of age. Histotype was found to independently influence the survival when analyzed in the total series of patients and

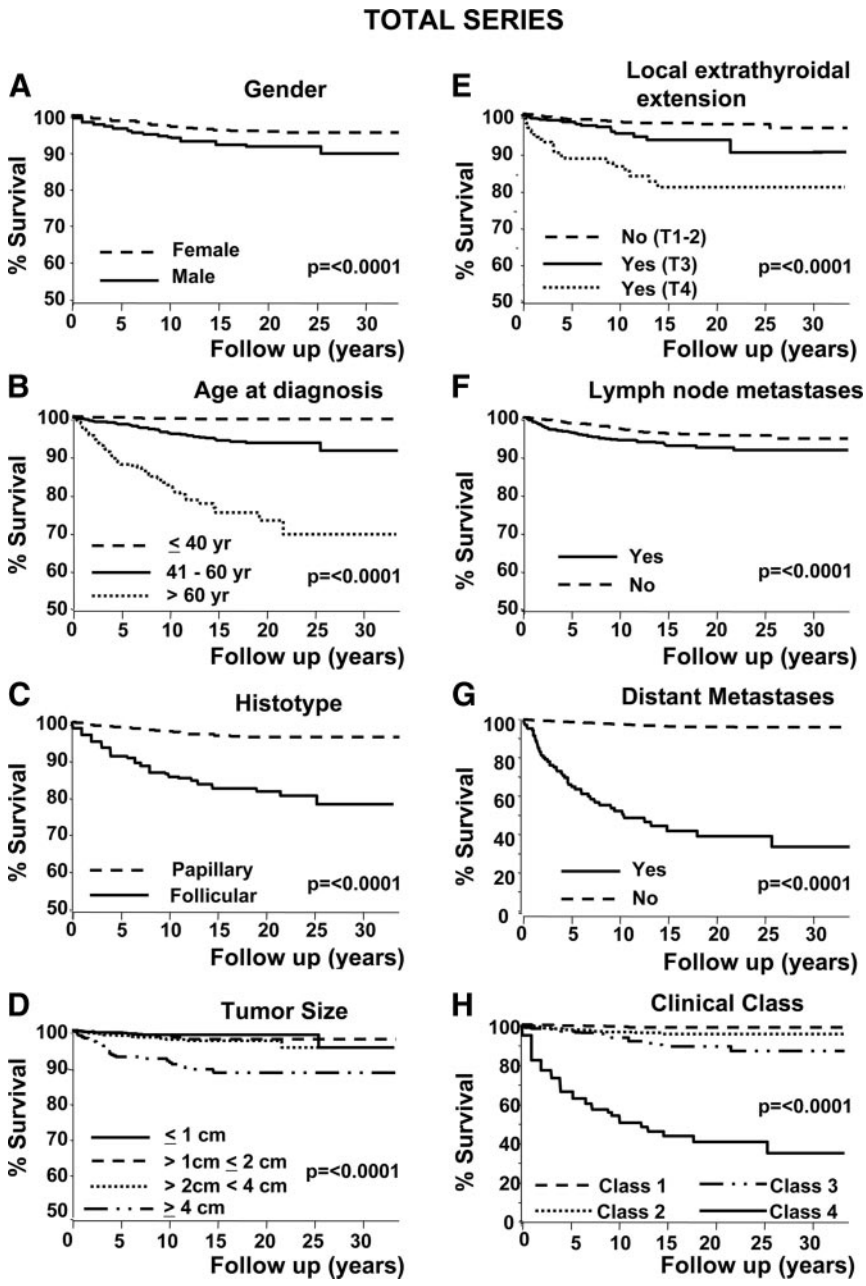

FIG. 3. Survival curves of the total series of 4187 patients with DTC according to the different clinical and pathological features: gender (A), age at diagnosis (B), histotype (C), tumor size (D), local extrathyroidal extension (E), lymph node metastases (F), distant metastases (G), and De Groot's clinical classification (H). All of these variables showed a significant prognostic role.

in group 1 but not in group 2. Local extrathyroidal extension barely reached a level of significance in the total series and in group 2 but was not significant in group 1. The same pattern was observed when T_3 and T_4 cases were separately analyzed (data not shown). The presence of lymph node metastases was found to have a significant negative influence on the survival rate both in the total series and, to a lesser extent, in the two groups. Class III and mainly class IV were associated with a markedly poor prognosis in all groups, whereas classes I and II were found to have no influence on the survival of DTC patients. Interestingly, in the multivariate analysis, the time period of the diagnosis (*i.e.* before 1990 or after 1990) was found to be an independent prognostic factor. Patients diagnosed after 1990 had a significantly higher survival rate when compared with those diagnosed before 1990.

