U.PORTO
FACULDADE DE MEDICINA
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MESTRADO INTEGRADO EM MEDICINA

2023/2024

Mariana Costa Caldeira

Aplicação dos novos critérios para Cancro da Tiróide Diferenciado de Alto Grau numa série de cancro da tiróide recorrente: um estudo retrospetivo de 138 casos.

Application of the new criteria for Differentiated High Grade Thyroid Carcinoma to a series of recurrent thyroid cancer: a retrospective study of 138 cases.

Fevereiro, 2024

Mariana Costa Caldeira

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Mestrado Integrado em Medicina

Área: Patologia
Tipologia: Dissertação

Trabalho efetuado sob a Orientação de:

Professora Doutora Ana Paula Soares Dias Ferreira

E sob a Coorientação de:

Professora Doutora Sule Canberk

Trabalho organizado de acordo com as normas da revista:
Diagnostics – an open access journal from MDPI (Multidisciplinary Digital Publishing Institute)

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DESIGNAÇÃO DA ÁREA DO PROJECTO

Patologia

TÍTULO DISSERTAÇÃO/MONOGRAFIA (riscar o que não interessa)

Application of the new criteria for Differentiated High Grade Thyroid Carcinoma to a series of recurrent thyroid cancer: a retrospective study of 138 cases.

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UC Dissertação - DECLARAÇÃO DE TRANSPARÊNCIA RELATIVAMENTE À UTILIZAÇÃO DE FERRAMENTAS DE CHATBOT GENERATIVO BASEADAS EM LARGE LANGUAGE MODELS

Eu, Mariana Costa Caldeira, abaixo assinado, nº mecanográfico 201807516, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro que:

☒ Não procedi à utilização de ferramentas de *chatbox* generativo baseadas em *large language models* para nenhuma das tarefas no contexto do meu trabalho de Dissertação ou Monografia

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Faculdade de Medicina da Universidade do Porto, 27/02/2024

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DEDICATÓRIA

À Professora Doutora Ana Paula Dias Soares Ferreira, muito obrigada por me ter recebido de braços abertos e dado esta oportunidade inestimável, que me deu muitos alicerces e decerto contribuirá para o meu futuro nesta área, que tanto me interessa e fascina.

To Professora Doutora Sule Canberk, thank you for accompanying me through this process, despite all the difficulties we came across. Everything you've taught me about your field of expertise, among so many other things has contributed to me being better as an individual, and future doctor and researcher.

À minha mãe, que apesar de todas as adversidades que ultrapassámos ao longo da vida, abdicou de tudo para me dar um futuro melhor. Apesar de à sua maneira, sempre me apoiou incondicionalmente, o que tem um valor imensurável. Nunca vou conseguir compensar-lhe pelo que me deu, mas passarei a minha vida a tentar.

Aos meus amigos e família que sabem bem quem são, obrigada por todo o apoio que me deram ao longo deste percurso e, sobretudo, por me aceitarem e incentivarem a ser como sou e crescer à minha maneira.

Aos Finalistas e à Casa de Medicina, muito obrigada por me integrarem e crescerem comigo nestes que foram e estão a ser os melhores 6 anos da minha vida.

À Tuna Feminina de Medicina do Porto, por me ter feito perceber que eu e todas nós somos capazes de muito mais que imaginávamos. Obrigada por todos os momentos e emoções inesquecíveis, amizades para a vida e o sentimento de pertencer a algo maior que nós. Enche-me de orgulho fazer parte deste grupo e vê-lo a crescer em todos os sentidos. Continuem a fazer o que estão a fazer, que está a correr incrivelmente.

A todos os que posso não me ter lembrado de mencionar,

Um grandessíssimo obrigado!

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Article

Application of the new criteria for Differentiated High Grade Thyroid Carcinoma to a series of recurrent thyroid cancer: a retrospective study of 138 cases.

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Abstract:

In the latest 5th WHO's Classification of Tumors of Endocrine Organs, a new term was created to identify those cases, at the time of pathological evaluation, which have a worse prognosis within differentiated follicular cell-derived thyroid carcinomas (DFCDTC): Differentiated High Grade Thyroid Carcinoma (DHGTC). In our work, we aimed to evaluate its frequency and clinicopathological features within a series of recurrent DFCDTC.

We gathered several clinicopathological characteristics of a retrospective cohort of 138 patients with primarily resected thyroid cancer which recurred after total thyroidectomy and adjuvant radioiodine therapy. We also reclassified them as DHGTC according to 5th WHO's criteria; compared DHGTC to non-high grade differentiated follicular cell-derived thyroid carcinomas (non-HG-DTC) and described the clinical behavior and pathological features of thyroid cancer with radioactive iodine refractoriness.

We found that DHGTC's prevalence is higher than what is described in studies without prior clinical selection. In comparison to non-HG-DTC, DHGTC cases were significantly associated to several adverse clinicopathological features: higher tumor size; tall-cell subtype of PTC; mitotic index ≥5; tumor necrosis; lymphovascular invasion; high AJCC 8th edition pT stage; distant metastasis; lung metastasis; synchronous metastasis and persistence of disease at the end of follow-up. Furthermore, the number of cases which developed RAIR (n=12 [8,7%]), despite low, is quite significant, as the estimated incidence of these forms of cancer is of 4-5 cases/year/million people, and we described their clinicopathological characteristics.

Our results strengthen the clinical usefulness of DHGTC classification by indicating patients at a higher risk for progression and highlight the need for tight surveillance in these forms of cancer.

Keywords: Thyroid cancer; Differentiated High Grade Thyroid Carcinoma; radioactive iodine refractoriness; prognosis

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Received: date
Revised: date
Accepted: date
Published: date

1. Introduction

In the latest decades, our understanding has grown considerably regarding the genetic landscape and biology of thyroid cancer (TC). This knowledge led to significant changes in its histopathological classification, which profoundly influenced clinical practice.

Histologic classification of follicular cell-derived thyroid cancer has been made according to tissue and cellular morphology, establishing a spectrum that goes from differentiated follicular cell-derived thyroid carcinomas (DFCDTC) [papillary (PTC); follicular (FTC) and oncocytic (OCA)], to the undifferentiated form, anaplastic carcinoma (ATC).

The last 4th and 5th editions of WHO's Classification of Tumors of Endocrine Organs have brought groundbreaking changes, such as the recognition of low-risk tumors (nonninvasive follicular thyroid neoplasm with papillary-like nuclear features [NIFTP]) and a new grading system for well-differentiated thyroid carcinoma.

The 5th edition of WHO's classification [1], uses evidence gathered in two defined criteria- Memory Sloan Kettering Cancer Center's (MSKCC) criteria and Turin consensus' criteria - to identify high-grade follicular cell-derived non-anaplastic thyroid carcinoma (HGFCDTC). [2].

This way, two subgroups have been recognized: differentiated high-grade thyroid carcinoma (DHGTC) and poorly differentiated thyroid carcinoma (PDTC), the former being the most recent addition to the classification.

In fact, there was the clinical perception that, in the group of DFCDTC as a whole, several discrepancies in prognosis exist. So, DHGTC's introduction appeared as a separate entity to identify those patients which, within DFCDTC, have bad prognosis, yet better prognosis compared to PDTC and ATC through pathological evaluation [3]. Thus, DHGTC is recognized as an intermediate category in terms of prognostic outcomes.

The initial management of patients diagnosed with thyroid cancer follows international guidelines which highlight the patient's biochemistry; ultrasound characteristics; cytopathology; risk stratification, among others, that do not distinguish high-grade tumors.

In fact, DHGTC and PDTC tumors can fit in different Bethesda categories [4] as well as in separate American Thyroid Association's (ATA) risk groups [5,6]. That can result in more conservative approaches, such as tight follow-up or lobectomy with isthmectomy [7,8,9] in patients at a higher risk for progression. [8,9]

The patients submitted to total thyroidectomy and RAI are indicated for life-time follow-up [8], and, during that period, recurrences can be documented. These recurrences can reflect genetic mutations and distortions of signaling pathways, which lead to radioactive iodine refractoriness (RAIR). RAIR patients can be therapeutically approached with new and effective targeted therapies, such as tyrosine kinase inhibitors (TKIs), sorafenib and lenvatinib, which act by inhibiting tumor growth and angiogenesis [10]. Upcoming therapies are being tested, such as the MEK and BRAF inhibitor combination, dabrafenib plus trametinib, with the aim of inducing redifferentiation of the tumors and response to further RAI treatments. [11]

According to the recent European Thyroid Association (ETA) guidelines,the proposed criteria to define RAI refractory disease are the following: (i) absence of RAI uptake in all lesions on scintigraphy; (ii) absence of RAI uptake in some but not in all lesions; (iii) progression despite RAI uptake; (iv) having reached the maximum recommended RAI activity [12].

On the other hand, the most recently published recommendations by the American and European Thyroid and Nuclear Medicine Societies combined define RAI refractoriness as: (i) no 131I uptake on a diagnostic 131I scan; (ii) no 131I uptake on a 131I scan performed several days after 131I therapy; (iii) 131I uptake is present in some but not all tumor foci; (iv) DTC metastasis(es) progress despite 131I uptake; (v) DTC metastasis(es) progress despite a cumulative 131I activity of >22.2 GBq (600 mCi) [13].

It has been shown that RAIR is more frequent in older patients, in patients with large metastases, in PDTC, and in tumors with high 18-fluordeoxyglycose (18-F-FDG) uptake on PET/CT. [14]

As DHGTC is such a recent addition to thyroid tumor's classification, studies of radioactive iodine refractory DHGTC have yet to be done. As these tumors display poorer prognosis in comparison to non-high-grade differentiated thyroid carcinoma (non-HG-DTC) [3], we hypothesize that a substantial part might develop RAIR and the rate at which that happens is yet to be defined.

In this study we gathered a retrospective cohort of 138 patients with resected thyroid cancer which recurred after total thyroidectomy and adjuvant RAI, of which a significant proportion developed RAIR. The tumor series was reclassified according to the 5th edition of WHO's criteria and several clinicopathological characteristics were collected.

