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External Validation of Three Available Grading Systems for Medullary Thyroid Carcinoma in a Single Institution Cohort

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Abstract

Medullary thyroid carcinoma (MTC) is a rare thyroid carcinoma with a variable clinical behavior. Potential clinical and pathological prognostic markers have been investigated, but studies are limited and controversial. In neuroendocrine neoplasms of various other sites, necrosis and proliferation (mitotic activity and/or Ki67 index) are integrated to provide a histological grade. Recently, an International Medullary Thyroid Carcinoma Grading System (IMTCGS) has been designed to define high- or low-grade MTC by combining proliferative activity and necrosis. This proposal integrates two previously published grading schemes by American (2-tiered grading, low- and high-grade MTC) and Australian authors (3-tiered grading, low-, intermediate-, and high-grade MTC). To validate the clinical role of these systems, their prognostic impact was evaluated in an independent cohort of 111 MTCs. Necrosis, which was the only parameter integrated into the 3 grading systems, proved to be individually correlate with tumor relapse, while no association was found with the proliferation (mitotic count and Ki67 index); however, by combining the different parameters according to all three grading systems, "high-grade" MTCs turned out to be significantly associated with the disease recurrence ($p < 0.005$) in all systems. In disease-free survival analysis, the IMTCGS stratification was the only one that demonstrated a significant impact at Cox regression analysis ($p = 0.004$), further confirmed by the Kaplan–Meier curves ($p = 0.002$). Similar findings were also reproduced when analysis was restricted to sporadic MTCs (68 cases). In conclusion, our results confirm the prognostic role of IMTCGS, supporting the importance of incorporating this information into

the pathology report. However, none of the systems proved to predict the overall survival in this validation cohort.

Introduction

Medullary thyroid carcinoma (MTC) is a rare epithelial neuroendocrine neoplasm originating from parafollicular calcitonin-producing C-cells of the thyroid, showing an incidence of 0.11 per 100,000. About 25% of cases show a hereditary transmission as part of multiple endocrine neoplasia (MEN)2A or MEN2B syndromes. Almost all the hereditary MTCs are associated with a mutation of the RET gene and share some distinctive clinicopathological features, being generally smaller, more frequently multifocal, and usually diagnosed in younger patients [1, 2]. RET somatic mutations are nonetheless present in about 50% of the sporadic forms.

It is generally considered an aggressive disease when compared with other thyroid tumors, although its clinical course may be variable, with long-term survivors and patients that rapidly relapse and progress to a fatal outcome [3]. Therefore, clinicians are constantly looking for prognostic markers that may help to identify high risk patients who may benefit of a more intensive treatment. Age, male sex, extrathyroidal extension, the presence of familial disease, and somatic RET and RAS mutations have been shown to impact survival in univariate analysis, but only age and stage resist in multivariate analysis [4,5,6,7,8]. Preoperative serum levels of calcitonin [9] and CEA [10] are routinely evaluated for prognostic purposes, but recently other circulating markers, such as serum levels of miR-375, have been under investigation [11]. Other studies have also explored the prognostic role of morphological, cytological, and architectural patterns, but the assessment of these parameters was biased by a high degree of subjectivity and led to contrasting results [1, 12].

So far, several investigators have assessed the value of necrosis and proliferation markers as prognostic tools in MTC. These factors are of particular importance in neuroendocrine neoplasms of other locations, and grading systems based on proliferation and necrosis (with some differences depending on the site of the neoplasm) are widely established in the routine neuroendocrine pathology [13,14,15]. However, in MTC, the usefulness of necrosis, mitotic rate, and Ki67 showed conflicting results [16,17,18,19,20,21,22].

Until recent years, a formal grading system was not described for MTCs, unlike other neuroendocrine neoplasms, in which grading generally relies on necrosis and proliferative activity (mitotic index and/or Ki67 index).

Considering this background, in 2020, two different groups independently developed and validated distinct but comparable MTC grading systems. Fuchs et al. from the Royal North Shore Hospital, Sydney, Australia, evaluated 76 cases and proposed a three-tiered scheme based on mitotic count (< 3/2 mm2, 3–20/2 mm2, > 20/2 mm2), Ki67 proliferative index (< 3%, 3–20%, > 20%), and necrosis (present/absent) (Sydney grading system) [23]. The second study, by Alzumaili et al. from the Memorial Sloan Kettering Cancer Center (MSKCC), New York, USA, separated their 144 MTC patients into 2 groups (low grade and high grade) combining only mitosis (< 5/10 high power fields (HPF), ≥ 5/10 HPF) and necrosis (present/absent) (MSKCC grading system) [24]. The Sydney grading system showed a significant stratification of patients according to the overall survival (OS), and the MSKCC grading system proved to be a predictor of disease

specific survival, local recurrence free survival, and distant metastasis free survival. About 1 year later, a multi-institutional study, including members of the two groups from New York and Sydney, examined several morphological criteria (including those of the two already published studies) and eventually selected a two-tier grading scheme that confirmed the relevance of the previously selected parameters (proliferation activity and necrosis) with a redefinition of the cut-offs [25]. According to this International Medullary Thyroid Carcinoma Grading System (IMTCGS), low-grade tumors are defined by < 5 mitoses/2 mm2, Ki67 proliferative index < 5%, and absence of tumor necrosis, whereas tumors with at least one criterion among ≥ 5 mitoses/2 mm2, Ki67 proliferative index ≥ 5%, or tumor necrosis are classified as high grade. The three grading systems are summarized in Table 1.

The aim of the present study is to validate the prognostic role of all three proposed grading systems in an independent MTC cohort collected from the two academic hospitals of the University of Turin, Italy.

Material and Methods

Patients

The study population was extracted from the files of the Pathology units of two academic hospitals, "Città della Salute e della Scienza" Hospital and "San Luigi Gonzaga" Hospital, both affiliated to the University of Turin. Inclusion criteria were a diagnosis of MTC occurred between 1st January 1986 and 1st June 2015 and the availability of material from the primary tumor, thus excluding patients with metastatic samples only. Pathological and clinical data have been collected from pathology reports and medical charts.

The study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and following the guidelines and regulations defined by the Research Ethics Committee of the University of Turin. Considering the retrospective nature of the study and that there was no impact on patient care, a specific written informed consent was not required. All cases were anonymized by a pathology staff member not involved in this project, and only coded data were used.

Histopathological Parameters

All cases have been revised by three senior pathologists with an experience in endocrine pathology (F.M., M.V., and M.P.). Diagnosis of MTC was confirmed for all the cases. Multifocal disease has been defined as the presence of more than one pathologic focus within the thyroid gland, either in the same lobe or the contralateral lobe. The mitotic rate has been reported as the number of mitoses per 2 mm2 (generally equivalent to 10 high power field in most microscopes) and the count have been performed in the area with the highest proliferative activity ("hot spot area") [25]. Tumor necrosis has been defined by the presence of degenerating cytoplasm and punctate karyorrhectic nuclear debris and has been reported as present/absent, irrespective of its extension. As suggested in a previous series [25], infarct-like necrosis ascribable to preoperative procedures (e.g., fine-needle aspiration, core biopsy) was not considered.

