Poorly differentiated carcinoma of the thyroid: validation of the Turin proposal and analysis of IMP3 expression

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The Turin Proposal algorithm defines poorly differentiated carcinoma on the basis of the presence of solid/trabecular/insular growth pattern, absence of conventional nuclear features of papillary carcinoma, and the presence of at least one of the following features: convoluted nuclei, mitotic activity $\geq$ 3/10 HPF, or tumor necrosis. IMP3 appears to have diagnostic and prognostic value in many solid tumors, including thyroid carcinomas. We examined a series of follicular-cell carcinomas with prominent solid patterns diagnosed at Mayo Clinic (56 cases) (Rochester, MN, USA) and at the University of Turin (96 cases) (Northern Italy) to validate the Turin consensus criteria defining poorly differentiated carcinoma of the thyroid and to evaluate the prevalence and prognostic behavior of this tumor. On this series, we analyzed the expression of conventional markers by immunohistochemistry and we investigated the expression of IMP3 by both immunohistochemistry and qRT-PCR. The prevalence of poorly differentiated carcinoma among the USA cases was 1.8% (56/3128) and that in the cases of Northern Italy was 6.7% (96/1442). Tumor characteristics were similar in the cases from the USA and from Italy except for extensive vascular invasion and a prevalent insular growth pattern (lower the former, higher the latter in the Italian series). In univariate analysis, the risk of death was higher for age $\geq$ 45, tumors $\geq$ 4 cm, and IMP3 positivity. Multivariate analysis showed that the risk of death from poorly differentiated carcinoma was higher for age $\geq$ 45. The Turin consensus criteria can reliably select poorly differentiated carcinomas. Tumors from the USA and from Italy showed similar overall survival, although the prevalence of poorly differentiated carcinoma was higher in Northern Italy. Expression of IMP3 appears to be an adverse prognostic factor for poorly differentiated thyroid carcinoma.

Poorly differentiated carcinoma of the thyroid is a worldwide recognized entity, but its incidence varies from reports of less than 1% of thyroid cancers in Japan[1] and 2–3% of thyroid cancers in North America, to 15% in Northern Italy.[2] These variations may either be real and related to geographic (environmental) influences or

could be due to differences in histopathological interpretation.

The term 'poorly differentiated carcinoma' of the thyroid gland was proposed more than 25 years ago to define a follicular-cell carcinoma showing limited evidence of structural follicular or papillary features, and having a clinical behavior intermediate between follicular or papillary and anaplastic carcinomas. Different terms have been used to refer to these tumors, including solid,[3] trabecular,[4] insular,[5,6] poorly differentiated,[3,7,8] intermediate-type,[9] primordial cell,[10] less well-differentiated carcinoma,[11] or follicular carcinoma with insular component.[12]

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In the latest World Health Organization (WHO) classification of Tumors of Endocrine Organs,[13] poorly differentiated carcinoma was introduced as a separate entity and defined as a follicular-cell neoplasm showing limited evidence of structural follicular cell differentiation, and morphologically and behaviorally at an intermediate position between differentiated (follicular and papillary carcinomas) and undifferentiated (anaplastic) carcinoma.

An international consensus meeting was held in Turin, on March 2006, to develop uniform diagnostic criteria for poorly differentiated carcinomas. An international panel of 12 thyroid pathologists recommended a diagnostic algorithm ('Turin Proposal criteria') on the basis of the interpretation of the WHO classification and suggesting a uniform approach to classify these tumors. The Turin proposal algorithm defines poorly differentiated carcinomas by the presence of solid/trabecular/insular growth pattern, absence of conventional nuclear features of papillary carcinoma, and the presence of at least one of the following features: convoluted nuclei, mitotic activity $\geq 3/10$ HPF, or tumor necrosis.[14]

Recently, Ito *et al*[15] in Japan compared the prognostic value of different histopathological definitions in a large series of thyroid malignancies and they concluded that in their series the subgroup defined according to the Turin consensus criteria was an uncommon but well-defined entity and included a subset of thyroid carcinomas associated with a more aggressive behavior. Volante *et al*[16] analyzed the prognostic impact of several clinicopathological parameters in a large series of poorly differentiated carcinomas classified according to the pattern of growth (trabecular, insular, and/or solid) and reported that age ≥ 45 years, presence of necrosis, and mitotic count $\geq 3 \times 10$ HPF were associated with poor outcome. In agreement with these findings, the study by Hiltzik *et al*,[17] reported that poorly differentiated carcinomas defined on the basis of high mitotic activity and/or tumor necrosis have a prognosis intermediate between well-differentiated thyroid carcinomas and anaplastic tumors, and also found that the growth pattern was not predictive of survival in this group of patients. More recently, poorly differentiated carcinomas with necrosis and/or mitotic index $\geq 5 \times 10$ HPF, irrespective of the growth pattern, were identified as radioactive iodine-refractory tumors.[18]

With this background, we retrieved a series of follicular-cell carcinomas of the thyroid with prominent solid pattern from Mayo Clinic (Rochester, MN, USA) and from University of Turin (Turin, Italy) to validate the histological features of poorly differentiated carcinoma using the Turin proposal criteria, to better define the prevalence and evolution of this entity, and to analyze the prognostic effect of previously reported morphological or genetic parameters. In addition, we investigated the expression and potential prognostic role in poorly differentiated carcinoma of insulin-like growth factor-II mRNA binding protein-3 (IMP3), also known as L523S or KOC (K-homologous domain-containing protein over expressed in cancer), which is a member of the IMP family[19] and has been reported as an important biomarker for several malignancies.[20] IMP3 expression has diagnostic and/or prognostic value in many malignant solid tumors.[20,21] In thyroid tumors, IMP3 expression has been proposed as a diagnostically useful parameter in differentiating malignant and benign follicular pattern lesions.[21,22] We sought to investigate whether IMP3 might have prognostic implications in poorly differentiated carcinomas.