The aims of the study were: (i) to describe the clinical behavior and pathological features of the cohort; (ii) evaluate the frequency of DHGTC and its clinical behavior in this series; (iii) evaluate the clinicopathological differences between DHGTC and non-HG-DTC and (iv) describe the clinical behavior and pathological features of cases with RAIR.

2. Materials and Methods

2.1. Tumor samples and data collection

Our study included a series of 138 formalin-fixed, paraffin embedded resection samples of human thyroid tissue from patients followed in Centro Hospitalar e Universitário de Coimbra, kept at the biobank of the Institute of Molecular Pathology and Immunology of the University of Porto. The samples of the patients were collected upon written informed consent of the patients, or their guardians, in the case of minors.

The clinicopathological data was collected from clinical records, with the approval of the Ethic Committee of the Faculty of Medicine of the University of Coimbra (n° 1309) and all the procedures described in this study were done in accordance with national ethical standards (Law n° 12/2005) and Helsinki declaration.

All the information was analyzed and stored according to local legal requirements, ensuring data protection.

2.1. Characteristics of the cohort and clinicopathological review

The patients were diagnosed between 1971 and 2010 with thyroid cancer and underwent total thyroidectomy and adjuvant therapy with radioactive iodine (131I) as an initial approach.

During the follow-up of the patients, radiological and/or biochemical recurrences were detected despite the adjuvant treatment, which often led to additional therapies being applied.

The histology of tumor samples was reviewed by a pathologist (SC), who updated the classification to match the 5th edition of WHO's classification, blinded to the clinical outcome to confirm the diagnosis.

In particular, DHGTC was classified according to the following criteria: mitotic count ≥5/2 mm^2 [6 HPFs in the microscope used- fields of 0,34 mm^2] and/or tumor necrosis. The mitotic count was determined within the most mitotically active area (the so-called 'hotspot').

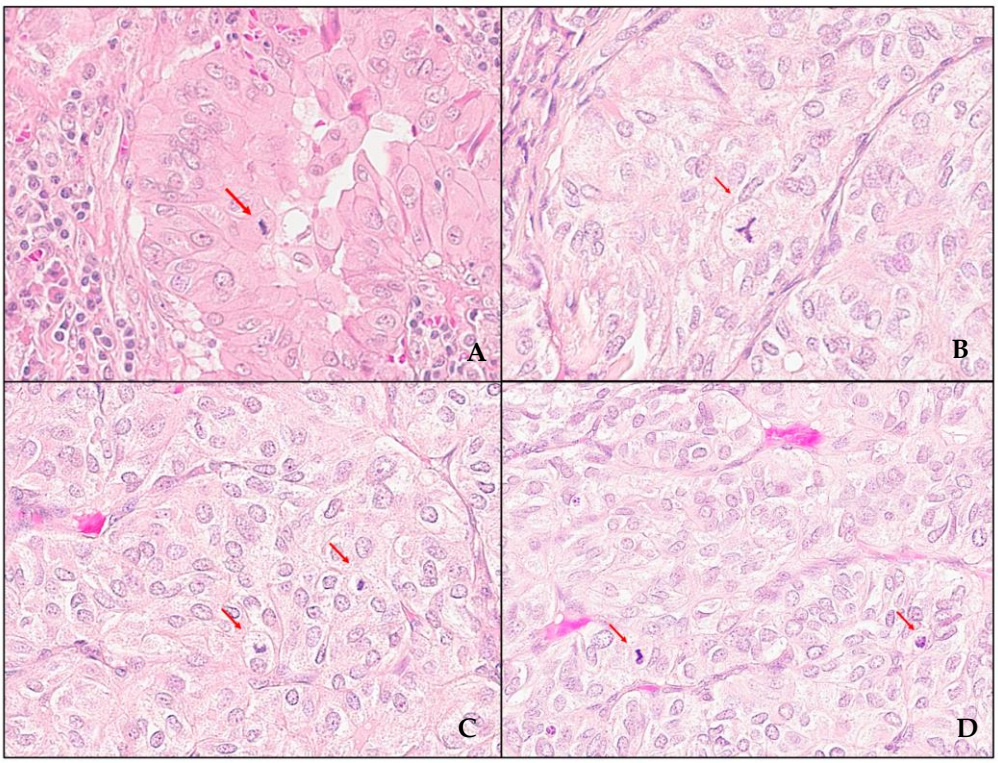

Figure 1. Selection of readily identifiable mitoses (arrows): in high grade papillary thyroid carcinoma, tall cell subtype (**A**) and high grade papillary thyroid carcinoma, solid subtype (**B,C,D**)- 400x amplification

Only true tumor necrosis was considered, as suggested by WHO's 5th edition, defined as the presence of clusters and sheets of ghost contours of dead tumor cells with karyorrhectic debris.

Data from genetic characterization of the tumors was obtained in previous works of the group which included part of the cases included in this series. [References 15 and 16 contain technical details.]

2.3. Clinical outcome and statistical analysis

Follow-up data was available for 133 patients. The outcomes collected included disease-free survival (DFS), disease specific survival (DSS) and persistence of disease at the end of follow-up. The follow-up was calculated from the date of primary resection.

DFS was defined as the time from primary resection to cancer recurrence, DSS as the time from primary resection to death due to thyroid cancer and persistence of disease was documented as proven structural disease.

All statistical analyses were performed with SPSS 29.0 (IBM Corporation, Armonk, NY, USA). Comparisons of clinicopathological features between subgroups were performed with the chi-square test or Fisher's exact test for categorical variables, and with Mann-Whitney U test for continuous variables. Univariate survival analysis was performed with the log rank test for categorical variables and with the Cox proportional hazards model for continuous variables. Factors that were significant in univariate analysis were subsequently subjected to multivariate analysis with the Cox proportional hazards model. *P*-values of <0,05 were considered to be statistically significant.

3. Results

3.1. Clinicopathological and genetic characteristics of the entire cohort

The clinical and pathological features of the entire cohort are summarized in Table 1.

The median age was 45 years (range, 13–81 years). There was a female predominance, with a female/male ratio of 3:1. The median tumor size was 2,65 cm (range, 0,5-9 cm).

In terms of diagnosis, 32 cases (23,2%) presented as HGFCDTC, of which 22 (15,9%) were diagnosed as DHGTC and 10 (7,2%) as PDTC.

Within the DHGTC cases, the most common subtypes were: tall-cell subtype of PTC (37,5%); classical subtype of PTC (22,7%) and infiltrative follicular subtype of PTC (13,6%).

Among the 106 non-HG-DTC cases (76,8%), the most common subtypes were: classical subtype of PTC (33%); infiltrative follicular subtype of PTC (22,6%); invasive encapsulated follicular subtype of PTC (8,5%) and oncocytic subtype of PTC (8,5%).

Regarding the mitotic index, 27 patients (19,6%) had a mitotic index of over 5/2 mm^2, of which 22 (81,5%) were diagnosed as DHGTC and 5 (18,5%) as PDTC .

Ten (7,6%) of the tumors present necrosis, 4 (40%) DHGTC cases and 6 (60%) PDTC.

With regard to capsule status, 80 cases (65%) were infiltrative and 43 (35%) invasive. Thirty-two cases (26%) were partially encapsulated; 108 (94,7%) had capsular invasion; 41 (33,9%) lymphovascular invasion and 49 (38,0%) microscopic extrathyroidal extension, more often to fibroadipose tissue (31,8%).

Concerning the AJCC 8th pT and pN staging of these tumors, 72 (57,1%) were high stage (T3/T4) and 43 (31,2%) presented with nodal metastasis (N1a/N1b).

Almost half of the tumors (48%) had the BRAF V600E mutation, and a significant proportion (14,8%) presented TERT promoter mutations. One case documented the Q61R mutation in NRAS.

DHGTC cases, as compared with non-HG-DTC patients, were significantly associated with the age ranges of ≤18 and ≥55 years old; higher tumor size, on average and in the range of >2 cm; tall-cell subtype of PTC; mitotic index ≥5; tumor necrosis; lymphovascular invasion; presence of distant metastasis; lung metastasis; synchronous metastasis; high AJCC 8th edition pT stage; submission to additional therapies; treatment with TKI; more administrations of additional RAI; higher median cumulative dose of RAI and persistence of disease at the end of follow-up, ($P < 0.05$; Table 1).

Table 1. Clinicopathologic characteristics of 138 patients with primarily resected recurrent thyroid cancer.

	All [n=138]	**DHGTC [n=22]**	**Non-HG-DTC [n=106]**	***P* value**
Clinicopathological parameters				
Age (years) [n=137]				
Median (range)	45 (13-81)	48 (13-81)	44,5 (17-74)	NS
≤18	4 (3,1%)	3 (13,6%)	1 (0,9%)	0,016
18-55	86 (62,3%)	8 (38,1%)	77 (72,6%)	<0,001
≥55	47 (34,1%)	10 (47,6%)	28 (26,4%)	<0,001
Sex				
Female	108 (78,3%)	16 (72,7%)	86 (81,1%)	NS
Male	30 (21,7%)	6 (27,3%)	20 (18,9%)	
Documented metastasis	85 (61,6%)	16 (72,7%)	62 (58,5%)	NS
Distant metastasis	36 (26,1%)	10 (45,5%)	19 (17,9%)	0,008
Types of distant metastasis				
Bone	13 (9,4%)	3 (13,6%)	8 (7,5%)	NS
Lung	26 (18,8%)	8 (36,3%)	12 (11,3%)	0,015
Brain	2 (1,4%)	1 (4,5%)	0 (0%)	NS
Kidney	1 (0,7%)	1 (4,5%)	0 (0%)	NS
Lymph node metastasis [n=135]	67 (49,6%)	12 (60%)	52 (49,5%)	NS
Synchronous metastasis [n=127]	27 (21,3%)	6 (35,3%)	14 (14%)	0,042
Tumour size (cms) [n=125]				
Median (range)	2,65 (0,5-9)	3,51 (0,8-8)	2,3 (0,5-9)	0,002
⩽2 cm	67 (56,3%)	5 (22,7%)	62 (58,5%)	
>2 cm	61 (47,7%)	17 (77,3%)	44 (41,5%)	
Main diagnosis				
Classical subtype of PTC	40 (29%)	5 (22,7%)	35 (33%)	NS
Hobnail subtype of PTC	6 (4,3%)	1 (4,5%)	5 (4,7%)	NS
Invasive encapsulated follicular subtype of PTC	9 (6,5%)	0 (0%)	9 (8,5%)	NS