Ki67 Immunohistochemistry

Tissue sections were immunostained using an automated platform (Ventana BenchMark AutoStainer, Ventana Medical Systems, Tucson, AZ, USA) with anti-Ki67 mouse monoclonal antibody (Clone 30–9, Ventana Medical System). Ki67 proliferation index was assessed as the percentage of cells with a weak to strong staining confined to the nuclei. A range of 500–2000 cells per tumor has been counted.

Grading

For each sample, tumor grade was calculated according to the 3 grading systems recently proposed [23,24,25] and summarized in Table 1.

Statistical Analyses

Statistical analyses were carried out using Stata 15.0 software (StataCorp, College Station, TX, USA.). The differences in the distribution of the variables evaluated based on clinical-pathological parameters were analyzed using parametric and non-parametric tests (Student's t test, Pearson's chi-square test and Bonferroni's correction, Wilcoxon's rank test). Time to relapse (disease-free survival (DFS)) was assessed from the date of diagnosis to the date of relapse or the date of the last checkup. OS was calculated from the date of diagnosis to the date of death or last check-up. All dead patients were considered as events. Survival curves were determined by the Kaplan–Meier method, and Mantel log-rank test was used to compare statistical differences. Cox proportional hazards regression univariate analysis to calculate HRs and 95% CIs was used to assess the independent role of different variables across DFS and OS. p values < 0.05 were considered significant.

Results

Study Population

We collected 151 MTC cases from the pathology files of the University of Turin between January 1st, 1986, and June 1st, 2015, including 133 cases from the "Città della Salute e della Scienza" Hospital and 18 cases from "San Luigi Gonzaga" Hospital. Forty cases were excluded due to incomplete clinical or pathological data. For the remaining 111 patients, clinical follow-up data were available, with a median follow-up time of 113.1 months (range 62.8–160.5). Clinical and pathological features of the 111 patients are reported in Table 2.

Patients included 67 females and 44 males, with a median age at diagnosis of 57 years (range 16–82). According to the last follow-up data, 27 relapses and 23 deaths were recorded in our cohort.

Data on the familial predisposition (sporadic versus hereditary MTCs) were available for 88 patients: 20 (22.7%) were affected by a familial syndrome associated to MTC (MEN2) and 68 tumors were sporadic (77.3%).

Most cases were histologically classified as pT1 (58.6%), and the median tumor size was 15 mm. Moreover, 23 patients (20.7%) presented multifocal disease: of these, 8 had a sporadic MTC (8/68, 11.7%), and 12 had a hereditary MTC (12/20, 60%) ($p < 0.001$); for the remaining 3 patients, the genetic status was not known.

Lymph node metastases were present in 43 patients (43.4%). Considering the eight edition of the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) TNM Classification of Malignant Tumors, 47 cases were assigned to stage I, 21 as stage II, 13 as stage III, and the remaining 30 cases as stage IV. Necrosis was observed in 26 tumors (23.4%).

Mitotic figures ranged from 0 to 9 mitoses/2 mm2 with a median of 0; 7 cases only showed a mitotic count ≥ 3/2 mm2, and only in 5 cases mitoses were ≥ 5/2 mm2.

The Ki67 proliferation index was assessed only for 101 cases, since for 10 cases leftover tumor material was insufficient to perform and evaluate immunohistochemical reactions (median = 2%, range: 0–20%). Fifty-one cases presented a Ki67 ≥ 3% but moving the cut-off to ≥ 5% only 15 cases showed a high Ki67 proliferation index.

Application of the Three Grading Systems

The MSKCC grading system was assessed in tumors from 111 patients, whilst only 101 tumors were graded according to the Sydney grading system and the IMTCGS, due to the lack of Ki67 data in 10 patients. According to the two-tiered MSKCC grading system, 85 cases were assigned to a low-grade disease, and 26 had high-grade MTC. The Sydney grading system classified 49 MTCs as low grade, 34 were assigned to an intermediate grade, and 18 tumors were high grade MTCs. Finally, 69 MTCs were defined as low grade and 32 as high grade using the IMTCGS (Fig. 1).

Then, possible correlations between the three grading systems and the clinical and pathological features of our study population were investigated (Table 2). As expected, we observed an association between all three grading systems and the mitotic activity ($p < 0.001$ for all the systems). Similarly, the Ki67 proliferation index was associated with the Sydney system ($p < 0.001$) and the IMTCGS ($p < 0.001$), but also with the MSKCC grade ($p = 0.001$) that actually does not include this parameter into its algorithm. No association was found with familial genetic status.

Analysis on All Sporadic and Familial Medullary Thyroid Carcinomas

A male gender ($p = 0.017$), high pT ($p < 0.001$), high tumor dimension ($p = 0.003$), lymph node metastasis ($p = 0.001$), AJCC/UICC stage IV ($p < 0.001$), and presence of necrosis ($p = 0.003$) were more common in patients with disease recurrence. Neither the number of mitoses per 2mm2 nor the Ki67 proliferation indices were individually associated with relapse (Table 3). However, all three grading systems were significantly associated with disease recurrence.

Among the MSKCC grading system defined high-grade MTCs, the relapse rate was 46.2% (12/26) compared to 17.6% (15/85) of low-grade MTCs ($p = 0.003$). Similarly, the Sydney grading system showed higher percentage of relapses among intermediate (11/34, 32.4%) and high-grade MTCs (8/18, 44.4%) compared to the low-grade MTCs (7/49, 14.3%) ($p = 0.024$), but considering the single categories, only high grade versus low grade was consistently different ($p = 0.036$). When the IMTCGS grading system was applied, 50%

of relapses were noticed in the high grade (16/32) and only 14.5% (10/69) in the low-grade MTCs ($p < 0.001$).

Thereafter, Cox regression analysis was performed to investigate potential pathological features associated to DFS (Table 4). Higher tumor dimensions (HR = 1.03; $p = 0.003$), higher pT (HR = 2.82; $p < 0.001$), and presence of lymph node metastasis (HR = 16.8; $p < 0.001$) turned out to be associated with a lower DFS. Considering the parameters integrated in the three grading systems, higher Ki67 proliferation indices were associated with a lower DFS (HR = 1.38; $p < 0.001$); however, no association was found for mitotic activity and presence of necrosis (HR = 2.21; $p = 0.087$). No significant associations were found for the MSKCC and Sydney grading systems (MSKCC HR = 2.22, $p = 0.087$; Sydney intermediate-grade HR = 2.89, $p = 0.057$; Sidney high-grade HR = 2.67, $p = 0.121$). On the contrary, the IMTCGS-defined high-grade MTC was associated with a shorter DFS (HR = 3.97; $p = 0.004$).

In order to correct for the potential role of familial/germline pathogenic variant status on the different grading systems, DFS Cox regression analysis was repeated adjusting the standard error for 2 clusters, sporadic and hereditary cases. Cases with unknown familial predisposition were excluded. The results confirmed the ability of IMTCGS to predict DFS (HR = 3.71; $p = 0.004$), regardless of the status of familial disease (germline inheritance).