Materials and methods

Patient Selection

Four thousand five hundred seventy primary thyroid malignancies from patients who underwent primary surgical treatment at Mayo Clinic (Rochester, MN, USA; 3128 cases) between 1955 and 2000, and at the Molinette Hospital & University of Turin (Northern Italy; 1442 cases), during the period 1974–2008 were reviewed. Specifically, one of the authors (S Asioli) spent 6 months (from 1 March 2009 to 31 August 2009) at Mayo Clinic (Rochester, MN, USA) as visiting clinician, funded by World Wide Style Projects, Fondazione CRT, University of Turin, Italy, and fulfilling the project she personally reviewed the slides of 3128 primary thyroid malignancies, extracting 148 cases of interest with a solid pattern, which were further reviewed with two authors (RV Lloyd, LA Erickson). The Italian cases (no. 1442) were all reviewed by two of the authors (M Volante and S Asioli) and the selected cases of interest were further checked by four authors (S Asioli, G Bussolati, RV Lloyd and LA Erickson). A total of 152 cases of poorly differentiated carcinoma were identified according to Turin consensus criteria from Mayo Clinic (Rochester, MN, USA; 56 cases) and the University of Turin (Italy; 96 cases).

The following selection criteria were used: correlation to the Turin consensus criteria,[14] including presence of well-differentiated component in less than 25% of the tumor (follicular or papillary type) and availability of clinicopathological and follow-up data, and of pathological material (slides and paraffin blocks). Some of these cases had been included in previous studies.[10,14,16,23]

Institutional Review Board Permission was obtained for the study.

The following features were analyzed: prevalence, age, sex, tumor size, necrosis, convoluted nuclei, mitoses, atypical mitoses, vascular invasion (either minimal: ≤ 3 foci of vascular invasion or extensive: 4 or more foci of vascular invasion), oncocytic features, pattern of growth.

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Disease persistence, recurrence, and metastases were evaluated by chart review, imaging studies, or histopathological examination of resected recurrent tumors and/or metastases.

Immunohistochemistry

Immunohistochemical analysis for IMP3 (L523; Dako, Carpinteria, CA, USA) thyroglobulin (2H11/6E1 cocktail; Zymed, San Francisco, CA, USA), TTF1 (8G7G3/1; Dako), HBME-1 (HBME-1; Dako), p53 (DO7; Dako), and Ki67 (MIB1; Dako) was performed on poorly differentiated carcinoma cases according to the standard automated immuno-histochemical procedure (Ventana XT autostainer; Ventana Medical Systems, Tucson, AZ, USA).

Interpretation of Immunoistochemical Stains

The immunohistochemical expression of IMP3 and the expression of p53 and HBME-1 were scored by considering both the intensity and the extent of the stain using a semi-quantitative scoring system. The criteria for intensity score were as follows: 0 for no staining; 1+ for weak staining; 2+ for moderate staining; and 3+ for strong staining. The criteria for scoring the extent of the stain were as follows: 0 for no staining; 1+ for staining <25% of neoplastic cells; 2+ for staining 25–50% of neoplastic cells; and 3+ for staining >50% of the neoplastic cells. The two scores were cumulated and a stain was considered positive with a combined immunohisto-chemical score higher than 2, whereas a final score of 2 or lower was considered negative.

Molecular Analysis

Molecular analysis for IMP3 and *RAS* mutations was focused on a subgroup of poorly differentiated carcinoma cases with a pure insular growth pattern. In particular, molecular analysis of IMP3 expression could be performed in 47 of these cases. All tumor samples were analyzed for IMP3 expression using quantitative RT-PCR (qRT-PCR) as described previously.[22] IMP3 mRNA expression was normalized with phosphoglycerokinase (PGK) mRNA expression and the IMP3 expression levels were expressed as relative fold change (fold) compared to normal thyroid RNA and TPC1 (calibrator).

Molecular analysis of *RAS* mutational status was carried out in 32 cases: 14 of these cases had already been analyzed in the recent study on *RAS* significance in poorly differentiated carcinomas;[23] to these, we added 18 new cases showing pure insular growth pattern. All tumor samples were analyzed for *NRAS* codon-61, *HRAS* codon-61, and *KRAS* codon-12 and 13 point mutations using PCR amplification and DNA sequencing as described previously.[23]