Infiltrative follicular subtype of PTC	27 (19,6%)	3 (13,6%)	24 (22,6%)	NS
Oncocytic subtype of PTC	9 (6,5%)	0 (0%)	9 (8,5%)	NS
Solid subtype of PTC	6 (4,3%)	2 (9,1%)	4 (3,8%)	NS
Tall-cell subtype of PTC	17 (12,3)	9 (37,5%)	8 (7,5%)	<0,001
Warthin-like subtype of PTC	7 (5,1%)	0 (0%)	7 (6,6%)	NS
Oncocytic carcinoma of the thyroid	2 (1,4%)	1 (4,5%)	1 (0,9%)	NS
Follicular thyroid carcinoma	5 (3,6%)	1 (4,5%)	4 (3,8%)	NS
Poorly differentiated thyroid carcinoma	10 (7,2%)	NA	NA	NA
Mitotic index (per 2 mm²)				
Median (range)	2 (0-8)	6 (5-8)	1 (0-4)	<0,001
<5	111 (80,4%)	0 (0%)	106 (100%)	<0,001
≥5	27 (19,6%)	22 (100%)	0 (0%)	
Tumor necrosis [n=132]				
Present	10 (7,6%)	4 (19%)	0 (0%)	<0,001
Absent	122 (92,4%)	17 (81%)	102 (100%)	
Encapsulation [n=123]				
Encapsulated	91 (74%)	11 (64,7%)	73 (75,3%)	NS
Partially encapsulated	32 (26%)	6 (35,3%)	24 (24,7%)	
Capsular invasion [n=114]				
Present	108 (94,7%)	16 (100%)	83 (93,3%)	NS
Absent	6 (5,3%)	0 (0%)	6 (6,7%)	
Lymphovascular invasion [n=121]				
Present	41 (33,9%)	9 (50%)	25 (26,6%)	0,039
Absent	80 (66,1%)	9 (50%)	69 (73,4%)	
Microscopic extrathyroidal extension [n=132]				
Present	52 (39,4%)	8 (38,1%)	42 (41,2%)	NS
Fibroadipose tissue	48 (36,3%)	7 (31,8%)	39 (36,8%)	NS
Skeletal muscle	7 (5,3%)	1 (5%)	6 (5,7%)	NS
Other organs	1 (0,8%)	0 (0%)	1 (0,9%)	NS
Capsule status [n=123]				
Infiltrative	80 (65%)	9 (52,9%)	69 (71,1%)	NS
Invasive	43 (35%)	8 (47,1%)	28 (28,9%)	
AJCC 8th pT stage [n=126]				
pT1/T2	54 (42,9%)	3 (15%)	50 (51%)	0,003
pT3/T4	72 (57,1%)	17 (85%)	48 (49%)	
AJCC 8th pN stage				
pN0/Nx	95 (73%)	16 (72,7%)	77 (72,6%)	NS
pN1a/N1b	43 (31,2%)	6 (27,3%)	37 (27,4%)	
Molecular alterations				

BRAF V600E mutated [n=123]	64 (48%)	9 (52,9%)	50 (51,5%)	NS
NRAS Q61R mutated [n=91]	1 (1,1%)	0 (0%)	1 (1,4%)	NS
TERT promoter mutated [n=108]	16 (14,8%)	5 (29,4%)	9 (11%)	NS
-124G>A mutation	10 (9,3%)	4 (23,5%)	6 (7,3%)	
-146G>A mutation	6 (5,6%)	1 (5,9%)	3 (3,7%)	
Follow-up and treatments [n=133]				
Deaths due to disease	17 (12,8%)	3 (13,6%)	11 (10,4%)	NS
Median follow-up time in years (range) [n=117]	6,54 (0,28-38,3)	4,34 (0,67-29,9)	6,57 (0,28-38,3)	NS
Median time until the first recurrence in months (range)	16 (1-313)	26,4 (0,83-312,8)	15,8 (0,07-93,2)	NS
Outcome at last observation				
Death due to disease	17 (12,3%)	3 (13,6%)	11 (10,4%)	NS
Alive with disease	48 (34,8%)	13 (59,1%)	31 (29,2%)	0,006
Alive without disease	81 (58,7%)	6 (27,3%)	71 (67%)	<0,001
Development of radioactive iodine refractoriness	12 (8,7%)	3 (13,6%)	5 (4,7%)	NS
Number of recurrences [n=117]				
1	62 (53%)	11 (64,7%)	45 (50%)	NS
2	55 (47%)	6 (35,3%)	45 (50%)	
Additional therapies [n=134]				
Yes	66 (52,8%)	12 (75%)	48 (48,5%)	0,043
Radioactive iodine therapy (RAI)	63 (47%)	13 (68,4%)	44 (41,9%)	NS
Radiation therapy	2 (1,6%)	1 (6,3%)	1 (1%)	NS
Tyrosine kinase inhibitors	2 (1,6%)	2 (12,5%)	0 (0%)	0,018
Surgery	35 (28%)	7 (43,8%)	22 (22,2%)	NS
Number of additional RAI administrations [n=134]				
0	71 (53%)	6 (31,6%)	61 (58,1%)	0,030
1 or more	63 (45,7)	13 (68,4%)	44 (41,9%)	
Median cumulative dose of RAI in millicuries [mCi] (range)	141 (30-34739,1)	366 (70-732)	127 (30-1146)	0,037

3.2. Outcomes and prognostic factors of the entire cohort

Documented metastasis was frequent, observed in 85 patients (61,6%), and 36 of them (42,3%) had distant metastasis. The most common sites of distant metastases were lung (n=26) and bone (n=13).

Lymph node metastasis was present in 67 cases (49,6%) and 27 (21,3%) had synchronous metastasis (defined as metastasis identified within 6 months after the diagnosis of the primary cancer [17]).

Seventeen (12,8%) patients died due to disease during follow-up, of which 4 (23,5%) were PDTC, 3 DHGTC (17,6%) and 11 (64,7%) non-HG-DTC. All the patients who died due to disease had documented metastasis; capsular invasion; AJCC pathological N1 stage and absence of NRAS mutations.

The results of univariate survival analysis on DSS and DFS are shown in Table 2, and selected Kaplan–Meier curves are shown in Figure 2.

Additional Kaplan–Meier curves for DSS and DFS are shown in Figure S1.

Follow-up data was available for 133 patients, with a median follow-up of 6,54 years (range, 0,28-38,30 years).

The overall 3-year; 5-year, 10-year and 20-year DSS rates in the whole series were: 98%; 98%; 96% and 93%. However, in the DHGTC population, these rates were the following: 91%; 91%; 86%; 86%.

No prognostic significance was found on univariate survival analysis of DFS and DSS within the following parameters: initial dose of RAI (as a continuous variable); treatment with TKI; size (as a continuous variable); mitosis (as a continuous variable <5/2 mm^2 vs ≥5/2 mm^2); necrosis; capsular invasion; lymphovascular invasion; AJCC 8th pT stage (T1/T2 versus T3/T4); capsule status (infiltrative vs invasive); BRAF mutations; TERT promoter mutations and RAS mutations; age (categorical and as a continuous variable); sex; DHGTC vs non-HG-DTC classification; treatment with radiotherapy;; documented metastasis; lymph node metastasis; encapsulation; AJCC 8th pN stage (N0/Nx versus N1a/N1b).

Prognostic factors identified on univariate analysis as impacting DFS were higher cumulative dose of RAI and microscopic ETE. Worse DSS was associated with tumor size >2 cm; presence of distant metastasis and absence of synchronous metastasis.

The results of multivariate survival analysis with the Cox proportional hazards model are shown in Table 3.

Independent prognostic factors for worse DFS were higher cumulative dose of RAI [P<0,001, HR=1,003, 95% CI (1,002-1,005)]; and microscopic ETE [P=0,007, HR=2,379, 95% CI (1,270-4,454)]. There were no significant independent prognostic factors for worse DSS.

Postoperative treatments such as radiotherapy and additional RAI administrations/ higher dosage were associated with decreased survival, owing to the selection bias of patients with aggressive disease receiving additional therapy.