Kaplan–Meier curves were also performed for all the grading schemes considering DFS (Fig. 2). The MSKCC grades showed a trend toward a prognostic relevance, since a higher DFS for patients affected by low-grade MTCs was observed, although this result did not reach the statistical significance ($p = 0.079$). Considering the Sydney grading system, Kaplan–Meier curves still showed a better survival for low grade tumors, but intermediate- and high-grade curves did not show a considerable different trend, and overall, the differences were not significative ($p = 0.115$). On the other hand, the IMTCGS clearly showed a longer DFS for low-grade MTCs compared to high-grade MTCs ($p = 0.002$).

We also analyzed the association between the three grading systems employing Cox regression analysis, but none of the grading systems predicted the OS (Table 5).

Analysis on Sporadic Medullary Thyroid Carcinoma Subgroup

To exclude a bias due to more favorable pathological features usually associated with hereditary cases and to investigate if the previous results could be reproduced in the subgroup of sporadic MTCs, we repeated all analyses in this subset of patients (n = 68).

Disease recurrence was more frequent in sporadic MTC patients with higher pT ($p < 0.001$), lymph node metastasis ($p < 0.001$), higher AJCC/UICC TNM stage ($p < 0.001$), and presence of necrosis ($p = 0.029$). Furthermore, in this subgroup, we also found an association with tumor relapse for multifocality ($p = 0.006$) and Ki67 proliferation index ($p = 0.037$). Tumor relapses were more frequent among patients affected by high-grade MTCs according to the MSKCC system ($p = 0.029$) and IMTCGS grading scheme ($p = 0.005$); conversely, the Sydney grades did not show any association with disease recurrence.

The DFS analysis showed a significant different hazard ratio for pT ($p < 0.001$), pN ($p = 0.003$) and AJCC/UICC TNM stage ($p = 0.002$). Data obtained from the whole cohort were partially confirmed, as higher Ki67 proliferation index was associated with a shorter DFS (HR = 1.34; $p = 0.008$), but not the mitotic count or presence of necrosis. Furthermore, the IMTCGS scheme stands out as the only grading system to predict a shorter DFS in the sporadic MTCs (HR = 2.87; $p = 0.050$).

Finally, Kaplan–Meier curves were plotted (Fig. 3), and a significant difference between the curves was found only applying IMTCGS ($p = 0.041$).

As already observed in the general analyses, the OS was not correlated with any grading systems.

Discussion

Both the rarity of MTC and the heterogeneity of its clinical behavior render prediction of outcome a difficult task: in some patients, the disease may pursue a very slow clinical course with prolonged patient survival, even in the presence of distant metastases, while in others, it exhibits a much more aggressive biological behavior.

According to the most recent guidelines of the American Thyroid Association (ATA) and the European Society of Medical Oncology (ESMO), postoperative MTC risk stratification and long-term follow-up are mainly based on histopathological data, including tumor stage, the presence of lymph node, and/or distant metastases (TNM classification), and on serum concentration of two biomarkers: calcitonin and CEA [8, 26]. In the past years, many studies aimed at investigating the potential prognostic roles of additional several parameters. Among them, proliferation-related markers (mitotic count and Ki67) and necrosis showed controversial results [23, 24]; this uncertainty has led experts of the 8th edition of the American Joint Committee on Cancer (AJCC) not to incorporate these histological features in their staging system [27].

In the last 2 years, three novel grading systems [23,24,25] had been proposed in order to predict the clinical behavior of MTC by combining proliferative activity (mitotic count and/or Ki67 proliferative index) and coagulative tumor necrosis. The aim of the present study was to test the three grading systems in our case-series of MTC, in order to find how they perform in an independent cohort of MTC, with the final aim to implement the adoption of these grading schemes into routine clinical practice, in line with the recommendations for other neuroendocrine neoplasms.

In the present study, male gender ($p = 0.017$), high pT ($p < 0.001$), lymph node metastasis ($p = 0.001$), and AJCC/UICC stage IV ($p < 0.001$) were more frequent in patients with disease recurrence, in line with the previous literature findings.

Multifocality (or multiple foci) was not associated with tumor relapse in sporadic and familial MTC cases taken together, while in the subgroup of sporadic MTC, a significant association was found ($p = 0.006$). In

sporadic MTC, multifocality is likely the result of tumor spread from the main tumor rather than a sign of germline susceptibility (as in hereditary MTC); this might reflect a more aggressive behavior and explain its association with tumor relapse, which was not observed in overall (sporadic and familial) MTC series. Necrosis turned out to be a predictor of disease recurrence ($p = 0.003$) but was not significantly related to DFS at univariate analysis. Neither the number of mitoses per 2 mm2 nor the Ki67 indices were associated with relapse when analyzed separately; however, at univariate analysis, the Ki67 was significantly related to DFS: a possible explanation for the different Ki67 significance is that DFS considers the role of time to relapse. In other words, the Ki67 index might predict early relapse, rather than relapse in general.

On the contrary, by combining these parameters, we found that the three grading systems were all significantly associated with disease recurrence. However, in terms of DFS, the three grading systems did not perform all the same. Cox regression analysis for DFS showed no significant associations for the MSKCC and Sydney grading systems, although a trend might be noticed for both. On the contrary, the IMTCGS-defined high-grade disease was significantly associated with a shorter DFS (HR = 3.97; $p = 0.004$). Similar results were obtained from Kaplan–Meier curves considering DFS for the three grading schemes. Nonetheless, none of the grading systems showed correlation with the OS; this may be due to different reasons: our series (probably unlike previous ones) is not affected by a significant referal selection bias, with all patients being operated and treated in the same hospital; this may influence the prevalence of other possible factors (different percentage of comorbidities and number of "death for causes other than MTC," different chemotherapy regimen) which may alter the results obtained in OS analysis.

In order to test whether the hereditary (germline pathogenic variants)/sporadic status may affect the performance of the three grading systems, a separate analysis on sporadic versus hereditary MTC was performed. The results indicated that there were no differences in the distribution of disease recurrences according to the germline inheritance/familial status. If Cox regression analysis was performed adjusting the standard error in the two subgroups (hereditary and sporadic), the IMTCGS stands out as the only grading scheme that identified subgroups of patients with a significantly different DFS (HR = 3.71; $p = 0.004$). Furthermore, the prognostic relevance of the IMTCGS was confirmed in the subgroup of sporadic cases, only.

Therefore, our results show that the two-tiered IMTCGS [25] performs better in our retrospective case-series of MTC than the other two grading schemes [23, 24]. Our data also support previous findings [25] that a three-tiered grading system is weaker than a two-tiered scheme. When applying the Sydney grading, statistical significance was found only if high-grade MTCs were compared to low-grade MTCs, while the intermediate grade had no prognostic significance. Moreover, none of the patients in our cohort showed a high proliferation activity (Ki67 > 20%, mitosis > 20/2 mm2) similarly to the cohorts analyzed by the Sydney team and the IMTCGS team, confirming that this condition is very rare and is of less clinical significance [23, 25]. Finally, a two-tiered grading system is simpler and more immediate to apply than a three-tiered grouping.