Statistical Analysis

Continuous data were compared between groups using Wilcoxon rank-sum tests and categorical data were compared between groups using χ^2 or Fisher's exact tests. The age of 45 years[24] was considered as the cut-off age for statistical analyses. Survival time was defined as time from the date of first surgery to the time of death (or the last follow-up). We also looked at four other events: disease-free survival, time to recurrence, time to lymph node metastasis, and time to distant metastasis. The time to events were each compared between groups initially using log-rank tests and illustrated using Kaplan–Meier curves. Survival was also compared between groups using Cox proportional-hazards regression from which hazard ratios were estimated. In models where the number of events was very small in the level of one of the predictors, confidence intervals were calculated using the profile likelihood method, and likelihood ratio tests were used to calculate *P*-values. In all other cases, the Wald method was used for confidence intervals and *P*-values. Multi-variable models were determined using stepwise variable selection. Associations between immuno-reactivity with IMP3 and clinicopathological para-meters were evaluated using the Yates corrected χ^2 test for 2×2 tables. A comparison between immunohistochemistry and qRT-PCR IMP3 results was performed using Student's *t*-test. A Spearman correlation was used to look for a relationship between IMP3 molecular analysis results and the follow-up data. *P*-values less than 0.05 were con-sidered statistically significant. All analyses were performed using SAS version 9 (Cary, NC, USA) and figures were generated in Splus (Palo Alto, CA, USA).

Results

The prevalence of poorly differentiated carcinomas in the Mayo Clinic series was 1.8% (56/3128) whereas in the University of Turin (Italy) series (a single Institution in Northern Italy) it was 6.7% (96/1442).

A summary of the associations between the clinicopathological parameters of the 152 cases between the two sites is provided in Table 1.

Clinical Features

The mean patient age was 60.6 years (median 61.4; range: 14.1–89.7). A female predominance was observed (female:male ratio, 1.6:1). Radioiodine therapy was administered (1–8 doses of ^{131}I between 3700 and 7400 MBq) after total thyroidectomy in 74 patients for whom post-surgical treatment informa-tion was available.

Clinically aggressive tumors, including those with recurrences or metastatic spread and/or patient

Table 1 Clinical and pathological features of 152 cases of poorly differentiated carcinoma of the thyroid gland

Characteristics	Mayo clinic (56 cases)	University of Turin (96 cases)	Total (152 cases)	P-value
Gender (female; male)	29F; 27M	65F; 31M	94F; 58M	0.0560
Age (years) (mean)	58.6	61.7	60.6	0.2773
Tumor size (cm) (mean)	5.8	6	5.9	0.7140
Predominant insular patter of growth	23/56 (41%)	65/96 (68%)	88/152 (58%)	0.0013
Presence of necrosis	40/56 (71%)	67/96 (70%)	107/152 (70%)	0.8311
Mitosis ≥3/10 high power fields	54/56 (96%)	89/96 (93%)	143/152 (94%)	0.3485
Atypical mitosis	10/56 (18%)	20/96 (21%)	30/152 (20%)	0.6565
Convoluted nuclei	17/56 (30%)	32/96 (33%)	49/152 (32%)	0.7049
Extensive vascular invasion	46/56 (84%)	55/96 (57%)	101/152 (67%)	0.0009
Presence of oncocytic features	19/56 (34%)	29/96 (30%)	48/152 (32%)	0.4412
Presence of well differentiated component papillary carcinoma (≤25%)	3/56 (5%)	13/96 (13%)	16/152 (11%)	0.113
Presence of well differentiated component follicular carcinoma (≤25%)	13/56 (23%)	23/96 (24%)	36/152 (24%)	1
Overall survival: 5-year overall survival rate (%)	68.6% (95% CI: 57.0–82.6%)	73.6% (95% CI: 64.0–84.6%)	71.6% (95% CI: 64.0–80.1%)	0.89*
10-year overall survival rate (%)	45.9% (95% CI: 33.9–62.2%)	45.7% (95% CI: 33.4–62.5%)	46.3% (95% CI: 37.4–57.3%)	

*P-value for difference in overall survival between Mayo Clinic cases and Turin cases was not significant ($P = 0.89$).

death, accounted for 99 of the 152 tumors (65%). Local recurrence was observed in 19 cases, metastases to lymph nodes in 22 cases, and to metastases to distant organs in 58 cases.

The estimated 5-year survival rate was 71.6% (95% CI: 64.0–80.1%) and the 10-year survival rate was 46.3% (95% CI: 37.4–57.3%) (Table 1).

Pathological Features

The mean tumor size was 5.9 cm (range: 2–13 cm). Most patients had vascular invasion, which was either minimal (≤3 foci of vascular invasion) in 50/152 (33%) or extensive (4 or more foci of vascular invasion) in 101/152 (66%) cases. Vascular invasion was identified in all but one patient who had, however, extensive full-thickness capsular penetration by the tumor. The type of growth pattern of the tumors included a predominantly insular pattern found in 58% (88/152) of the tumors and a solid-trabecular architecture in 42% (64/152) of the tumors. The presence of a well-differentiated component (≤25% of the tumor) as follicular carcinoma was found in 36 tumors (24%), whereas papillary thyroid carcinoma was identified in 16 tumors (11%). Predominant oncocytic features were identified in 48/152 tumors (32%). Convoluted nuclei were observed in 49/152 (32%) cases. Necrotic areas were present in 107/152 tumors (70%) (Figure 1a). A mitotic count ≥3 per 10 HPF was observed in 143/152 (94%) and atypical mitoses were present in 30/152 (20%) cases (Figure 1b).

On the basis of these distinct morphological features and considering that all cases were meeting the Turin consensus criteria, we divided the poorly differentiated carcinoma cases into four groups.