Table 2. Univariate survival analysis for disease-free survival (DFS), and disease specific survival (DSS) in recurrent thyroid cancer

Clinical parameters	**DFS**	**DSS**
Cumulative dose of RAI (as a continuous variable)	<0,001	NS
Size (in 3 categories)	NS	0,024
Distant metastasis	NS	0,005
Synchronous metastasis	NS	0,008
Microscopic ETE	0,014	NS

Table 3. Multivariate survival analysis using Cox proportional hazards model in recurrent thyroid cancer

DFS	***P* value**	**Hazard ratio (95% CI)**
Cumulative dose of RAI (as a continuous variable)	**<0,001**	1,003 (1,002-1,005)
Microscopic ETE	**0,007**	2,379 (1,270-4,454)
DSS	**P value**	**Hazard ratio (95% CI)**
Size (>2 cms)	0,185	4,399 (0,491-39,411)
Size (≤2 cm)	0,185	0,227 (0,025-2,037)
Distant metastasis	0,701	2,755 (0,016-483,456)
Synchronous metastasis	0,513	5,677 (0,031-1033,097)

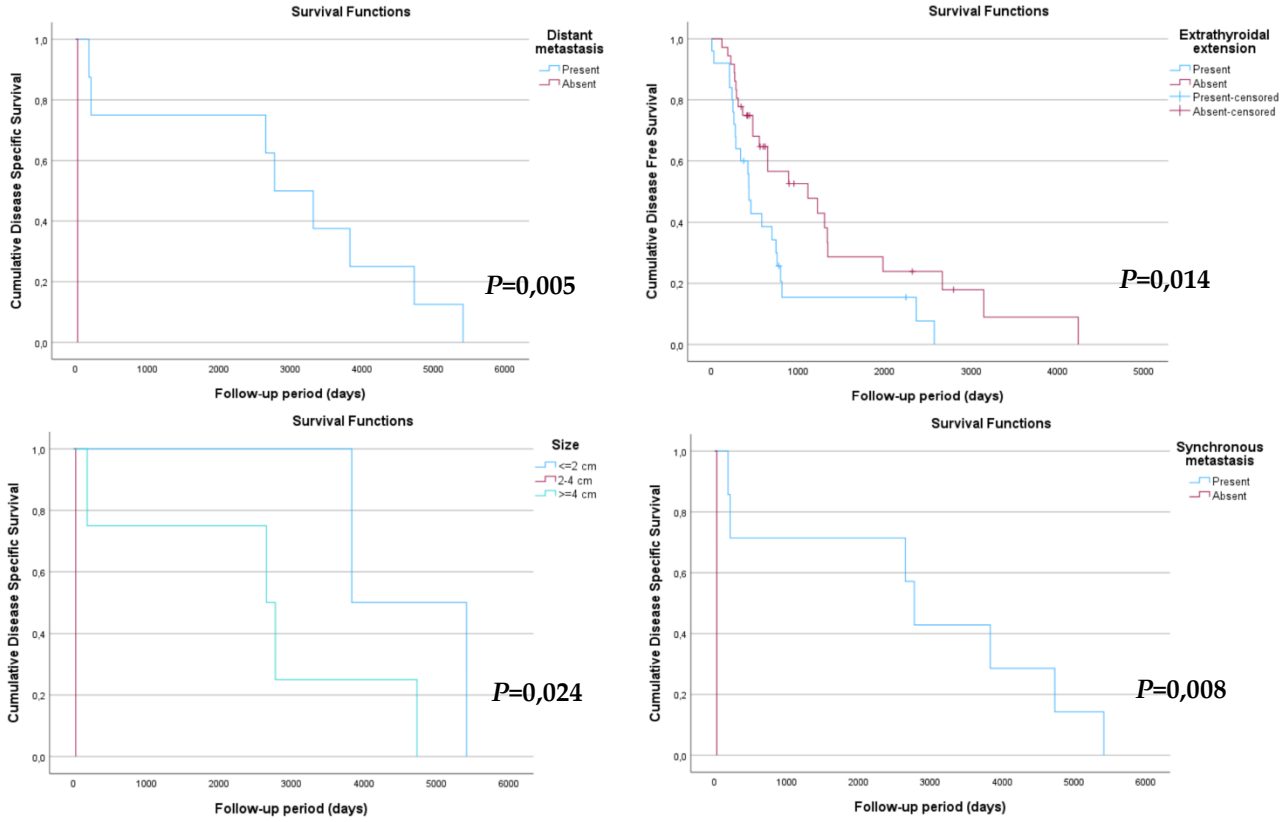

Figure 2. Selected Kaplan–Meier curves for DFS and DSS in recurrent thyroid cancer

3.3. Clinicopathological features, outcomes and prognostic factors of patients with DHGTC

Patients with DHGTC have a significantly higher proportion of cases which developed distant metastasis (45,5%) and synchronous metastasis (35,3%), in comparison to non-HG-DTC. All the DHGTC cases had capsular invasion and did not harbor NRAS mutations.

Additionally, the deadly cases were all ≥55 years old; were not submitted to therapy with TKI; had documented metastasis; lymph node metastasis; AJCC pathological T3/T4 stage and did not have TERT promoter mutations.

The results of univariate survival analysis on DFS and DSS within the DHGTC subgroup are shown in Table 4. Kaplan–Meier curves for DFS and DSS are shown in Figure S2 and the results of multivariate survival analysis with the Cox proportional hazards model are shown in Table 5.

No prognostic significance was found on univariate survival analysis of the following parameters: age (categorical); age (as a continuous variable); sex; initial dose of RAI (as a continuous variable); treatment with radiotherapy; treatment with TKI; cumulative dose of RAI (as a continuous variable); size (categorical); size (as a continuous variable); mitosis (as a continuous variable); documented metastasis; distant metastasis; lymph node metastasis; synchronous metastasis; necrosis; encapsulation; capsular invasion; lymphovascular invasion; AJCC 8th pT stage (T1/T2 versus T3/T4); AJCC 8th pN (N0/Nx versus N1a/N1b); BRAF mutations; TERT promoter mutations and RAS mutations.

Prognostic factors identified on univariate analysis as disclosing worse DFS in DHGTC cases were microscopic ETE and infiltrative capsule status, but in multivariate analysis no significant independent prognostic factors for worse DFS were found.

Table 4. Univariate survival analysis for disease-free survival (DFS), and disease specific survival (DSS) in recurrent DHGTC

Pathological parameters	DFS	DSS
Microscopic ETE	**0,012**	NS
Capsule status (infiltrative vs invasive)	**0,046**	NS

Table 5. Multivariate survival analysis using Cox proportional hazards model in recurrent DHGTC

DFS	***P* value**	**Hazard ratio (95% CI)**
Microscopic ETE	0,184	6,506 (0,411-103,063)
Capsule status	0,619	1,357 (0,407-4,519)

3.4. Clinicopathological features and outcomes of patients with RAIR

The clinical and pathological features of the 12 cases with RAIR (8,7%) are summarized in Table S1.

The median age was 47,5 years (range, 15–74 years). There was a female predominance, with a female/male ratio of 5:1. The median tumor size was 2,5 cm (range, 1,5-8 cm). The most frequent diagnosis was PDTC, with 4 cases (33,3%), followed by classical and infiltrative follicular subtype of PTC, with 2 cases each (16,7%). The remaining diagnostics were: oncocytic subtype of PTC; warthin-like subtype of PTC, oncocytic carcinoma of the thyroid; and follicular thyroid carcinoma, with 1 case (8,3%) each.

Regarding the mitotic index, 6 cases (50%) displayed a mitotic index of over 5/2 mm^2, of which 3 (50%) were PDTC and the remaining 3 (50%) DHGTC. Tumor necrosis was present in 2 of these cases (16,7%), corresponding to PDTC. Two cases (18,2%) were partially encapsulated; all of them had capsular invasion; 8 (72,7%) presented with lymphovascular invasion; 7 (63,6%) had microscopic extrathyroidal extension, more often to fibroadipose tissue (85,7%); 8 (72,7%) had infiltrative and 3 (27,3%) invasive capsule status; 8 (72,7%) presented with high AJCC 8th pT stage (T3/T4); 4 (33,3%) had pN1a/N1b AJCC 8th pN stage; 6 (54,4%) presented with the BRAF V600E mutation and 4 (33,3%) with TERT promoter mutations, of which 2 had the -124G>A mutation and the remaining the -146G>A mutation.

Within the 12 cases with RAIR, all of them presented with distant metastasis, more often to the lungs (75%), but also to bone (33,3%), brain (8,3%) and kidney (8,3%). Seven

(58,7%) had lymph node metastasis and 10 (83,3%) synchronous metastasis. Eleven patients (91,7%) had persistence of disease at the end of follow-up, of which 3 (27,2%) died due to disease.

There were no statistically significant differences in the clinicopathological features between the DHGTC and non-HG-DTC subgroups within the RAIR population.

Within the DHGTC-RAIR subgroup (n= 3), the diagnostics were classical subtype of PTC (33,3%); infiltrative follicular subtype of PTC (33,3%) and oncocytic carcinoma of the thyroid (33,3%).

4. Discussion

The 5th WHO classification of thyroid tumors includes a new entity that is DHGTC, however the clinical characterization of that entity remains largely unknown.

We evaluated DHGTC in the setting of recurrent thyroid cancer, in a retrospective study composed by a large selection of these cases (the reported prevalence of recurrence is around 5% in PTC [18]).

We found that the prevalence of DHGTC in a series of recurrent thyroid cancer is higher than what is described in studies without prior clinical selection (17,2% vs 7,2%) [19].

Curiously, the same is not true in relation to PDTC. Its prevalence (7,2%) is within the reported prevalence of 2-15% [20] in unselected series. This result suggests that DHGTC category encloses cases that might have a higher tendency to recur, recognizing DHGTC as an intermediate category in terms of prognostic outcomes.

The most frequent subtypes of PTC in the whole series are classical and infiltrative follicular PTC, which don't necessarily correspond to the subtypes considered to be aggressive [22, 23]. Within the DHGTC population, nonetheless, the most frequent subtype is tall-cell, which is indeed shown to be aggressive, coherent with these tumors' poorer prognosis [3].

We also addressed adverse prognostic clinicopathological factors for the outcome of the patients (DFS and DSS) in the whole series. Several clinicopathological factors were found to be adverse in univariate analysis: higher cumulative dose of RAI (for DFS); microscopic ETE (for DFS); tumor size >2 cm (for DSS); presence of distant metastasis (for DSS) and absence of synchronous metastasis (for DSS).

In the subsequent multivariate analysis only microscopic ETE (for DFS) and higher cumulative dose of RAI (for DFS) remain significantly associated to higher risk of recurrence.

The impact of microscopic ETE in the patients' outcome is intriguing. Microscopic ETE was recently excluded from the AJCC's 8th edition TNM staging [24], since it resulted in a stage upgrade in numerous patients with low grade tumors. We must stress that we are evaluating a series of recurrent tumors and these findings should be verified in more representative series.

We didn't identify factors independently associated with worse DSS, but that could be because we had a limited sample size (n=17), as even recurrent thyroid cancer rarely leads to death, despite often leading to persistent disease. Low numbers in the analysis

of subgroups and interactions between factors often lead to lack of significance in multivariate analysis, a reality which is difficult to avoid.