To our knowledge, this is the first European/Italian series which compares the three grading systems on an independent set of MTCs demonstrating the superiority of IMTCGS in predicting clinical behavior of MTC, irrespective of the hereditary predisposition status. We suggest the implementation of the IMTCGS scheme in routine clinical practice: one of the main advantages of this grading scheme is that both mitoses and

tumor necrosis are relatively well-defined and objective histologic features, less prone to interobserver variability than other parameters (for example, vascular invasion or cell morphology). This grading system is easy to adopt in routine clinical practice since the three parameters are already widely used for grading other neuroendocrine neoplasms. In addition, it is easy to use and cheap and does not require the use of expensive or scarcely available molecular testing.

The identification of these parameters still requires the skills of a well-trained pathologist: mitoses should be carefully looked for in hot spots, counting unequivocal mitotic figures rather than pyknotic or degenerating nuclear fragments. Ki67 index should be assessed according to the same methods proposed for gastrointestinal neuroendocrine tumors [14]; moreover, caution should be taken in distinguishing tumor necrosis (so-called “comedo-like” necrosis) from other type of necrosis (for example, the one related to fine needle aspiration or to infarct-like degenerative phenomena) [25].

Furthermore, some issues still need to be addressed: the present grading scheme does not consider the effect of somatic RET or RAS mutation on prognosis. In that regard, data are in part conflicting: some authors have found that somatic RET mutation status correlated with a worse outcome [20], while others did not find such a relationship [28, 29]. Moura et al. found that MTC with RET mutations in exons 15 and 16 had a higher number of nodal metastasis than those with other types of RET mutations; however, no statistically significant survival differences between the three groups of patients were found [28]. Ciampi et al. found a correlation between RAS mutation and better outcome in MTC, but it did not reach statistical significance [30]. Interestingly, the recent study by Najdawi et al. [31] found that there was no correlation between the Sydney and MSKCC systems [23, 24] and also with respect to the RET or RAS mutation status. Specifically, neither RET mutations in general nor high-risk RET mutations in exons 15 and 16 were enriched in high-grade tumors. These findings suggest that grade and genotype may give independent prognostic information. The explanation for these conflicting results is that probably larger studies using multivariate analysis are needed to assess the additive prognostic value of molecular profiling in sporadic MTC.

Moreover, the molecular findings may gain further significance in the era of targeted therapy including RET inhibition [32], and further studies considering data on treatment modalities may be needed. In this regard, the histological grade should be considered in future clinical trials for experimental adjuvant therapies [19].

Conclusion

In conclusion, our data support the importance of including the IMTCGS, based on mitosis, Ki67 index, and necrosis, in the pathology report in order to provide a reliable risk stratification for clinical decisions. While the lack of association with the OS is an area of interest in this series, the IMTCGS may be used to pave the way to a customized patient follow-up and therapy, by enabling the treating clinician to better counsel the patient, design the optimal follow-up, and better select those individuals who may benefit from systemic therapy.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available due to privacy reasons but are available from the corresponding author on reasonable request.

References

1. Abraham DT, Low TH, Messina M, Jackson N, Gill A, Chou AS, Delbridge L, Learoyd D, Robinson BG, Sidhu S, Sywak M (2011) Medullary thyroid carcinoma: Long-term outcomes of surgical treatment. Ann Surg Oncol 18:219–225. https://doi.org/10.1245/s10434-010-1339-y
2. Pacini F, Castagna MG, Brilli L, Pentheroudakis G (2012) Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 23:vii110–vii119. https://doi.org/10.1093/annonc/mds230
3. Van Veelen W, De Groot JWB, Acton DS, Hofstra RMW, Höppener JWM, Links TP, Lips CJM (2009) Medullary thyroid carcinoma and biomarkers: Past, present and future. J Intern Med 266:126–140. https://doi.org/10.1111/j.1365-2796.2009.02106.x
4. Schröder S, Böcker W, Baisch H, Bürk CG, Arps H, Meiners I, Kastendieck H, Heitz PU, Klöppel G (1988) Prognostic factors in medullary thyroid carcinomas. Survival in relation to age, sex, stage, histology, immunocytochemistry, and DNA content. Cancer 61:806–16.
5. Kebebew E, Ituarte PH, Siperstein AE, Duh QY, Clark OH (2000) Medullary thyroid carcinoma: clinical characteristics, treatment, prognostic factors, and a comparison of staging systems. Cancer 88:1139–48.
6. Elisei R, Cosci B, Romei C, Bottici V, Renzini G, Molinaro E, Agate L, Vivaldi A, Faviana P, Basolo F, Miccoli P, Berti P, Pacini F, Pinchera A (2008) Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: A 10-year follow-up study. J Clin Endocrinol Metab 93:682–687. https://doi.org/10.1210/jc.2007-1714
7. Moura MM, Cavaco BM, Leite V (2015) RAS proto-oncogene in medullary thyroid carcinoma. Endocr Relat Cancer 22:R235–R252. https://doi.org/10.1530/ERC-15-0070
8. Wells SA, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, Lee N, MacHens A, Moley JF, Pacini F, Raue F, Frank-Raue K, Robinson B, Rosenthal MS, Santoro M, Schlumberger M, Shah M, Waguespack SG (2015) Revised American thyroid association guidelines for the management of medullary thyroid carcinoma. Thyroid 25:567–610. https://doi.org/10.1089/thy.2014.0335
9. Park H, Park SY, Park J, Choe JH, Chung MK, Woo SY, Choi JY, Kim SW, Chung JH, Kim TH (2021) Prognostic Value of Preoperative Serum Calcitonin Levels for Predicting the Recurrence of Medullary Thyroid Carcinoma. Front Endocrinol (Lausanne) 12:1–8. https://doi.org/10.3389/fendo.2021.749973
10. Machens A, Ukkat J, Hauptmann S, Dralle H (2007) Abnormal carcinoembryonic antigen levels and medullary thyroid cancer progression: a multivariate analysis. Arch Surg 142:289–93; discussion 294 . https://doi.org/10.1001/archsurg.142.3.289
11. Censi S, Bertazza L, Piva I, Manso J, Benna C, Iacobone M, Mondin A, Plebani M, Faggian D, Galuppini F, Pennelli G, Barollo S, Mian C (2021) Serum miR-375 for Diagnostic and Prognostic Purposes in Medullary Thyroid Carcinoma. Front Endocrinol (Lausanne) 12:1–7. https://doi.org/10.3389/fendo.2021.647369
12. Erovic BM, Kim D, Cassol C, Goldstein DP, Irish JC, Asa SL, Mete O (2012) Prognostic and predictive markers in medullary thyroid carcinoma. Endocr Pathol 23:232–242. https://doi.org/10.1007/s12022-012-9225-8
13. Lloyd R, Osamura R, Kloppel G, Rosai J (2017) WHO Classification of Tumours of Endocrine Organs, 4th Ed. International Agency for Research on Cancer (IARC), Lyon
14. Who Classification of Tumours Editorial Board (2019) Digestive System Tumours, 5th Ed. International Agency for Research on Cancer (IARC), Lyon
15. Who Classification of Tumours Editorial Board (2021) Thoracic Tumours, 5th Ed. International Agency for Research on Cancer (IARC), Lyon