Group-1 included 52/152 (34%) poorly differentiated carcinoma cases that showed a pure insular growth pattern and absence of even focal conventional nuclear features of papillary carcinoma. Group-2 included 16/152 (10%) poorly differentiated carcinoma cases that showed foci (<25% of the tumor) of well-differentiated papillary thyroid carcinoma. Group-3 included 36/152 (24%) poorly differentiated carcinoma cases that showed foci of well-differentiated follicular carcinoma in less than 25% of the tumors. Group-4 was composed of 48/152 (32%) poorly differentiated carcinoma cases that showed a solid/trabecular/insular growth pattern with oncocytic features in more than 75% of the tumors.

Tumor characteristics were similar between the Mayo Clinic and the University of Turin poorly differentiated carcinoma cases with the exception of extensive vascular invasion (84% at Mayo Clinic *versus* 57% at University of Turin, $P = 0.0009$) and insular pattern (41% at Mayo Clinic *versus* 68% at University of Turin, $P = 0.001$) (Table 1).

Immunohistochemical Analysis

Immunohistochemical analysis was feasible in 103 cases (68%) because of the limited quality (very old tissue blocks) of the tissue in 49 cases. Thyroglobulin was positive in 95 of 103 (92%) cases and showed a peculiar pattern of dot-like paranuclear staining in most cases (73/95) (77%) (Figure 1c). TTF1 immunostaining showed diffuse nuclear positivity in all 103 poorly differentiated carcinoma cases. HBME-1 showed focal membrane positivity in 50 of 103 cases (49%). P53 immunostaining showed nuclear positivity focally in 39 of 103 cases (38%)
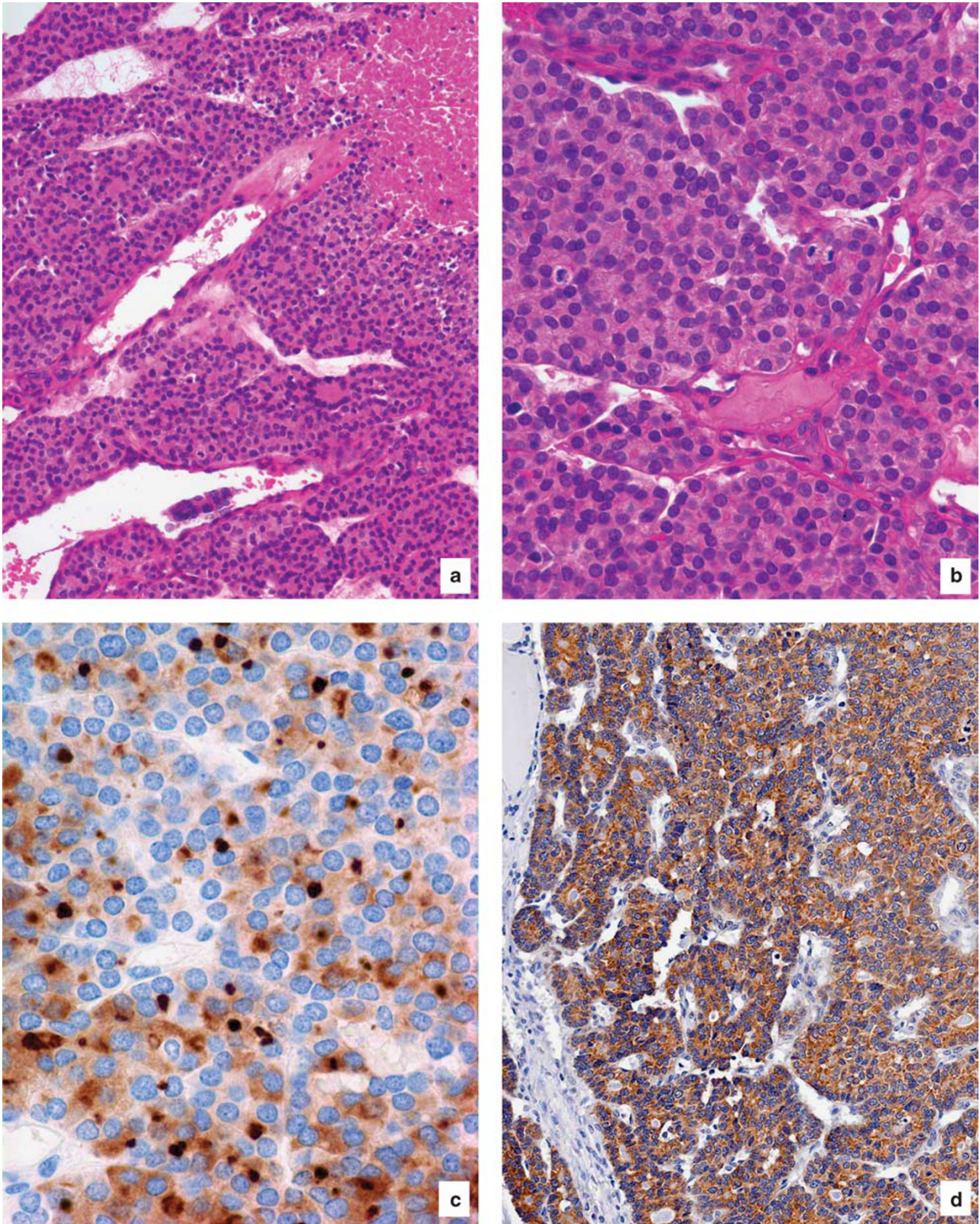
Figure 1 An example of a poorly differentiated carcinoma case on the H&E showing a prominent insular growth pattern, necrosis (**a**, ×10), and high mitotic count (**b**, ×20). The peculiar pattern of dot-like paranuclear staining for thyroglobulin is observed in the majority of poorly differentiated carcinoma cells (**c**, ×20). A prevalent cytoplasmic staining for IMP3 is present in the poorly differentiated carcinoma cells whereas the adjacent normal thyroid cells are negative (**d**, ×10).

and diffuse nuclear positivity in 46 of 103 cases (45%). The Ki67 proliferative index ranged from 3 to 40%, with a mean of 13% (9.5 s.d.).