All the patients who died due to disease had documented distant metastasis; capsular invasion; AJCC pathological N1 stage and absence of NRAS mutations, discoveries that support the adverse prognostic value of all the parameters, except the absence of NRAS mutations, which could be found at various steps of tumorigenesis leading to thyroid cancer [21].

In the evaluation of DHGTC group we verified that recurrent DHGTC is more frequent in older ages (≥55 years) and younger ages (≤18 years), in comparison to non-HG-DTC, which justifies the 8th Edition of AJCC's change in the age cutoff for risk stratification of DTC from 45 to 55 years old. Our results also highlight the higher prevalence of DHGTC in the pediatric group. Pediatric patients are not considered in the 8th Edition of AJCC's risk stratification system, due to the fact that more often than not, pediatric tumors behave favorably. In our series, one of these patients developed RAIR. We suggest more studies should be done to evaluate pediatric thyroid cancer patients, despite their rarity, as we found cases with marked aggressivity.

DHGTC cases were also significantly associated with distant metastasis; lung metastasis; synchronous metastasis; higher tumor size, on average and in the range of >2 cm; lymphovascular invasion; high AJCC 8th edition pT stage and persistence of disease at the end of follow-up. All the DHGTC cases had capsular invasion, a finding which also verifies the adverse prognostic value of that parameter. These observations indicate these patients' higher risk for progression and strengthen the DHGTC classification, highlighting the need for tight surveillance in these forms of cancer. Of note, within the comparison between DHGTC and non-HG-DTC, the two cases with brain or kidney distant metastasis were classified as DHGTC.

In accordance with their increased aggressiveness, patients with DHGTC were submitted to more additional therapies, more administrations of additional RAI and, consequently, a higher cumulative dose of RAI. This indicates that these patients are more resistant to conventional therapies, and have a higher risk to develop other features, such as distant metastasis during follow-up. It's worth mentioning that only two DHGTC cases were indicated for treatment with TKI, and perhaps if poor prognosis features were found earlier, more could benefit from that.

We think that DHGTC classification would contribute to the recognition of these tumors right after the first surgery, so they are indicated for earlier and more effective treatments, leading to better outcomes.

In the univariate survival analysis restricted to DHGTC, microscopic ETE and infiltrative capsule status were adverse factors in DFS, but in the subsequent multivariate analysis, no independent adverse clinicopathological factors were found.

All the DHGTC cases who died due to disease were categorized as over 55 years old; weren't submitted to therapy with TKI; had documented metastasis; lymph node metastasis; AJCC pathological T3/T4 stage and didn't have TERT promoter mutations. This also validates the adverse prognostic factors forementioned, except for the absence of TERT promoter mutations, a genetic alteration that is associated with progressive tumors [25]. The fact that none of the deadly DHGTC cases was submitted to therapy with TKI could be because the recurrences were found so late that they progressed to be untreatable. We cannot exclude the possibility that these therapies have not yet been available, since our study includes cases diagnosed over the last few decades.

The number of cases that developed RAIR (n=12), despite low, is quite significant, as the estimated incidence of these forms of cancer is of 4-5 cases/year/million people [14]. The most frequent diagnosis in RAIR thyroid cancer is PDTC, coherent with these tumors documented poor prognosis. All the RAIR cases present distant metastasis, and they represent some of the most aggressive forms in our series (with brain and kidney metastases). They also often display other adverse clinicopathological features (capsular invasion; lymphovascular invasion; microscopic extrathyroidal extension; infiltrative and invasive capsule status; high AJCC pT stage; BRAF V600E mutations; TERT promoter mutations, lymph node metastasis; synchronous metastasis; etc). We could not perform survival analysis or compare DHGTC-RAIR to non-HG-DTC- RAIR cases, as the figures were too low for statistical analysis. More studies should be done to properly classify RAIR cases and define their real incidence, as right now it is only estimated, because the criteria to define RAIR thyroid cancer are not fully established.

5. Conclusions

In summary, this retrospective study assessed the clinicopathological features and outcomes of a series of recurrent thyroid cancer in light of the reclassification as DHGTC.

We found that the DHGTC fulfilling the 5th WHO criteria revealed to be more aggressive since DHGTC cases were significantly associated to several adverse clinicopathological parameters, such as higher tumor size, presence of distant metastasis, synchronous metastasis, microscopic ETE, tall-cell subtype of PTC, lymphovascular invasion, high AJCC 8th edition pT stage, persistence of disease at the end of follow-up, among others.

This is of value because it can assist in clinical decision making, namely in patients' selection to be submitted to more aggressive therapies in order to prevent recurrences, use of targeted therapies or suggest recommendations of tighter follow-up intervals in patients diagnosed with DHGTC.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Clinicopathologic characteristics of 138 patients with primarily resected RAIR thyroid cancer; Figure S1: Kaplan–Meier curves for DSS and DFS in the whole population; Figure S2: Kaplan–Meier curves for DFS and DSS within the DHGTC subgroup

Author Contributions: Conceptualization, S.C. and P.S.; methodology, M.C.; software, M.C.; validation, M.C., S.C. and P.S.; formal analysis, M.C.; investigation, M.C.; resources, M.M.; data curation, M.C.; writing—original draft preparation, M.C.; writing—review and editing, M.S., S.C. and P.C.; visualization, S.C. and P.S.; supervision, P.S.; project administration, P.S.; funding acquisition, P.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received funding for SC, in the framework of a Ph.D. grant (SFRH/BD/147650/2019) supported by Portuguese funds through Fundação para a Ciência e a Tecnologia (FCT). This study is part of the project "Institute for Research and Innovation in Health Sciences" (UID/BIM/04293/2019) and the project "The Porto Comprehensive Cancer Center" ref. NORTE-01-0145-FEDER-072678—Consórcio PORTO.CCC—Porto. Comprehensive Cancer Center Raquel Seruca.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Faculty of Medicine of the University of Coimbra (protocol code 1309).

Informed Consent Statement: Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: Data is not publicly available due to privacy and ethical restrictions.

Acknowledgments: The authors wish to acknowledge Sofia Macedo [PhD student at Instituto de Investigação e Inovação em Saúde (i3S) and Institute of Molecular Pathology and Immunology of

the University of Porto (Ipatimup)] for sharing her invaluable knowledge and contribution in immunohistochemistry staining of this selection of cases, which wasn't included in the study, due to suboptimal results. The authors would also like to acknowledge all the members of the Cancer Signaling and Metabolism research group at IPATIMUP/i3S for their input on different topics of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

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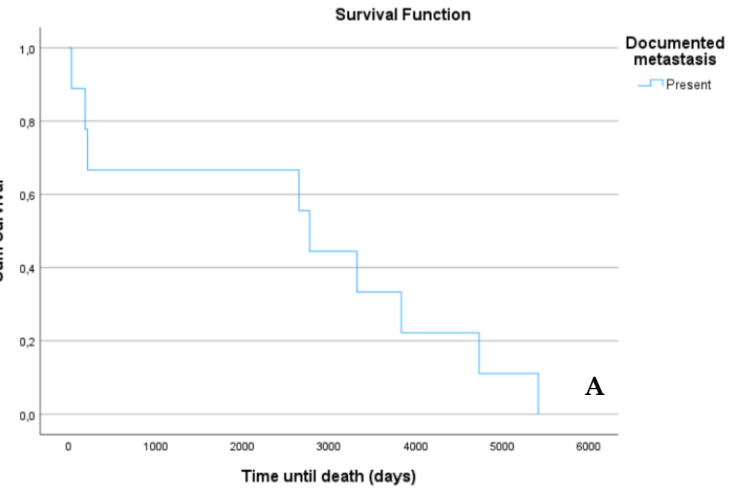
Supplementary materials:

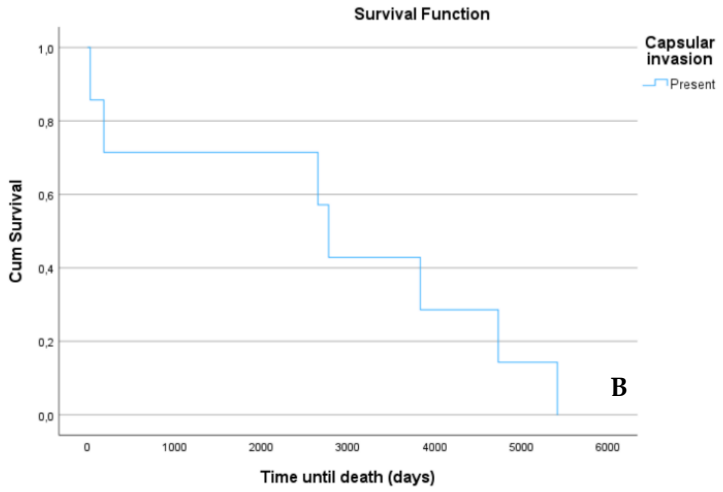
Table S1. Clinicopathologic characteristics of 138 patients with primarily resected RAIR thyroid cancer