16. Ríos A, Rodríguez JM, Acosta JM, Balsalobre MD, Torregrosa N, Sola J, Pérez-Flores D, Parrilla P (2010) Prognostic value of histological and immunohistochemical characteristics for predicting the recurrence of medullary thyroid carcinoma. Ann Surg Oncol 17:2444–2451. https://doi.org/10.1245/s10434-010-1021-4
17. Franc B, Rosenberg-Bourgin M, Caillou B, Dutrieux-Berger N, Floquet J, Houcke-Lecomte M, Justrabo E, Lange F, Labat-Moleur F, Le Bodic MF, Patey M, Beauchet A, Saint-André JP, Hejblum G, Viennet G (1998) Medullary thyroid carcinoma: Search for histological predictors of survival (109 proband cases analysis). Hum Pathol 29:1078–1084. https://doi.org/10.1016/S0046-8177(98)90417-8
18. Raue F, Kotzerke J, Reinwein D, Schröder S, Röher HD, Deckart H, Höfer R, Ritter M, Seif F, Buhr H (1993) Prognostic factors in medullary thyroid carcinoma: evaluation of 741 patients from the German Medullary Thyroid Carcinoma Register. Clin Investig 71:7–12 . https://doi.org/10.1007/BF00210956
19. Dottorini ME, Assi A, Sironi M, Sangalli G, Spreafico G, Colombo L (1996) Multivariate analysis of patients with medullary thyroid carcinoma. Prognostic significance and impact on treatment of clinical and pathologic variables. Cancer 77:1556–65.
20. Mian C, Pennelli G, Barollo S, Cavedon E, Nacamulli D, Vianello F, Negro I, Pozza G, Boschin IM, Pelizzo MR, Rugge M, Mantero F, Girelli ME, Opocher G (2011) Combined RET and Ki-67 assessment in sporadic medullary thyroid carcinoma: A useful tool for patient risk stratification. Eur J Endocrinol 164:971–976. https://doi.org/10.1530/EJE-11-0079
21. Pezzani R, Bertazza L, Cavedon E, Censi S, Manso J, Watutantrige-Fernando S, Pennelli G, Galuppini F, Barollo S, Mian C (2019) Novel Prognostic Factors Associated with Cell Cycle Control in Sporadic Medullary Thyroid Cancer Patients. Int J Endocrinol 2019. https://doi.org/10.1155/2019/9421079
22. Tisell LE, Oden A, Muth A, Altiparmak G, Mõlne J, Ahlman H, Nilsson O (2003) The Ki67 index a prognostic marker in medullary thyroid carcinoma. Br J Cancer 89:2093–2097. https://doi.org/10.1038/sj.bjc.6601453
23. Fuchs TL, Nassour AJ, Glover A, Sywak MS, Sidhu SB, Delbridge LW, Clifton-Bligh RJ, Gild ML, Tsang V, Robinson BG, Clarkson A, Sheen A, Sioson L, Chou A, Gill AJ (2020) A Proposed Grading Scheme for Medullary Thyroid Carcinoma Based on Proliferative Activity (Ki-67 and Mitotic Count) and Coagulative Necrosis. Am J Surg Pathol 44:1419–1428. https://doi.org/10.1097/PAS.0000000000001505
24. Alzumaili B, Xu B, Spanheimer PM, Tuttle RM, Sherman E, Katabi N, Dogan S, Ganly I, Untch BR, Ghossein RA (2020) Grading of medullary thyroid carcinoma on the basis of tumor necrosis and high mitotic rate is an independent predictor of poor outcome. Mod Pathol 33:1690–1701. https://doi.org/10.1038/s41379-020-0532-1
25. Xu B, Fuchs TL, Ahmadi S, Alghamdi M, Alzumaili B, Bani M-A, Baudin E, Chou A, De Leo A, Fagin JA, Ganly I, Glover A, Hartl D, Kanaan C, Khneisser P, Najdawi F, Nigam A, Papachristos A, Repaci A, Spanheimer PM, Solaroli E, Untch BR, Barletta JA, Tallini G, Al Ghuzlan A, Gill AJ, Ghossein RA (2022) International Medullary Thyroid Carcinoma Grading System: A Validated Grading System for Medullary Thyroid Carcinoma. J Clin Oncol 40:96–104 . https://doi.org/10.1200/jco.21.01329
26. Filetti S, Durante C, Hartl D, Leboulleux S, Locati LD, Newbold K, Papotti MG, Berruti A, ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org (2019) Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol Off J Eur Soc Med Oncol 30:1856–1883.https://doi.org/10.1093/annonc/mdz400
27. Mahul B. Amin, Edge S, Greene F, Byrd DR, Brookland RK, Washington MK, Gershenwald JE, Compton CC, Hess KR, Sullivan DC, Jessup JM, Brierley JD, Gaspar LE, Schilsky RL, Balch CM, Winchester DP, Asare EA, Madera M, Gress DM, Meyer LR (2017) AJCC cancer staging manual, 8th ed. Springer International Publishing, New York

28. Moura MM, Cavaco BM, Pinto AE, Domingues R, Santos JR, Cid MO, Bugalho MJ, Leite V (2009) Correlation of RET somatic mutations with clinicopathological features in sporadic medullary thyroid carcinomas. Br J Cancer 100:1777–1783. https://doi.org/10.1038/sj.bjc.6605056
29. Simbolo M, Mian C, Barollo S, Fassan M, Mafficini A, Neves D, Scardoni M, Pennelli G, Rugge M, Pelizzo MR, Cavedon E, Fugazzola L, Scarpa A (2014) High-throughput mutation profiling improves diagnostic stratification of sporadic medullary thyroid carcinomas. Virchows Arch 465:73–78. https://doi.org/10.1007/s00428-014-1589-3
30. Ciampi R, Mian C, Fugazzola L, Cosci B, Romei C, Barollo S, Cirello V, Bottici V, Marconcini G, Rosa PM, Borrello MG, Basolo F, Ugolini C, Materazzi G, Pinchera A, Elisei R (2013) Evidence of a low prevalence of ras mutations in a large medullary thyroid cancer series. Thyroid 23:50–57. https://doi.org/10.1089/thy.2012.0207
31. Najdawi F, Ahmadi S, Capelletti M, Dong F, Chau NG, Barletta JA (2021) Evaluation of grade in a genotyped cohort of sporadic medullary thyroid carcinomas. Histopathology 79:427–436. https://doi.org/10.1111/his.14370
32. Wirth LJ, Sherman E, Robinson B, Solomon B, Kang H, Lorch J, Worden F, Brose M, Patel J, Leboulleux S, Godbert Y, Barlesi F, Morris JC, Owonikoko TK, Tan DSW, Gautschi O, Weiss J, de la Fouchardière C, Burkard ME, Laskin J, Taylor MH, Kroiss M, Medioni J, Goldman JW, Bauer TM, Levy B, Zhu VW, Lakhani N, Moreno V, Ebata K, Nguyen M, Heirich D, Zhu EY, Huang X, Yang L, Kherani J, Rothenberg SM, Drilon A, Subbiah V, Shah MH, Cabanillas ME (2020) Efficacy of Selpercatinib in RET -Altered Thyroid Cancers. N Engl J Med 383:825–835 . https://doi.org/10.1056/nejmoa2005651