Sixty-one of 103 (59%) poorly differentiated carcinoma cases showed prevalent cytoplasmic staining for IMP3 (Figures 1d, 2d). Forty-two of 103 (41%) cases were considered negative because tumor cells were completely negative for IMP3 or showed immunohistochemical cumulative score less than 2. Tumor characteristics were compared between poorly differentiated carcinoma cases that were positive ($n = 61$) *versus* those that were negative ($n = 42$) for IMP3. No association could be established between IMP3 expression and sex, age, tumor size, angioinvasion, oncocytic features, presence of convoluted nuclei, pattern of growth, necrosis, and number of mitoses or atypical mitoses.

Molecular Analysis

IMP3 mRNA quantitative analysis

IMP3 mRNA quantitative analysis was only performed for poorly differentiated carcinoma cases of group-1 to avoid the risk of contamination by RNA belonging to well-differentiated tumor areas (papillary in group-2 and follicular in group-3) or oncocytic tumor cells (group-4). IMP3 was detected in 42 of 47 poorly differentiated carcinoma cases tested, with the remaining five cases having insufficient RNA. The relative IMP3 expression in 42 cases of poorly differentiated carcinoma was on the

average 17.5 (14.6 s.d.)-fold higher than the normal thyroid RNA (calibrator). The results of Spearman correlation suggest that the relationship between IMP3 molecular analysis results and follow-up data is statistically significant ($\rho = 0.4683$, $P = 0.0018$).

RAS mutation analysis

RAS mutations were identified in 6 of 32 cases (19%), including 14 cases reported previously.[23] All but one mutation were in the *NRAS* gene, codon-61, with the Gln-to-Arg (Q61R, CAA_CGA) substitution. The remaining tumor had the *NRAS* Q61K mutation (Q61K, CAA_AAA).

No mutation was detected in the *KRAS* and *HRAS* genes.

Three poorly differentiated carcinoma cases showing *NRAS* Q61R and the case with *NRAS* Q61K mutations were positive for IMP3 by immunohistochemistry and had an average of 20.8 (15.8 s.d.) *IMP3* fold changes in qRT-PCR whereas the remaining two mutated cases were negative for *IMP3* at immunohistochemistry and had an average of 8.6 IMP3 fold changes (6.8- and 10.4-fold changes, respectively).

Survival analysis

Univariate analysis showed that the risk of death was higher for patients with poorly differentiated carcinoma whose age was ≥ 45 years (HR = 4.26, $P < 0.01$) and for those with tumors ≥ 4 cm (HR = 1.93, $P = 0.04$) (Figures 2a, b). There was no
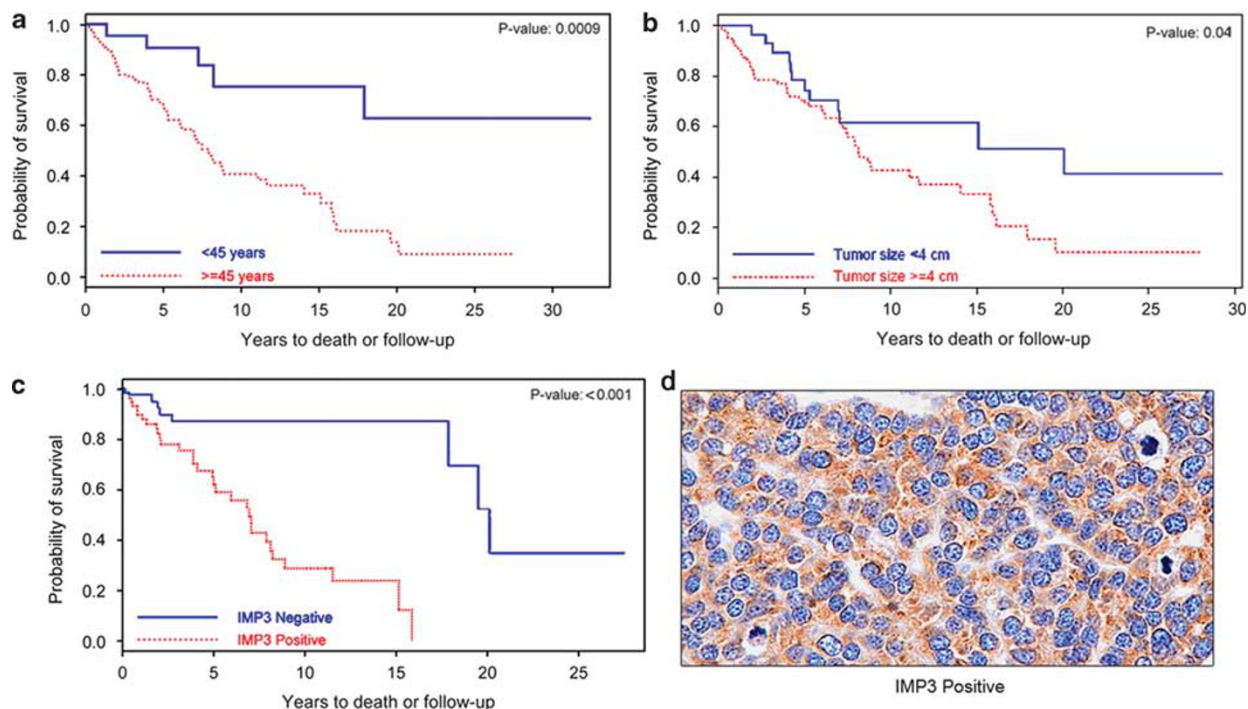
Figure 2 Overall survival distribution on the basis of the Kaplan–Meier product-limit estimates of poorly differentiated carcinoma according to age (**a**), tumor size (**b**), and IMP3 expression (**c**). There is prevalent cytoplasmic staining for IMP3 in this poorly differentiated carcinoma (**d**).