Discussion

We analyzed clinical and pathological features and prognostic patterns of 4187 consecutive DTC patients referred to our institution between October 1969 and December 2004. To our knowledge, this is the largest series of DTC patients treated at a single institution using standardized therapeutic and follow-up protocols and having a remarkably low loss at follow-up. We felt it would be interesting to subdivide the total series into two groups according to the time period of diagnosis: group 1 with 1215 patients and group 2 with 2972 patients observed before and after 1990, respectively. The rationale to choose the year 1990 was related to the widespread use of neck ultrasound and the introduction of other sensitive diagnostic tools that happened around that year.

We found a similar age at diagnosis in the two groups, and this finding was consistent with those of Farahati *et al.* (5) and Chow *et al.* (9), who after subdividing their series into three groups according to the year of diagnosis, failed in finding differences in the mean age at diagnosis. Still in keeping with our results, they also observed a slight increase in the percentage of DTC in males over the years. Such an increase in males could be partially explained by the fact that thyroid nodules are clinically not easy to be appreciated in males, whereas they can be found by ultrasound (32). Indeed, the incidental discovery of a thyroid nodule by Doppler sonography performed during the work-up of hypertension or other cardiovascular diseases is the most common medical context in which thyroid nodules are found in middle-aged men (33).

We observed an increase of PTC accompanied by a relevant decrease of FTC in group 2. This pattern is con-