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Table 1 Medullary thyroid carcinoma grading system according to the Royal North Shore Hospital, Sydney [23], the Memorial Sloan Kettering Cancer Center (MSKCC), New York [24], and the International Medullary Thyroid Carcinoma Grading System (IMTCGS) [25]

From: External Validation of Three Available Grading Systems for Medullary Thyroid Carcinoma in a Single Institution Cohort

Sydney grading system (Fuchs et al.)			
Mitotic count (mitoses/2 mm^2)	**Ki67 proliferation index** (%)	**Tumor necrosis**	**Grade**
< 3	< 3	Absent	Low grade
< 3	< 3	Present	Intermediate grade
3–20	3—20	Absent	Intermediate grade
3–20	3—20	Present	High grade
> 20	> 20	Present or absent	High grade
MSKCC grading system (Alzumaili et al.)			
Mitotic count (mitoses/10 HPF)		**Tumor necrosis**	**Grade**
< 5		Absent	Low grade
Any		Present	High grade
≥ 5		Any	High grade
IMTCGS (Xu et al.)			
Mitotic count (mitoses/2 mm^2)	**Ki67 proliferation index** (%)	**Tumor necrosis**	**Grade**
< 5	< 5	Absent	Low grade
Any	≥ 5	Any	High grade
≥ 5	Any	Any	High grade
Any	Any	Present	High grade

Table 2 Distribution of clinico-pathological features and correlations according to the three grading systems

From: External Validation of Three Available Grading Systems for Medullary Thyroid Carcinoma in a Single Institution Cohort

			MSKCC grading system		Sydney grading system	IMTCGS
		Total (111 cases)	*p*	Total (101 cases)	p	*p*
Gender	Female	67	0.438	62	0.272	0.470
	Male	44		39		
Age	Median	57	0.460	57	0.221	0.053
	(interval)	(16–82)		(16–82)		
Hereditary pathogenesis	Sporadic	68	0.557	65	0.313	0.626
	Hereditary	20		18		
pT	1	65	0.285	57	0.217	0.056
	2	28		26		
	3	13		13		
	4	5		5		
pN*	0	56	0.318	50	0.379	0.164
	1	43		43		
Stage	I	47	0.504	39	0.565	0.359
	II	21		19		
(AJCC/UICC)	III	13		13		
	IV	30		30		
Multifocality	No	88	0.735	80	0.381	0.855
	Yes	23		21		
Ki67	Median	2	**0.001**	2	**< 0.001**	**< 0.001**
	(interval)	(0–20)		(0–20)		
Tumor Dimension	Median (mm)	15	0.065	16	0.224	0.177
(mm)	(interval)	(0.5–90)		(0.5–90)		
Mitotic count	Median	0	**< 0.001**	0	**< 0.001**	**< 0.001**
(mitoses/2 mm^2)	(Interval)	(0—9)		(0–9)		

MSKCC Memorial Sloan Kettering Cancer Center, *IMTCGS* International Medullary Thyroid Carcinoma Grading System, *AJCC/UICC* American Joint Committee on Cancer/Union for International Cancer Control

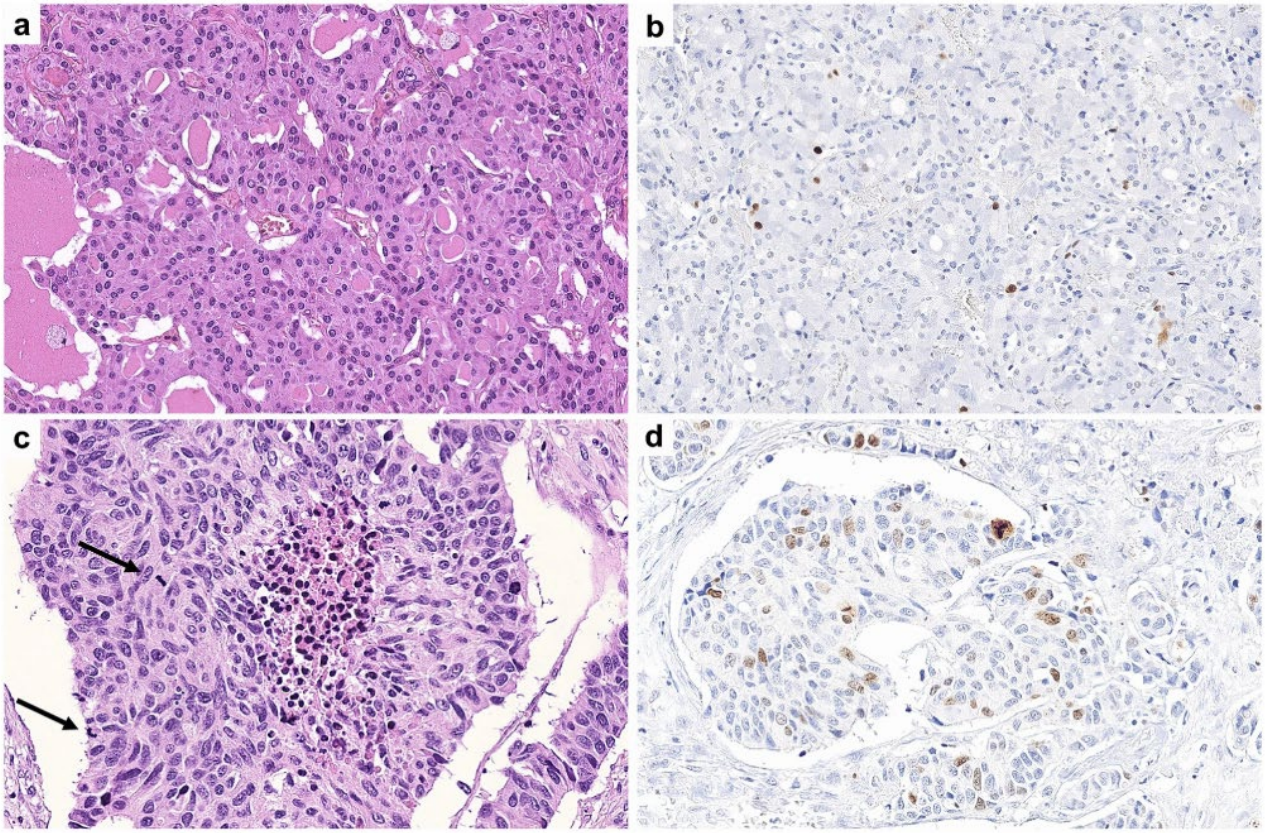
In bold the significative *p* values

[§]Hereditary pathogenesis (germline *RET* variant) status was unknown in 23/111 cases and 18/101 cases

[*]pN was assessed in 99 cases only (the lymph node status was not known in 12 cases) for the MSKCC grading and in 93 cases only (the lymph node status was not known in 8 cases) for the Sydney and IMTCGS schemes