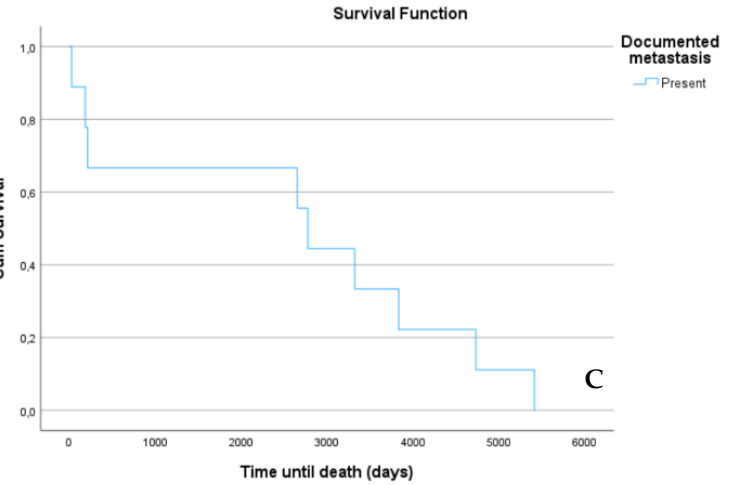
	All [n=12]	**DHGTC [n=3]**	**Non-HG-DTC [n=5]**
Clinicopathological parameters			
Age (years)			
Median (range)	47,5 (15-74)	44 (15-45)	63 (44-74)
≤18	1 (8,3%)	1 (33,3%)	0 (0%)
18-55	7 (58,3%)	2 (66,7%)	2 (40%
≥55	4 (33,3%)	0 (0%)	3 (60%)
Sex			
Female	10 (83,%)	3 (100%)	4 (80%)
Male	2 (16,7%)	0 (0%)	1 (20%)
Documented metastasis	12 (100%)	3 (100%)	5 (100%)
Distant metastasis	12 (100%)	3 (100%)	5 (100%)
Types of distant metastasis			
Bone	4 (33,3%)	0 (0%)	3 (60%)
Lung	9 (75%)	1 (33,3%)	1 (20%)
Brain	1 (8,3%)	1 (33,3%)	0 (0%)
Kidney	1 (8,3%)	1 (33,3%)	0 (0%)
Lymph node metastasis	7 (58,3%)	1 (33,3%)	4 (80%)
Synchronous metastasis	10 (83,3%)	3 (100%)	3 (60%)
Tumor size (cms) [n=9]			
Median (range)	2,5 (1,5-8)	3,25 (2,5-4)	2,00 (1,5-2,8)
≤2 cm	3 (25%)	0 (0%)	3 (60%)
>2 cm	6 (75%)	3 (100%)	2 (40%)
Main diagnosis			
Classical subtype of PTC	2 (16,7%)	1 (33,3%)	1 (20%)
Infiltrative follicular subtype of PTC	2 (16,7%)	1 (33,3%)	1 (20%)
Oncocytic subtype of PTC	1 (8,3%)	0 (0%)	1 (20%)
Warthin-like subtype of PTC	1 (8,3%)	0 (0%)	1 (20%)
Oncocytic carcinoma of the thyroid	1 (8,3%)	1 (33,3%)	0 (0%)
Follicular thyroid carcinoma	1 (8,3%)	0 (0%)	1 (20%)
Poorly differentiated thyroid carcinoma	4 (33,3%)	NA	NA
Mitotic index (per 2 mm^2)			
Median (range)	4,5 (1-8)	6 (5-6)	2 (1-3)
<5	6 (50%)	0 (0%)	5 (100%)
≥5	6 (50%)	3 (100%)	0 (0%)
Tumor necrosis [n=11]			
Present	2 (16,7%)	0 (0%)	0 (0%)
Absent	9 (81,8%)	3 (100%)	5 (100%)

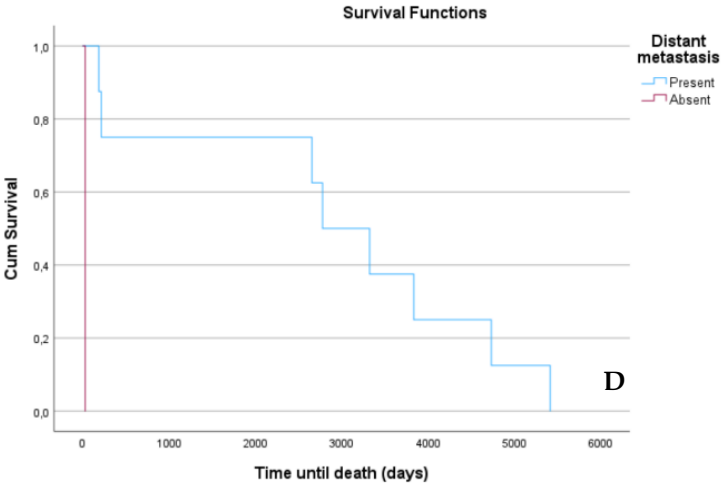
Encapsulation [n=11]			
Encapsulated	9 (81,8%)	3 (100%)	4 (80%)
Partially encapsulated	2 (18,2%)	0 (0%)	1 (20%)
Capsular invasion [n=11]			
Present	11 (100%)	3 (100%)	5 (100%)
Lymphovascular invasion [n=11]			
Present	8 (72,7%)	3 (100%)	2 (40%)
Absent	3 (27,3)	0 (0%)	3 (60%)
Microscopic extrathyroidal extension [n=11]			
Present	7 (63,6%)	2 (66,7%)	3 (60%)
Fibroadipose tissue	6 (54,5%)	2 (66,7%)	3 (60%)
Skeletal muscle	1 (9,1%)	1 (33,3%)	1 (20%)
Unspecified	1 (9,1%)	NA	NA
Capsule status [n=11]			
Infiltrative	8 (72,7%)	2 (66,7%)	4 (80%)
Invasive	3 (27,3%)	1 (33,3%)	1 (20%)
AJCC 8th pT stage [n=11]			
pT1/T2	3 (27,3%)	0 (0%)	2 (40%)
pT3/T4	8 (72,7%)	3 (100%)	3 (60%)
AJCC 8th pN stage			
pN0/Nx	8 (66,7%)	3 (100%)	3 (60%)
pN1a/N1b	4 (33,3%)	0 (0%)	2 (40%)
Molecular alterations			
BRAF V600E mutated [n=11]	6 (54,5%)	0 (0%)	3 (60%)
NRAS Q61R mutated [n=11]	0 (0%)	0 (0%)	0 (0%)
TERT promoter mutated	4 (33,3%)	2 (66,7%)	1 (20%)
-124G>A mutation	2 (16,7%)	1 (33,3%)	1 (20%)
-146G>A mutation	2 (16,7%)	1 (33,3%)	0 (0%)
Follow-up and treatments			
Median follow-up time in years (range)	10 (2,60-22,7)	3,53 (2,60-22,7)	9,97 (6,35-12,76)
Median time until the first recurrence in months (range)	10,2 (0,83-88,9)	9,3 (0,83-43,5)	9,10 (7,37-21-60)
Outcome at last observation			
Death due to disease	3 (25%)	0 (0%)	2 (40%)
Alive with disease	8 (66,7%)	3 (100%)	2 (40%)
Alive without disease	1 (8,3%)	0 (0%)	1 (20%)
Number of recurrences [n=11]			
1	11 (100%)	2 (100%)	5 (100%)
Additional therapies			
None	0 (0%)	0 (0%)	0 (0%)
Radioactive iodine therapy (RAI)	12 (100%)	3 (100%)	5 (100%)

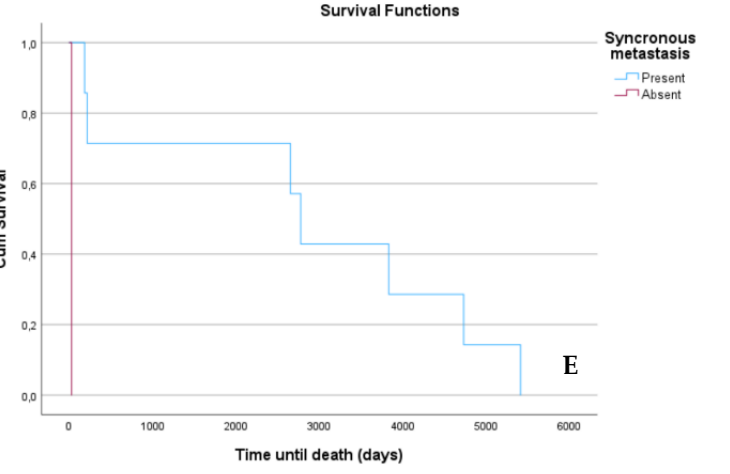
Radiation therapy	0 (0%)	0 (0%)	0 (0%)
Tyrosine kinase inhibitors	2 (16,7%)	2 (66,7%)	0 (0%)
Surgery	11 (91,7%)	3 (100%)	4 (80%)
Number of additional RAI administrations			
1	1 (8,3%)	0 (0%)	1 (20%)
2	2 (16,7%)	1 (33,3%)	0 (0%)
3	5 (41,7%)	2 (66,7%)	3 (60%)
4	4 (33,3%)	0 (0%)	1 (20%)
Median cumulative dose of RAI in millicuries [mCi] (range)	728,5 (241-1094)	501 (498-659)	812 (241-1094)