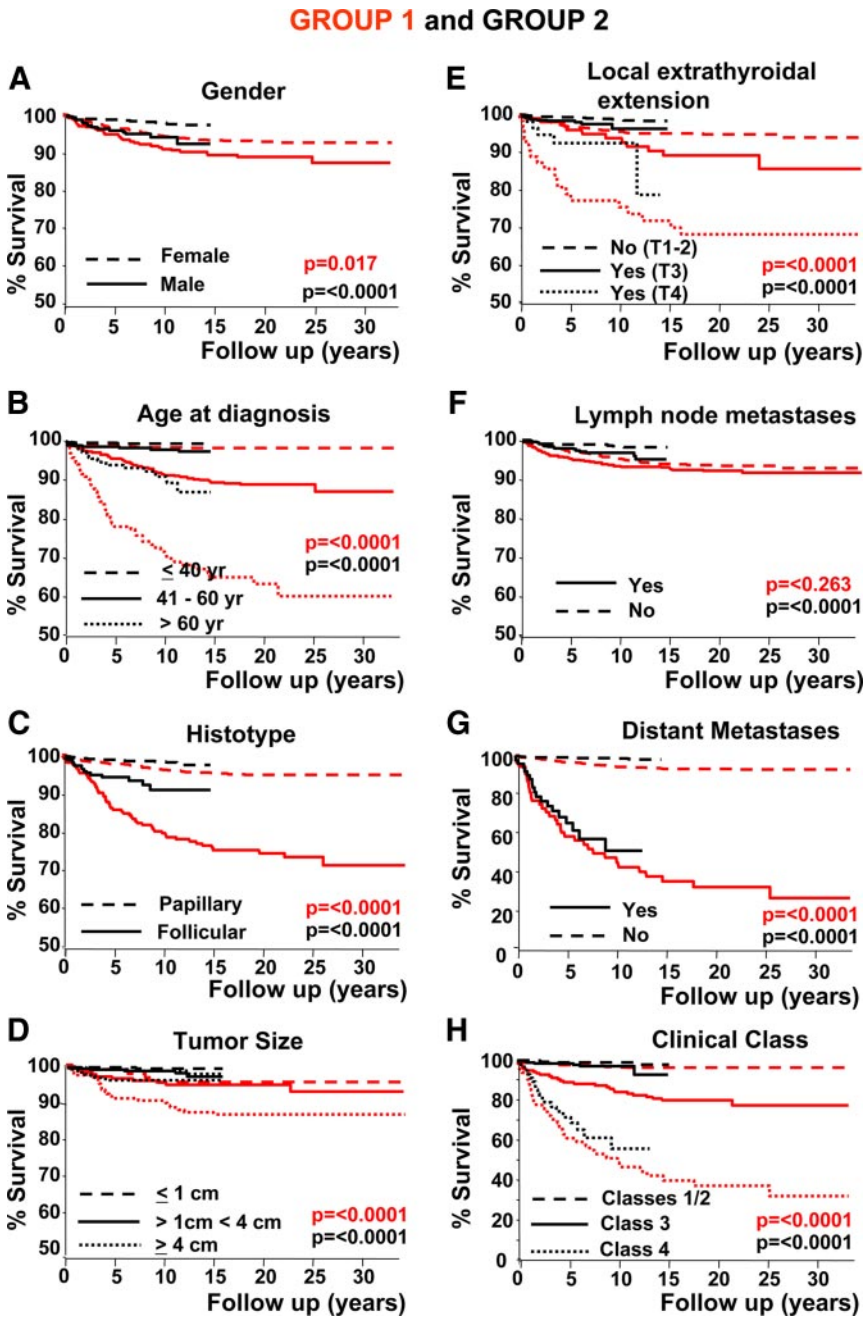

FIG. 4. Survival curves of group 1 (1969–1989, *red lines*) and group 2 (1990–2004, *black lines*) patients (univariate analysis) according to their clinical and pathological features. To a variable extent, survival rate was more affected in group 1 for all prognostic factors except for the presence of lymph node metastases, which had a small but significant influence on survival in group 2 but not in group 1 (F).

sistent with previous reports (1–3, 6), and several explanations may be offered for its occurrence. A shift in the PTC/FTC ratio in favor of PTC is a well-recognized effect of iodine prophylaxis (34, 35). This phenomenon was also observed by Belfiore *et al.* (36) in Sicily. Because a significant decrease of iodine deficiency has been observed in Italy in the recent years (37), we can assume that it could have contributed to the increase of the PTC to FTC ratio. Regarding this, it is worth noting that, recently, an association between a high prevalence of BRAF V600E mutation, which is the most common genetic alteration in PTC (38), and an elevated iodine intake has been reported and interpreted as a possible link between iodine exposure and the development of PTC (39). Another explanation of this increased ratio could be the greater attention to the histological pattern of the follicular variant of PTC. Albores-Saavedra *et al.* (40) reported that the rate of this variant increased by 173% between 1973 and 2003. They considered this as the major contributing factor underlying the increase of PTC in the last decades. We made an effort to reduce this bias by reassessing the histotype of the tumors diagnosed before the middle of the 1980s, but unfortunately, this could not be done in all cases. Finally, the relative fall in the percentage of FTC cases in group 2 could be the result of the increased detection of small thyroid cancer, most of which are PTC (2).

The presence of a coexisting thyroid disease, in particular of a nodular goiter and thyroid autoimmunity, was more frequently observed in group 2. Regarding this, the role of the neck ultrasound appears of great relevance for the possibility of detecting both uni- or multinodular small nodular goiter and performing echo-guided FNA of these small nodules as opposed to the clinical approach that limited FNA to the dominant nodule (41). Neck ultrasound played also an important role in the diagnosis of thyroiditis, which has been demonstrated to have a specific hypoechoic ultrasound pattern (42), not clinically identifiable. Another possible explanation for the higher percentage of thyroiditis in group 2 is the increased attitude to measure serum thyroid antibodies during the work-up of thyroid diseases. However, there are data showing also a true increase of thyroiditis that has been attributed to environmental factors, including an increase in iodine intake (43), which, as aforementioned, has happened also in our country in the recent years (37).