Fig. 1

From: External Validation of Three Available Grading Systems for Medullary Thyroid Carcinoma in a Single Institution Cohort



Examples of a low-grade medullary thyroid carcinoma (MTC) and a high-grade MTC according to the 3 grading systems. **A–B** Low-grade MTC shows typical neuroendocrine morphology, absence of necrosis and mitoses, and a low Ki67 proliferation index. **C–D** High-grade MTC IS characterized by areas of "comedo-like" necrosis and increased mitoses (black arrows) and a high percentage of Ki67-positive cells

Table 3 Distribution of clinicopathological features, including the 3 grading systems, according to disease recurrence

From: External Validation of Three Available Grading Systems for Medullary Thyroid Carcinoma in a Single Institution Cohort

			Disease recurrence		
		Total (111 cases)	No (84 cases)	Yes (27 cases)	*p*
Gender	Female	67	56	11	**0.017**
	Male	44	28	16	
Hereditary pathogenesis§	Sporadic	68	52	16	0.740
	Hereditary	20	16	4	
pT	1[a]	65	57	8	**< 0.001** (c vs a < 0.001 d vs a 0.001 c vs b 0.002 d vs b 0.011)[$]
	2[b]	28	22	6	
	3[c]	13	4	9	
	4[d]	5	1	4	
pN*	0	56	54	2	**0.001**
	1	43	20	23	
Stage (AJCC/UICC)	I[a]	47	45	2	**< 0.001** (a vs d < 0.001 b vs d < 0.001 c vs d 0.001)[$]
	II[b]	21	19	2	
	III[c]	13	10	3	
	IV[d]	30	10	20	
Multifocality	No	88	70	18	0.063
	Yes	23	14	9	
Necrosis	No	85	70	15	**0.003**
	Yes	26	14	12	
Ki67	Median (interval)	2 (0—20)	2 (0—7)	4 (1—20)	0.130
Tumor dimension (mm)	Median (interval)	15 (0.5—90)	12 (0.5—80)	25 (6—90)	**0.003**
Mitotic count (mitoses/2 mm^2)	Median (interval)	0 (0—9)	0 (0—7)	0 (0—9)	0.210
MSKCC grading system	Low grade	85	70	15	**0.003**
	High grade	26	14	12	
Sydney grading system (101 cases)	Low grade[a]	49	42	7	**0.024** (c vs a 0.036)[$]
	Intermediate grade[b]	34	23	11	
	High grade[c]	18	10	8	
IMTCGS (101 cases)	Low grade	69	59	10	**< 0.001**
	High grade	32	16	16	

[$]Bonferroni's correction

MSKCC Memorial Sloan Kettering Cancer Center, *IMTCGS* International Medullary Thyroid Carcinoma Grading System, *AJCC/UICC* American Joint Committee on Cancer/Union for International Cancer Control

In bold the significative *p* values

[§]Hereditary pathogenesis (germline *RET* variant) status was not known in 23 cases

[*]pN was assessed in 99 cases only (the lymph node status was not known in 12 cases)

Table 4 Disease-free survival univariate analysis using Cox regression model

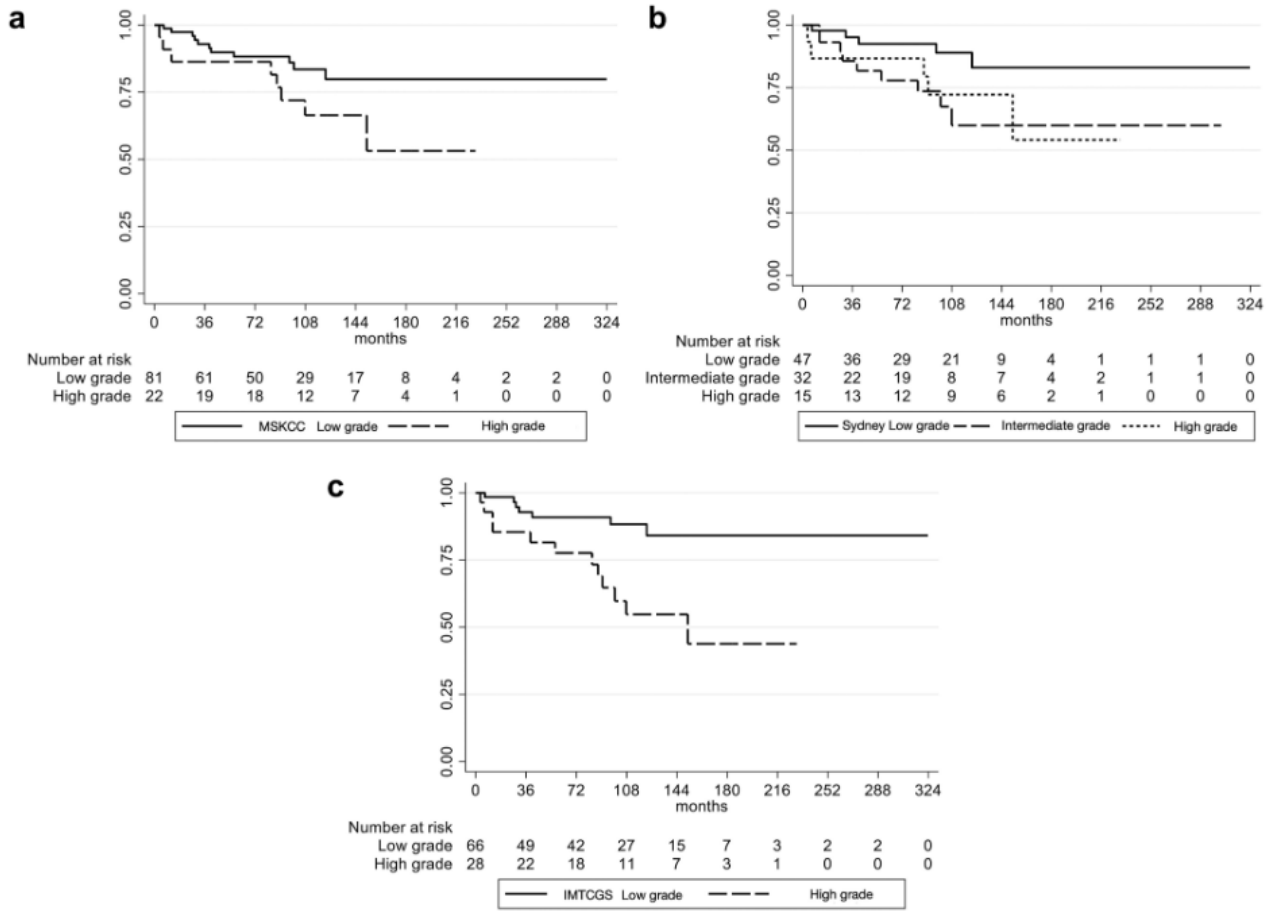
From: External Validation of Three Available Grading Systems for Medullary Thyroid Carcinoma in a Single Institution Cohort