significant difference in the risk of death between poorly differentiated carcinoma cases from Mayo Clinic *versus* those from University of Turin. The presence of atypical mitoses was associated with higher risk of recurrence (HR = 2.74, $P = 0.03$) and risk of lymph node metastasis (HR = 2.69, $P = 0.03$). Age ≥ 45 was associated with higher risk of distant metastasis (HR = 2.99, $P = 0.02$). No significant differences in the risk of recurrence or metastases were found between the Mayo Clinic and University of Turin poorly differentiated carcinoma cases by univariate analysis. No significant differences in overall survival, disease-free survival, and metastasis (lymph nodes and distant organs)-free survival were found between the four groups by univariate analysis (Table 2).

IMP3 and Ki67 stains were analyzed statistically among the 103 poorly differentiated carcinoma cases.

IMP3 positivity was associated with an increased risk of death (HR for positive *versus* negative = 6.97, $P < 0.01$) (Figure 2c), metastases (HR for lymph node metastasis = 7.09, $P = 0.02$; HR for distant metastases = 15.65, $P < 0.01$), and with a decrease of disease-free survival.

MIB-1/Ki67 was marginally associated with an increased risk of recurrence (HR for 5 unit increase = 1.59, $P = 0.048$) and of lymph node metastases (HR = 2.06, $P = 0.04$).

The results of multivariate analysis are summarized in Table 3.

By multivariate analysis, age ≥ 45 (HR = 4.81, $P = 0.001$) was associated with death of disease whereas presence of convoluted nuclei (HR = 0.52, protective, $P = 0.02$) was associated with a better overall survival. Furthermore, the risk of distant metastasis was higher for patients with age ≥ 45 (HR = 3.85, $P = 0.005$) and was lower for cases with convoluted nuclei (HR = 0.39, $P = 0.005$).

Among the 103 poorly differentiated carcinoma cases in which IMP3 immunohistochemical analysis was available, IMP3 positivity was associated with higher risk of distant metastases (HR positive *versus* negative for distant metastases = 36.7, $P < 0.001$). IMP3 positivity was also associated with decreased overall survival (HR for IMP3 positive *versus* negative = 8.96, $P < 0.001$) and decreased disease-free survival (HR for IMP3 positive *versus* negative = 56.08, $P < 0.001$).

Multivariate Cox regression analysis found IMP3 expression to be a strong independent prognostic factor followed by presence of necrosis (Table 3).

qRT-PCR analyses were performed for 42 cases of poorly differentiated carcinoma for which IMP3 immunohistochemical studies had also been performed. The average number IMP3 mRNA transcripts detected in 14 cases of poorly differentiated carcinoma negative for IMP3 by immunohistochemistry was 9.4 (s.d. 6.6)-fold, whereas the average number of IMP3 mRNA transcripts in 28 cases of poorly differentiated carcinoma showing IMP3 positivity by immunohistochemistry was 21.4 (s.d. 14.3)-fold ($P = 0.02$, Student's *t*-test).

Discussion

This study shows that the Turin consensus criteria can reliably diagnose poorly differentiated carcinomas in a large series of primary thyroid malignancies from different countries, and this definition has clinical, prognostic, and possible pathogenetic significance. We have validated on a large retrospective case series from the USA and Italy the algorithmic approach proposed by the Turin Consensus Meeting and have verified that the application of these criteria to daily practice is a sound approach for diagnosing poorly differentiated carcinomas. The prevalence of poorly differentiated carcinomas was higher in the mountainous areas of Northern Italy than in the USA (6.7 *versus* 1.8%, respectively), the prevalence in USA being close to the $< 1\%$ prevalence reported in Japan.[1] These findings indicate geographic differences in the prevalence of this tumor type, in agreement with a higher incidence in areas having iodine deficiency.

Poorly differentiated carcinomas are clinically intermediate between well-differentiated follicular or papillary carcinomas and anaplastic carcinomas: poorly differentiated carcinoma-affected patients had a mean 5-year survival of 71.6% and a 10-year survival of 46.3%. Furthermore, our results indicate that the inclusion of these tumors into a distinct category, comprehensive also of cases with oncocytic features, appears justified. It is noteworthy that the oncocytic variant of poorly differentiated carcinoma was not originally considered in the Turin Consensus Meeting despite the fact that thyroid carcinomas having prevalent oncocytic features and meeting the diagnostic criteria for poorly differentiated carcinomas had been reported in the literature.[25,26]

In our study, no significant differences in overall survival, disease-free survival, and metastasis-free survival were found between the poorly differentiated carcinomas with oncocytic features and the other groups by univariate analysis. However, detailed studies on this variant are still lacking and would be useful to better clarify the histogenesis of such tumors.