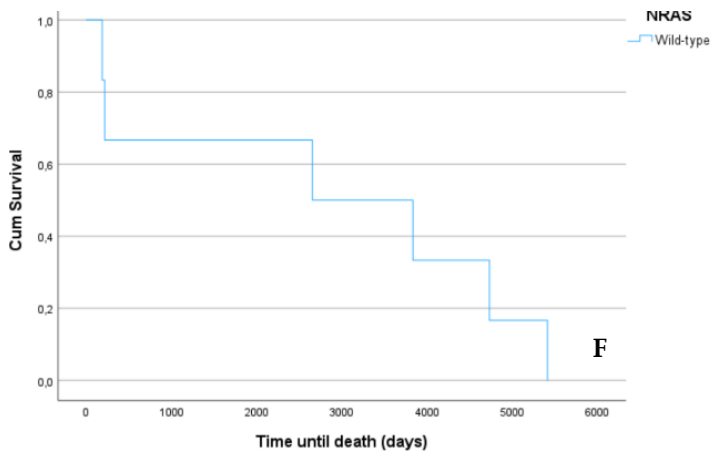


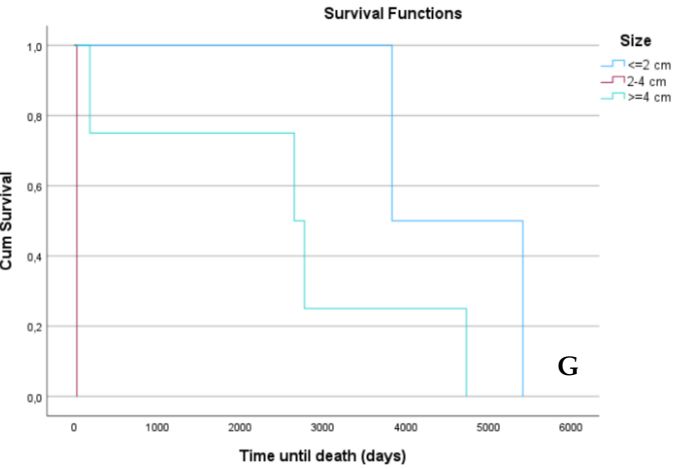












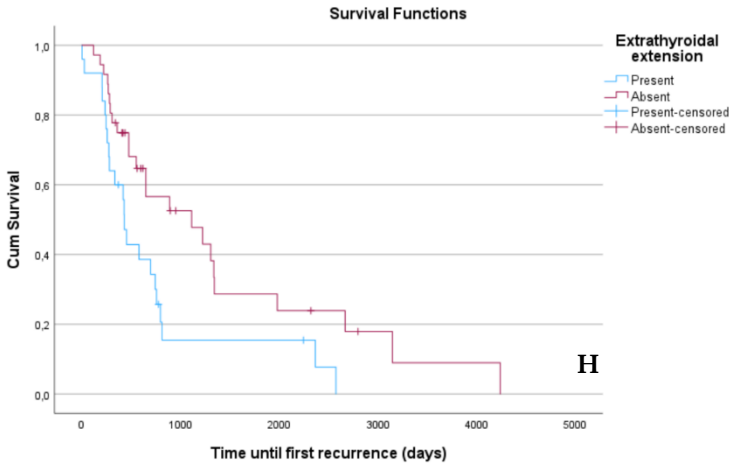
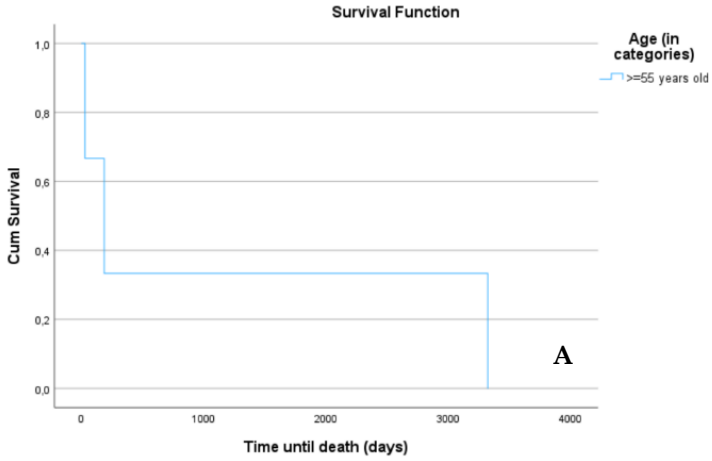
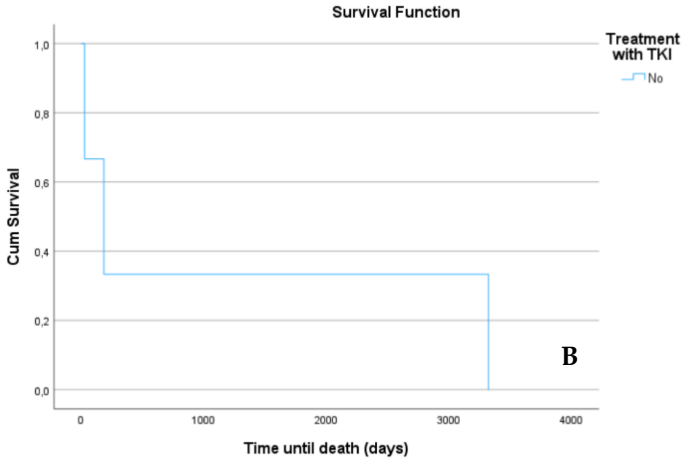
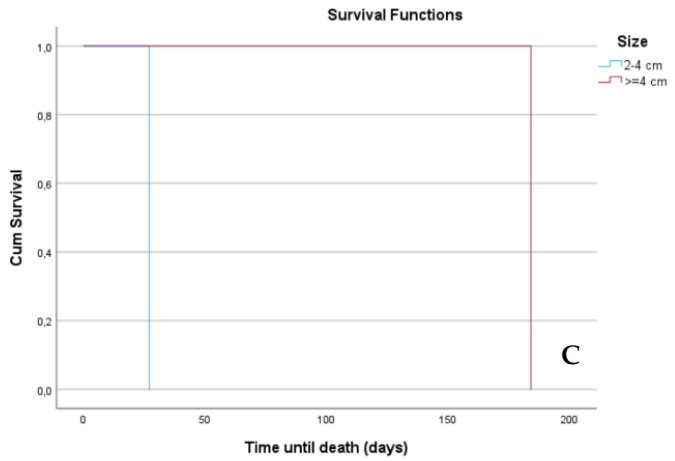
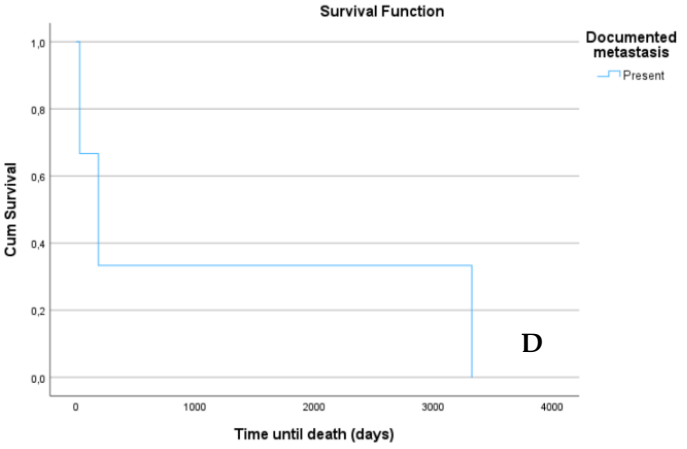


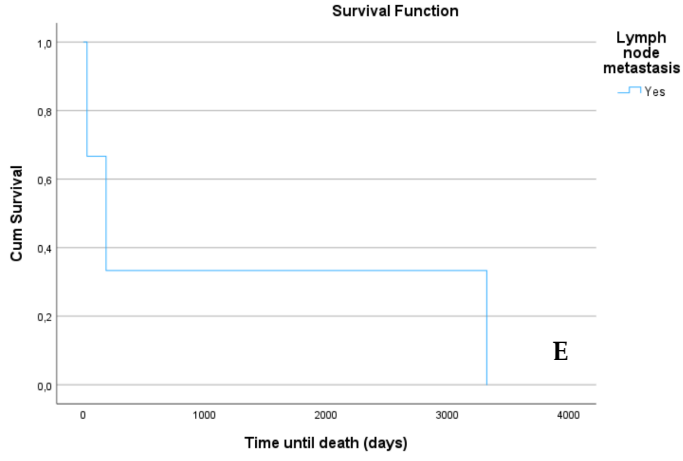
Figure S1: Kaplan–Meier curves for DSS **(A-G)** and DFS **(H)** in the whole population

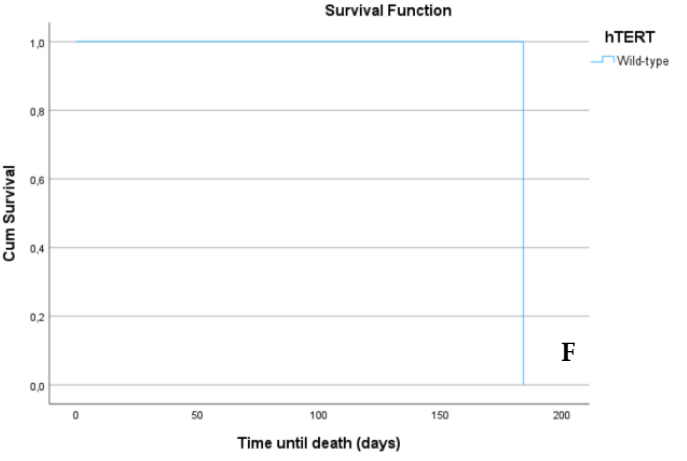












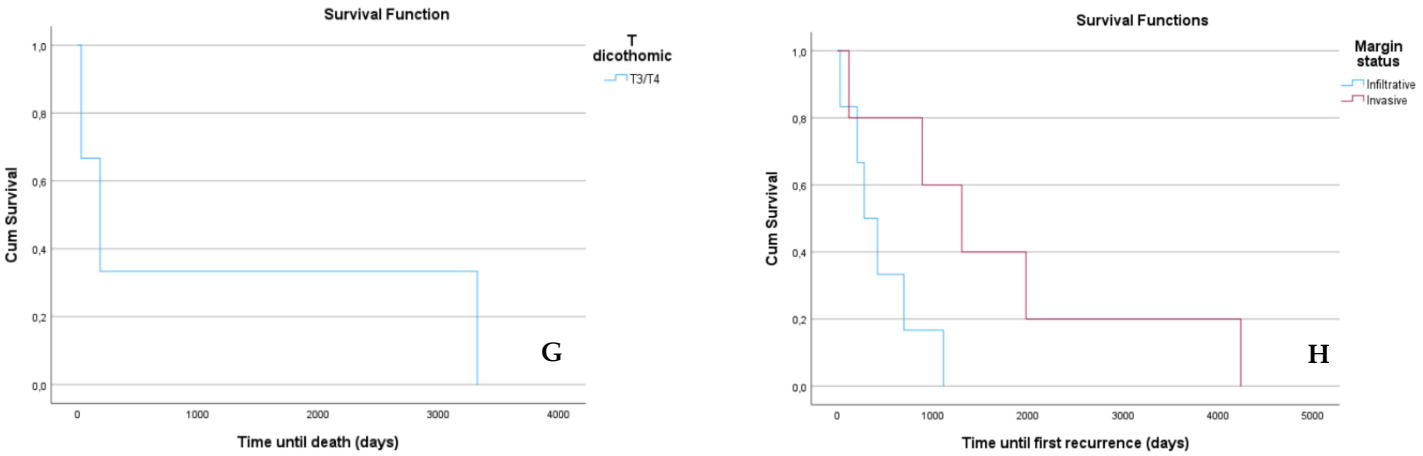


Figure S2: Kaplan–Meier curves for DSS **(A-G)** and DFS **(H)** within the DHGTC subgroup