DFS		HR	CI	p
Gender	**Male vs Female**	2.26	0.92–5.59	0.076
Age	**linear**	1.00	0.97–1.04	0.590
Hereditary pathogenesis	**Hereditary vs sporadic**	0.42	0.10–1.87	0.258
Tumor dimension (mm)	**linear**	1.03	1.01–1.06	**0.003**
pT	**linear**	2.82	1.88–4.23	**< 0.001**
pN	**0 vs 1**	16.8	3.87–72.9	**< 0.001**
Stage (AJCC/UICC)	**I**	1		
	II	2.60	0.16–41.6	0.499
	III	10.2	0.92–112.6	0.058
	IV	35.5	4.68–268	**0.001**
Multifocality	**Present vs absent**	1.72	0.65–4.54	0.270
Necrosis	**Present vs absent**	2.21	0.89–5.53	0.087
Mitotic count (mitoses/2 mm^2)	**Linear**	1.13	0.89–1.44	0.287
Ki67 (101 cases)	**linear**	1.38	1.21–1.59	**< 0.001**
MSKCC grading system	**High vs Low**	2.22	0.89–5.53	0.087
Sydney grading system (101 cases)	**Low**	1		
	Intermediate	2.89	0.97–8.66	0.057
	High	2.67	0.77–9.24	0.121
IMTCGS (101 cases)	**High vs Low**	3.97	1.57–10.1	**0.004**

DFS Disease-free survival, *HR* Hazard ratio, *CI* Confidence interval, *MSKCC* Memorial Sloan Kettering Cancer Center, *IMTCGS* International Medullary Thyroid Carcinoma Grading System, *AJCC/UICC* American Joint Committee on Cancer/Union for International Cancer Control
In bold the significative p-values

Fig. 2

From: External Validation of Three Available Grading Systems for Medullary Thyroid Carcinoma in a Single Institution Cohort



Kaplan–Meier curves for disease-free survival on all medullary thyroid carcinoma (MTC) cases according to **A** the Memorial Sloan Kettering Cancer Center (MSKCC) grading system ($p = 0.079$); **B** the Sydney grading system ($p = 0.115$); **C** the International Medullary Thyroid Carcinoma Grading System (IMTCGS) ($p = 0.002$)

Table 5 Overall survival univariate analysis using Cox regression model

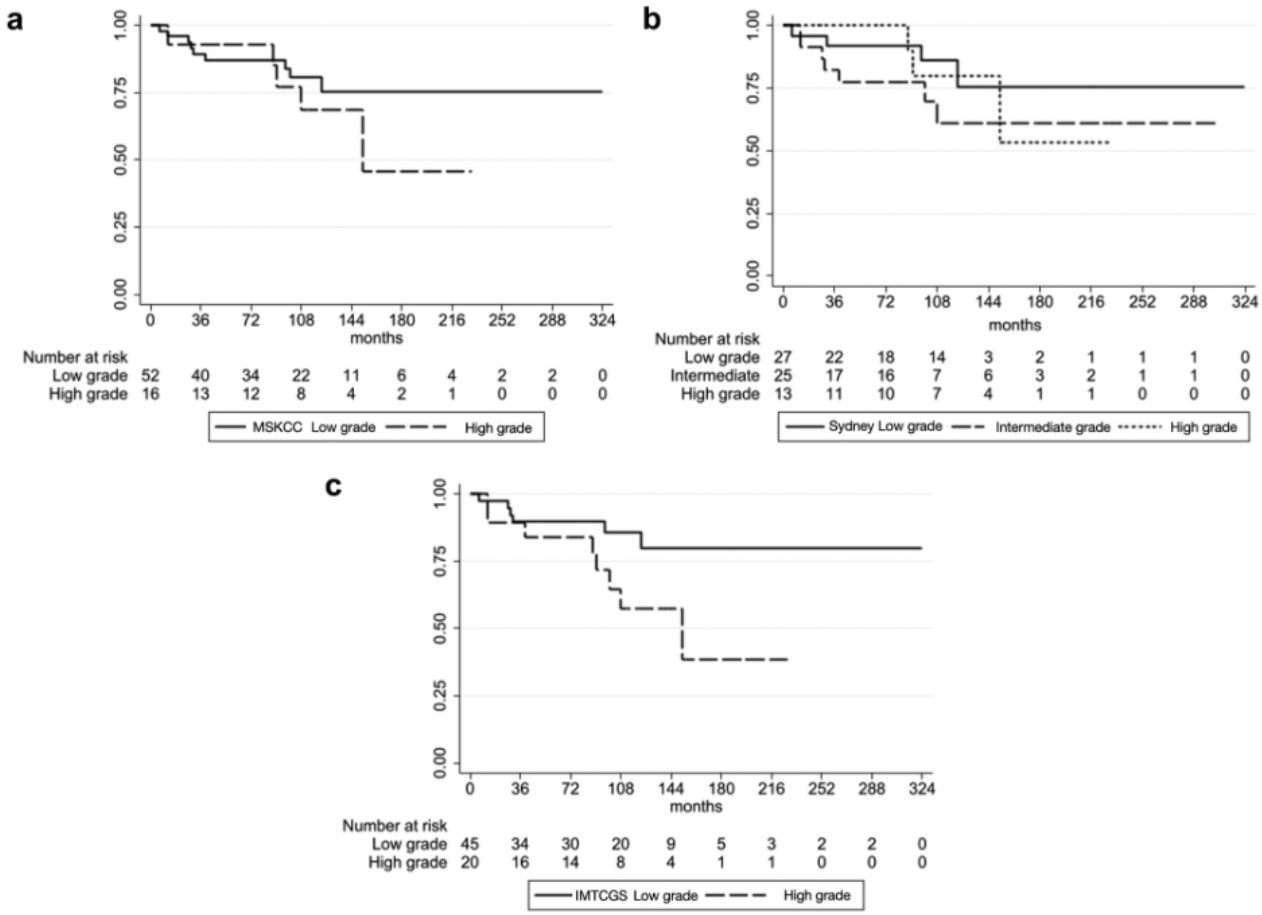
From: External Validation of Three Available Grading Systems for Medullary Thyroid Carcinoma in a Single Institution Cohort

OS		HR	CI	*p*
MSKCC grading system	**High vs Low**	1.49	0.59—3.75	0.393
Sydney grading system	**Low**	1		
(101 cases)	**Intermediate**	0.94	0.31—2.90	0.923
	High	2.20	0.75—6.46	0.152
IMTCGS (101 cases)	**High vs Low**	1.61	0.64—4.09	0.314

OS overall survival, *HR* hazard ratio, *CI* confidence interval, *MSKCC* Memorial Sloan Kettering Cancer Center, *IMTCGS* International Medullary Thyroid Carcinoma Grading System

Fig. 3

From: External Validation of Three Available Grading Systems for Medullary Thyroid Carcinoma in a Single Institution Cohort



Kaplan–Meier curves for disease-free survival on sporadic manifestations (absence of pathogenic germline *RET* variant) only according to **A** the Memorial Sloan Kettering Cancer Center grading system ($p = 0.343$); **B** the Sydney grading system ($p = 0.518$); **C** the International Medullary Thyroid Carcinoma Grading System ($p = 0.041$)