Clearly, the Turin proposal is only focused on solid/trabecular/insular tumors and does not refer to non-solid tumors showing tumor necrosis/high mitotic activity. One such examples is represented by aggressive variants of papillary thyroid carcinoma.[27]

The diagnosis of poorly differentiated carcinoma is primarily made on morphological criteria in H&E-stained sections. Immunohistochemistry is usually not necessary to classify a lesion in this group, but can be helpful in the confirmation of follicular-cell derivation. In fact, the solid/trabecular/insular growth pattern in poorly differentiated carcinomas might be a source of difficulties in differential diagnosis from

Table 2 Kaplan–Meyer univariate analyses for clinical and pathologic variables

Predictor	HR (CI) for overall survival	P-value	HR (CI) for disease-free survival	P-value	HR (CI) for recurrence-free survival	P-value	HR (CI) for MTS LN-free survival	P-value	HR (CI) for MTS distant-free survival	P-value
All cases (n = 152)										
Male	1.15 (0.71, 1.87)	0.56	1.14 (0.68, 1.92)	0.61	1.94 (0.79, 4.79)	0.15	1.88 (0.81, 4.33)	0.14	1.40 (0.83, 2.36)	0.21
Age ≥45 years	4.26 (1.69, 10.76)	<0.01	4.04 (1.46, 11.20)	<0.01	0.88 (0.29, 2.65)	0.81	1.45 (0.43, 4.94)	0.55	2.99 (1.18, 7.56)	0.02
Tumor size ≥4 cm	1.93 (1.02, 3.65)	0.04	1.63 (0.84, 3.16)	0.15	2.98 (0.68, 13.01)	0.15	7.12 (0.94, 53.80)	0.06	1.47 (0.73, 2.94)	0.28
Necrosis	1.51 (0.88, 2.60)	0.13	1.57 (0.88, 2.79)	0.12	0.92 (0.36, 2.34)	0.86	1.83 (0.67, 4.96)	0.24	0.90 (0.52, 1.54)	0.69
Convoluted nuclei	0.61 (0.35, 1.05)	0.08	0.46 (0.24, 0.86)	0.02	0.64 (0.23, 1.77)	0.39	0.47 (0.16, 1.38)	0.17	0.51 (0.27, 0.97)	0.04
Mitoses ≥3	1.08 (0.39, 2.98)	0.88	1.35 (0.42, 4.31)	0.62	NA[a]	0.10	NA[b]	0.06	1.40 (0.44, 4.47)	0.57
Atypical mitoses	1.19 (0.67, 2.12)	0.55	1.22 (0.66, 2.26)	0.53	2.74 (1.08, 6.98)	0.03	2.69 (1.13, 6.42)	0.03	0.81 (0.41, 1.59)	0.54
Vascular invasion	1.33 (0.77, 2.28)	0.30	1.69 (0.91, 3.14)	0.09	2.42 (0.70, 8.35)	0.16	1.55 (0.57, 4.21)	0.39	1.50 (0.82, 2.74)	0.19
Insular growth pattern	1.21 (0.75, 1.95)	0.44	1.12 (0.67, 1.88)	0.65	0.71 (0.29, 1.76)	0.47	1.00 (0.43, 2.31)	1.00	0.94 (0.56, 1.58)	0.82
Oxyphilic features	1.27 (0.77, 2.09)	0.34	1.33 (0.78, 2.27)	0.29	1.19 (0.45, 3.13)	0.73	1.93 (0.83, 4.47)	0.13	0.93 (0.53, 1.63)	0.80
Groups 2–4 vs 1	1.17 (0.71, 1.93)	0.54	1.11 (0.65, 1.89)	0.70	0.94 (0.37, 2.39)	0.90	1.85 (0.68, 5.03)	0.23	1.08 (0.62, 1.87)	0.79
Group 4 vs 1	1.29 (0.72, 2.29)	0.39	1.28 (0.70, 2.34)	0.43	1.08 (0.36, 3.22)	0.89	2.36 (0.80, 6.92)	0.12	1.01 (0.53, 1.93)	0.98
Turin vs Mayo	0.97 (0.59, 1.57)	0.89	0.79 (0.47, 1.33)	0.37	0.73 (0.29, 1.84)	0.51	0.94 (0.40, 2.21)	0.89	0.61 (0.36, 1.04)	0.07
WHO criteria	1.51 (0.88, 2.60)	0.13	1.57 (0.88, 2.79)	0.12	0.92 (0.36, 2.34)	0.86	1.83 (0.67, 4.96)	0.24	0.90 (0.52, 1.54)	0.69
Immunohistochemistry cases (n = 103)										
Turin vs Mayo	1.45 (0.59, 3.53)	0.41	1.36 (0.55, 3.37)	0.51	0.58 (0.11, 3.13)	0.52	0.88 (0.14, 5.70)	0.89	0.99 (0.37, 2.66)	0.98
IMP3 positive	6.97 (2.66, 18.28)	<0.01	29.38 (7.08, 121.85)	<0.01	6.55 (1.33, 32.40)	0.02	7.09 (1.42, 35.43)	0.02	15.65 (3.69, 66.36)	0.01
MIB-1/Ki67 (continuous)	1.02 (0.96, 1.07)	0.59	1.02 (0.97, 1.08)	0.45	1.10 (1.00, 1.20)	0.05	1.16 (1.01, 1.32)	0.04	1.03 (0.97, 1.09)	0.38

[a]All recurrences had mitoses ≥3; unable to estimate the hazards ratio.
[b]All metastasis to lymph nodes had mitoses ≥3; unable to estimate the hazards ratio.