External radiation to the head and neck was frequently used for the treatment of benign diseases in children and adolescents up until the early 1960s. This was before the

TABLE 5. Multivariate analysis of prognostic variables in the entire group (total series) and separately in patients diagnosed before 1990 (group 1) and after 1990 (group 2)

Variables	Total series				Group 1				Group 2			
	β	SE	*P*	OR	β	SE	*P*	OR	β	SE	*P*	OR
Gender												
Male *vs.* female	0.44	0.26	0.095	1.55	0.57	0.36	0.113	1.77	0.43	0.42	0.300	1.55
Age (yr)												
41–60 *vs.* ≤40	2.42	0.62	<0.0001	11.26	2.95	1.04	0.005	19.20	1.93	0.80	0.016	6.92
>60 *vs.* ≥40	3.89	0.61	<0.0001	49.20	4.53	1.03	<0.0001	93.56	3.11	0.79	<0.0001	22.52
Histotype												
PTC *vs.* FTC	−0.95	0.27	0.001	0.38	−1.44	0.35	<0.0001	0.23	0.05	0.56	0.919	1.06
Tumor size (cm)												
1–2 *vs.* ≥1	−0.85	0.45	0.062	0.42	−0.40	0.66	0.543	0.66	−1.51	0.83	0.069	0.22
2–4 *vs.* ≥1	−0.24	0.41	0.562	0.78	−0.10	0.63	0.870	0.90	0.01	0.59	0.978	1.01
>4 *vs.* ≥1	0.71	0.39	0.068	2.03	0.97	0.60	0.104	2.65	0.79	0.56	0.170	2.21
Local extrathyroidal extension (with *vs.* without)	−1.09	0.54	0.045	0.33	−0.89	0.89	0.316	0.40	−1.47	0.73	0.045	0.22
Lymph node metastases (with *vs.* without)	1.50	0.37	<0.0001	4.51	1.08	0.52	0.041	2.9	1.42	0.57	0.014	4.1
De Groot's class												
2 *vs.* 1	0.14	0.54	0.979	1.01	0.45	0.74	0.538	1.58	0.24	0.87	0.778	1.27
3 *vs.* 1	1.78	0.59	0.003	5.96	1.80	0.90	0.045	6.05	2.21	0.90	0.015	9.17
4 *vs.* 1	2.89	0.38	<0.0001	18.07	2.38	0.47	<0.0001	10.84	3.98	0.73	<0.0001	53.54
Year of diagnosis: >1990 *vs.* <1990	−0.88	0.26	0.001	0.41								

recognition of the associated risk of radiation-induced thyroid cancer (44). The relatively high percentage of patients with a positive history of radiation exposure to the neck in group 1 (6.1%) was consistent with the rather long latency period of radiation-induced thyroid cancer (45). In patients of group 2, however, a much lower percentage was found (1.9%). This was related to the discontinuation of external radiation use in children and adolescents.

The majority of patients in both groups presented with thyroid nodules, but it is worth noting that as many as 20% of those referred after 1990 found the nodule during diagnostic procedures (usually a neck ultrasound) performed for reasons other than thyroid-related disorders including an extension of gynecological and mammary gland work-up, metabolic assessment preceding a weight loss program, or color Doppler evaluation of the neck for evaluation of carotid vascular disease.

Larger tumors, lymph nodes, and/or distant metastases were less frequent in group 2 than in group 1. Classes III and IV, which included the most severe cases, were also significantly less represented in group 2. Similar results have been observed by Chow *et al.* (9), who found a decrease in the percentage of patients with positive lymph nodes and/or distant metastases in the more recent period of observation. An earlier diagnosis, probably due to the increased use of more sensitive diagnostic procedures such as neck ultrasound and Tg measurement in FNA (17), is the most reasonable explanation of this finding.

In the present study, the reduced percentage of more aggressive tumors observed in group 2 was associated with a marked increase in small PTCs. In particular, mPTC accounted for almost 30% in group 2, as opposed to 8% in group 1. An increase of small tumors has been reported in other series recently published, and an increased incidence of mPTC has been specifically stressed by several authors (1–3, 10). The question remains as to whether the reduced aggressiveness and size of tumor more recently diagnosed are due to diagnosis in an early phase of development or whether it is due to the identification of a small thyroid carcinoma that would have otherwise never become clinically evident. Although further studies are needed to clarify this issue, it may be noted that in this series, as well as in the majority of series reported on mPTC (46), both the mean and median age of the two groups of patients were essentially the same. This finding contradicts the simple hypothesis of an early diagnosis and is consistent with the concept that many, albeit not all, small thyroid carcinomas may remain silent over the entire lifespan, as indicated by the finding of small thyroid tumors at autopsy in many subjects who had died due to unrelated causes (47, 48). Nevertheless, the significantly lower percentage of patients with lymph node and/or distant metastases at the time of diagnosis in group 2 strongly support the hypothesis of an anticipation of diagnosis at least of advanced cases that, in the past, would have been found at a much later point with physical examination or chest radiography.