REPORTING GUIDELINES- CHECKLIST

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	3
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	5
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	5
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	5-8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	5-8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6
Discussion			
Key results	18	Summarise key results with reference to study objectives	12-14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

REPORTING GUIDELINES- EXAMPLES

1- "We gathered several clinicopathological characteristics of a retrospective cohort of 138 patients 25 with primarily resected thyroid cancer which recurred after total thyroidectomy and adjuvant radioiodine therapy. We also reclassified them as DHGTC according to 5th WHO's criteria; compared 27 DHGTC to non-high grade differentiated follicular cell-derived thyroid carcinomas (non-HG-DTC) and described the clinical behavior and pathological features of thyroid cancer with radioactive iodine refractoriness."
2- "Histologic classification of follicular cell-derived thyroid cancer has been made according to tissue and cellular morphology, establishing a spectrum that goes from differentiated follicular cell-derived thyroid carcinomas (DFCDTC) [papillary (PTC); follicular (FTC) and oncocytic (OCA)], to the undifferentiated form, anaplastic carcinoma (ATC)."
3- "The aims of the study were: (i) to describe the clinical behavior and pathological features of the cohort; (ii) evaluate the frequency of DHGTC and its clinical behavior in this series; (iii) evaluate the clinicopathological differences between DHGTC and non-HG-DTC and (iv) describe the clinical behavior and pathological features of cases with RAIR."
4- "In this study we gathered a retrospective cohort of 138 patients with resected thyroid cancer which recurred after total thyroidectomy and adjuvant RAI, of which a significant proportion

developed RAIR. The tumor series was reclassified according to the 5th edition of WHO's criteria and several clinicopathological characteristics were collected."

5- "Our study included a series of 138 formalin-fixed, paraffin embedded resection samples of human thyroid tissue from patients followed in Centro Hospitalar e Universitário de Coimbra, kept at the biobank of the Institute of Molecular Pathology and Immunology of the University of Porto. The samples of the patients were collected upon written informed consent of the patients, or their guardians, in the case of minors. (…) The patients were diagnosed between 1971 and 2010 with thyroid cancer and underwent total thyroidectomy and adjuvant therapy with radioactive iodine (131I) as an initial approach."

6- "The patients were diagnosed between 1971 and 2010 with thyroid cancer and underwent total thyroidectomy and adjuvant therapy with radioactive iodine (131I) as an initial approach."

7- "Follow-up data was available for 133 patients. The outcomes collected included disease-free survival (DFS), disease specific survival (DSS) and persistence of disease at the end of follow-up. The follow-up was calculated from the date of primary resection. DFS was defined as the time from primary resection to cancer recurrence, DSS as 158 the time from primary resection to death due to thyroid cancer and persistence of dis-159 ease was documented as proven structural disease."

8- "The clinicopathological data was collected from clinical records, with the approval of the Ethic Committee of the Faculty of Medicine of the University of Coimbra (n° 1309) and all the procedures described in this study were done in accordance with national ethical standards (Law n° 12/2005) and Helsinki declaration."

9- The series is intentionally biased by the selection of cases with recurrence/poorer prognosis.

10- The study size was determined by the number of cases available.

11- "In particular, DHGTC was classified according to the following criteria: mitotic 139 count ≥5/2 mm2 [6 HPFs in the microscope used- fields of 0,34 mm2] and/or tumor necro-140 sis. The mitotic count was determined within the most mitotically active area (the so-141 called 'hotspot')."

12- "All statistical analyses were performed with SPSS 29.0 (IBM Corporation, Armonk, NY, USA). Comparisons of clinicopathological features between subgroups were performed with the chi-square test or Fisher's exact test for categorical variables, and with Mann-Whitney U test for continuous variables. Univariate survival analysis was performed with the log rank test for categorical variables and with the Cox proportional hazards model for continuous variables. Factors that were significant in univariate analysis were subsequently subjected to multivariate analysis with the Cox proportional hazards model. P-values of <0,05 were considered to be statistically significant."

13- "Follow-up data was available for 133 patients. The outcomes collected included disease-free survival (DFS), disease specific survival (DSS) and persistence of disease at the end of follow-up. The follow-up was calculated from the date of primary resection."

14- The clinical and pathological features of the entire cohort are summarized in Table 1. The median age was 45 years (range, 13–81 years). There was a female 173 predominance, with a female/male ratio of 3:1. The median tumor size was 2,65 cm 174 (range, 0,5-9 cm). In terms of diagnosis, 32 cases (23,2%) presented as HGFCDTC, of which 22 (15,9%) were diagnosed as DHGTC and 10 (7,2%) as PDTC.

15- "Documented metastasis was frequent, observed in 85 patients (61,6%), and 36 of them (42,3%) had distant metastasis. The most common sites of distant metastases were lung (n=26) and bone (n=13). Lymph node metastasis was present in 67 cases (49,6%) and 27 (21,3%) had synchronous metastasis (defined as metastasis identified within 6 months after the diagnosis of the primary cancer [17]). Seventeen (12,8%) patients died due to disease during follow-up, of which 4 (23,5%) were PDTC, 3 DHGTC (17,6%) and 11 (64,7%) non-HG-DTC. All the patients who died due to disease had documented metastasis; capsular invasion; AJCC pathological N1 stage and absence of NRAS mutations."

16- There were no confounder-adjusted estimates in our study.
17- "DHGTC cases, as compared with non-HG-DTC patients, were significantly associated with the age ranges of ≤18 and ≥55 years old; higher tumor size, on average and in the range of >2 cm; tall-cell subtype of PTC; mitotic index ≥5; tumor necrosis; lymphovascular invasion; presence of distant metastasis; lung metastasis; synchronous metastasis; high AJCC 8th edition pT stage; submission to additional therapies; treatment with TKI; more administrations of additional RAI; higher median cumulative dose of RAI and persistence of disease at the end of follow-up, ($P < 0.05$; Table 1)."
18- "We found that the prevalence of DHGTC in a series of recurrent thyroid cancer is higher than what is described in studies without prior clinical selection (17,2% vs 7,2%) [19]. Curiously, the same is not true in relation to PDTC. Its prevalence (7,2%) is within the reported prevalence of 2-15% [20] in unselected series. This result suggests that DHGTC category encloses cases that might have a higher tendency to recur, recognizing DHGTC as an intermediate category in terms of prognostic outcomes."
19- "We must stress that we are evaluating a series of recurrent tumors and these findings should be verified in more representative series."
20- "In accordance with their increased aggressiveness, patients with DHGTC were submitted to more additional therapies, more administrations of additional RAI and, consequently, a higher cumulative dose of RAI. This indicates that these patients are more resistant to conventional therapies, and have a higher risk to develop other features, such as distant metastasis during follow-up. It's worth mentioning that only two DHGTC cases were indicated for treatment with TKI, and perhaps if poor prognosis features were found earlier, more could benefit from that."
21- "We found that the DHGTC fulfilling the 5th WHO criteria revealed to be more aggressive since DHGTC cases were significantly associated to several adverse clinicopathological parameters, such as higher tumor size, presence of distant metastasis, synchronous metastasis, microscopic ETE, tall-cell subtype of PTC, lymphovascular invasion, high AJCC 8th edition pT stage, persistence of disease at the end of follow-up, among others. This is of value because it can assist in clinical decision making, namely in patients' selection to be submitted to more aggressive therapies in order to prevent recurrences, use of targeted therapies or suggest recommendations of tighter follow-up intervals in patients diagnosed with DHGTC."
22- "This research received funding for SC, in the framework of a Ph.D. grant (SFRH/BD/147650/2019) supported by Portuguese funds through Fundação para a Ciência e Tecnologia (FCT). This study is part of the project "Institute for Research and Innovation in Health Sciences" (UID/BIM/04293/2019) and the project "The Porto Comprehensive Cancer Center" ref. NORTE-01-0145-FEDER-072678—Consórcio PORTO.CCC—Porto. Comprehensive Cancer Center Raquel Seruca."

Diagnostics (MDPI) Author guidelines

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- *Protocol:* Protocols provide a detailed step-by-step description of a method. They should be proven to be robust and reproducible and should accompany a previously published article that uses this method. Any materials and equipment used should be explicitly listed. Conditions, quantities, concentrations, etc., should be given. Critical timepoints and steps, as well as warnings, should be emphasized in the text. The structure should include an Abstract, Keywords, Introduction, Experimental Design, Materials and Equipment, Detailed Procedure, and Expected Results, with a suggested minimum word count of 4000 words.
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- **Research manuscripts** should comprise:
  - **Front matter**: Title, Author list, Affiliations, Abstract, Keywords.
  - **Research manuscript sections**: Introduction, Materials and Methods, Results, Discussion, Conclusions (optional).
  - **Back matter**: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, **References**.

Front Matter

These sections should appear in all manuscript types

- **Title:** The title of your manuscript should be concise, specific and relevant. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used. Please do not include abbreviated or short forms of the title, such as a running title or head. These will be removed by our Editorial Office.
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- **Abstract:** The abstract should be a total of about 200 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts, but without headings: 1) Background: Place the question addressed in a broad context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used; 3) Results: Summarize the article's main findings; and 4) Conclusion: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article: it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.
- **Keywords:** Three to ten pertinent keywords need to be added after the abstract. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

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- **Introduction:** The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance, including specific hypotheses being tested. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the main conclusions. Keep the introduction comprehensible to scientists working outside the topic of the paper.
- **Materials and Methods:** They should be described with sufficient detail to allow others to replicate and build on published results. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited. Give the name and version of any software used and make clear whether computer code used is available. Include any pre-registration codes.
- **Results:** Provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.
- **Discussion:** Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible and limitations of the work highlighted. Future research directions may also be mentioned. This section may be combined with Results.
- **Conclusions:** This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.
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Back Matter

- **Supplementary Materials:** Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.
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Research Ethics

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