Table 3 Results of multivariate Cox regression analysis

Predictor	HR (CI) for overall survival	P-value	HR (CI) for disease-free survival	P-value	HR (CI) for MTS distant-free survival	P-value
All cases (n = 152)	(n = 144)		(n = 144)		(n = 129)	
Age ≥45 years	4.81 (1.90, 12.23)	0.001	4.70 (1.68, 13.12)	0.003	3.85 (1.49, 9.91)	0.005
Convoluted nuclei	0.52 (0.30, 0.91)	0.02	0.40 (0.21, 0.75)	0.005	0.39 (0.20, 0.76)	0.005
Immunohistochemistry cases (n = 103)	(n = 103)		(n = 103)		(n = 89)	
Necrosis	4.47 (1.34, 14.90)	0.02	2.64 (1.05, 6.66)	0.04	4.22 (1.12, 15.86)	0.03
IMP3 positive	8.96 (3.11, 25.84)	<0.001	56.08 (11.47, 274.14)	<0.001	36.70 (6.68, 201.79)	<0.001

Final models using only the predictors found significant through stepwise models. Only those subgroups/outcomes with >1 significant predictor are shown.

other malignant lesions within the thyroid, including medullary thyroid carcinoma, parathyroid neoplasms, and metastatic tumors. In this respect, when a suspicion is raised by the morphological appearance, immunohistochemistry is advisable to avoid possible pitfalls. In particular, a peculiar pattern of thyroglobulin staining as dot-like paranuclear deposits (as originally described by some of us[28]) was observed in most of the poorly differentiated carcinoma cases of the present series (77%).

In our series, immunohistochemical markers thought by some to be diagnostic of malignancies, such as HBME-1 and p53, were often positive in poorly differentiated carcinomas, although the distribution of the former marker was more focal than in well-differentiated thyroid carcinomas. Thus, the usefulness of these markers in poorly differentiated carcinoma diagnosis is very limited. Similarly, an increase of proliferation index is associated with decrease of differentiation in thyroid tumors,[29] and Martyniak *et al*[30] suggested that p53 and Ki67 stains can assist in cases where diagnosis of poorly differentiated carcinoma is suspected on the basis of growth pattern, but lacking convoluted nuclei, increased mitotic count, or necrosis. In our experience, Ki67 might thus replace mitotic count as a more objective means of evaluating cell proliferation, but in well-preserved tissues, only.

In our study, IMP3 expression can significantly predict overall survival (HR for IMP3 positive *versus* negative = 8.96, P<0.001), disease-free survival (HR for IMP3 positive *versus* negative = 56.08, P<0.001), and metastasis-free survival (HR positive *versus* negative = 36.7, P<0.001) in poorly differentiated carcinoma of the thyroid. The identification of the IMP3 expression as a prognostic parameter segregating aggressive from more indolent poorly differentiated carcinoma cases may bear important clinical implications. IMP3 can be evaluated by immunohistochemistry on cytological specimens and could potentially be incorporated into the routine practice.

Recently, Volante *et al*[23] showed that *RAS* mutations are present in a fraction of poorly differentiated carcinomas and may be clinically relevant for the prognosis of patients affected by poorly differentiated carcinoma. Our study, comprehensive of 32 cases (14 already reported in a previous study[23]), supports and extends the above finding: in fact *NRAS* gene mutations in codon-61 were found in 19% of the current poorly differentiated carcinoma cases. In addition, it is interesting that 4 of 6 poorly differentiated carcinoma cases showing *NRAS* gene mutations were immunoreactive for IMP3, with a high IMP3 mRNA fold change at qRT-PCR.

In conclusion, poorly differentiated carcinomas of the thyroid are clinically and histologically intermediate between well-differentiated follicular or papillary carcinomas and anaplastic carcinomas. This study of a large tumor series selected on the basis of the Turin consensus criteria shows that poorly differentiated carcinoma is present in different geographical areas and has a biological behavior that is distinct from well-differentiated and anaplastic carcinomas of the thyroid. Poorly differentiated carcinomas in the USA and Italy showed similar overall survival, although the prevalence of poorly differentiated carcinoma is higher in the mountainous areas of Northern Italy. In addition, the variant of poorly differentiated carcinoma with oncocytic features shares the same behavior as other poorly differentiated carcinomas. Therefore, the classification of poorly differentiated carcinoma into a distinct entity, which also encompasses oncocytic poorly differentiated carcinomas, appears justified.

Furthermore, identification of the prognostic parameters differentiating highly aggressive from rather indolent cases has important clinical implications. Interestingly, our study identified a potential new prognostic marker: in fact IMP3 was a predictor at poor prognosis of poorly differentiated carcinoma, in both univariate and multivariate analysis, and detection of IMP3 by immunohistochemistry appears to be a useful prognostic factor for poorly differentiated carcinoma and it has potential for application on cytological specimens.

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Disclosure/Conflict of interest

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