In contrast with a significant increase of intrathyroidal tumors (*i.e.* class I or stage I) and the decrease of both lymph node (*i.e.* class II or stage II and III) and distant metastases (*i.e.* class IV or stage IVC), we did not observe

a corresponding decline of the locally advanced DTC (*i.e.* class III or stage IVA and IVB) in group 2. This finding is similar to that reported by Enewold *et al.* (10) and evokes the question of why the prevalence of DTC with local invasiveness did not change during the time studied. A possible simplified explanation is that the cellular growth rate of these groups of DTC is so high that it is almost impossible to anticipate their diagnosis. Only a complete molecular characterization of DTC tumors with different biological behavior could better explain these clinical observations (49).

Local extrathyroidal extension showed no difference between the two groups of patients. This finding is in apparent contrast with data indicating that most indices of severity of thyroid cancer were less frequent in group 2. According to data reported by both Gemsenjäger *et al.* (50) and Hu *et al.* (51) macro (*i.e.* T_4) but not micro (*i.e.* T_3) extrathyroidal extension represents a risk factor for a lower survival. When we analyzed separately T_3 and T_4 in our two groups of patients, we found that macro-extrathyroidal extension was, as expected, significantly less frequent in group 2.

Both multifocality and bilaterality were more frequent in group 2. This finding could be related to the changed methodology used in the pathological assessment, with particular regard to the greater number of samplings performed after the 1980s. Nevertheless, neither bilaterality nor multifocality appeared to influence the survival of our patients. This finding is in keeping with other reports (52–54) and confirms our previous observation that both multifocality and bilaterality may affect the local recurrence but not the survival (55).

Gender, age, histotype, tumor size, local macro-extrathyroidal extension, lymph nodes, and distant metastases as well as clinical classes were found to have a significant negative influence on survival in univariate analysis of the total series. When these prognostic factors were analyzed separately in group 1 and group 2, similar qualitative results were obtained. However, in the multivariate analysis, only age and clinical class were found to retain their independent prognostic values. As shown in Table 5, patients older than 60 yr or those with a class IV tumor, which included all cases with distant metastases, showed the higher risk in terms of OR value.

Of interest is the finding that the year of diagnosis showed a significant independent influence on the survival rate in the multivariate analysis. Patients diagnosed and treated after 1990 had a better outcome. The two most reasonable explanations of this more favorable outcome are the early diagnosis and the more effective treatment developed in the recent years. However, as stated in the introductory section, in our series, the therapeutic strategy was very similar during the years studied, and we prefer the hypothesis that an earlier identification of both the primary tumor and recurrence is the reason of the better outcome of group 2 patients.

In conclusion, the present study, although based on cases referred to our institution and not on all Italian DTC cases, confirms and extends data indicating a marked change in clinical and pathological features of DTC occurring in recent years. The most relevant aspect is the sharp increase in the proportion of PTCs of smaller size, especially mPTC, in patients diagnosed after 1990. In parallel, a relative reduction of more advanced cases was also observed in this group. The smaller proportion of patients free from other thyroid disorders, but particularly of those in whom the thyroid lesion was an incidental finding of diagnostic procedures for other conditions, is consistent with the suggestion that the increasing use of ultrasound procedures is largely responsible for the recognition of clinically silent thyroid diseases, especially thyroid nodules of small/medium size. Taken together, these observations may suggest that the increased attention and larger use of sensitive diagnostic techniques, such as neck ultrasound, result in the detection of tumors at an earlier stage. This interpretation is not consistent with the finding that the mean age at diagnosis of patients observed before and after year 1990 was unchanged. However, we can explain the two apparently conflicting results with the introduction of neck ultrasound and the general improvement of diagnostic tools that determined both the discovery of a great number of clinically silent mPTC and the early diagnosis of metastases and recurrences. Irrespective of these very important changing features and the improved general outcome of DTC patients diagnosed in the recent years, older age and advanced stage still remain the two most unfavorable prognostic factors for a lower survival and must be taken into consideration in planning the therapeutic strategy.

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A.P. has access to all of the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis. F.P. was in Pisa at the Department of Endocrinology until October 2002.

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