

Multinucleated Giant Cells' Incidence, Immune Markers, and Significance: A Study of 172 Cases of Papillary Thyroid Carcinoma

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Abstract Multinucleated giant cells (MGCs) are often detected in cases of papillary thyroid carcinoma (PTC). Their origin and significance, however, has not been established. One possibility is that they form in response to injury induced by fine needle aspiration biopsy (FNAB). Other hypotheses are that the chemically-altered colloid produced by PTC induces MGCs to act as colloidophages, or else MGCs are a non-specific immune response ingesting neoplastic follicle cells. We assigned 172 cases of PTC a semi-quantitative score for MGCs. Cases with “many” MGCs were immunohistochemically stained for AEI/AE-III, CD68, and CD163 to assess for epithelial vs histiocytic differentiation, and for thyroglobulin and TTF-1 to assess for MGC ingestion of colloid or thyroid follicle cells respectively. Overall, we identified MGCs in 100/172 (58.1%) PTC specimens; in 45 (26.2%), “many” MGCs were found, while in 55 (31.9%) MGCs were “few.” The mean sizes of PTC in cases with many as opposed to rare/no MGCs was 2.50 cm vs 1.8 [$P = 0.003$]. The cases of PTC with many MGCs had higher multifocality (26/45 vs 51/127 [$P = 0.06$]), extrathyroidal extension (21/45 vs 36/127 [$P = 0.03$]), and recurrence (8/45 vs 9/127 [$P = 0.08$]), than did cases with rare or no MGCs. The

majority of patients both with and without numerous MGCs had previous histories of FNA or hemilobectomy: 40/45 and 99/127 respectively ($P = 0.062$). The majority of MGCs were positive for CD68 (45/45), CD163 (44/45), thyroglobulin (34/45) and negative for AEI/AEIII (44/45) and TTF-1 (44/45). These results indicate that MGCs in PTC are of histiocytic origin. Cases of PTC with many MGCs have a significantly greater likelihood of extrathyroidal extension and greater tumor size than cases with few/no MGCs. MGCs appear to be functioning largely as colloidophages.

Keywords Multinucleated giant cells · Papillary thyroid carcinoma · Thyroglobulin · Colloid

Introduction

Multinucleated giant cells (MGCs) are often associated with papillary thyroid carcinoma (PTC). While their presence has been well-documented in cytologic preparations, there are relatively few studies of MGCs in histologic sections of PTC. Estimates of the prevalence of MGCs in histologic specimens vary widely from 46% to 100%, and their origin has not been well-established [1–3]. One possible explanation for the presence of MGCs in PTC is that they form as a response to prior fine needle aspiration biopsy (FNAB): patients with thyroid malignancies commonly undergo a diagnostic FNAB prior to surgery, and MGCs may be induced by the subsequent degeneration and inflammation of the surrounding follicular epithelium. Another explanation is that MGCs form in response to the abnormal colloid produced by PTCs. Previous studies have suggested that the colloid secreted by PTC is biochemically different than that secreted by the normal thyroid [4, 5]. A

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third possibility is that the MGCs evolve as a non-specific immune response to the carcinoma itself, and act as phagocytes ingesting the malignant follicle cells. MGCs in the thyroid are not specific for PTC: they can be found in benign thyroid diseases such as Hashimoto's, De Quervain's, or palpation thyroiditis, as well as in other malignancies such as follicular and anaplastic carcinoma [1, 2, 6, 7]. The prognostic significance of MGCs in PTC is uncertain. In the current study, we evaluated 172 cases of PTC for the presence of MGCs, scoring them in a semi-quantitative fashion. Those cases with many MGCs were correlated with clinicopathologic parameters including patient age at diagnosis, tumor median size, extrathyroidal extension, recurrence, multifocality to assess for possible prognostic significance of MGCs in PTC. We also performed a panel of immunohistochemical stains to better elucidate the origin and nature of MGCs in PTC, including antibodies to epithelial, histiocytic, and thyroid markers.

Materials and Methods

Study Population and Histopathological Evaluation

Having obtained University of Vermont Institutional Review Board approval, a total of 172 cases of papillary thyroid carcinoma were retrieved following computer diagnostic search from the archives of Fletcher Allen Health Care. The cases were diagnosed between the years of 1989–2003 on either complete thyroidectomy or thyroid lobectomy specimens from a total of 168 patients. Five patients had initial lobectomies or hemilobectomies, and subsequent completion surgeries. In four of these cases, we were able to examine the initial as well as final surgical specimens. Patients ranged in age from 9 to 82 years with a male to female ratio of 51:117. The histologic diagnosis was rendered on the basis of previously well-established nuclear criteria, including enlargement, elongation, crowding, irregular contours, grooves, pseudoinclusions, chromatin clearing with peripheral margination, and

multiple macronucleoli, as well as architectural features [8, 9]. No histopathological variants of papillary thyroid carcinoma were excluded. All cases with diagnoses other than papillary carcinoma or follicular variant of papillary carcinoma were carefully reviewed; in order to be designated as a variant, tumors were required to predominantly manifest variant histopathologic features as specified by the 2004 World Health Organization criteria [9]. Ultimately, cases included a total of 43 follicular variants, two oncocyctic variants, one diffuse sclerosing variant, and one columnar cell variant. The remaining 125 cases were simply diagnosed as papillary thyroid carcinoma. An average of four hematoxylin and eosin stained slides were examined per case by two pathologists concurrently. When assessing slides for the presence of MGCs, certain strict histologic criteria were employed (Fig. 1).

1. MGCs needed to be located within cystic spaces.
2. They must have glossy dense eosinophilic cytoplasm (either with or without hemosiderin or vacuoles) [6, 10].
3. There must be at least three randomly assorted nuclei.

Important exclusionary criteria for MGCs included the presence of fibrovascular cores or any nuclear features suggestive of PTC, such as nuclear grooves or chromatin clearing with peripheral margination (Fig. 2). Any one of these features was considered indicative of a sloughed papillae and was deemed sufficient cause to exclude the entity from consideration as an MGC.

A semi-quantitative score was assigned as follows: 0 MGCs per slide = none, 1–2 MGCs per slide in areas of greatest density = few, ≥ 3 MGCs per slide in area of greatest density = many.

Immunohistochemistry

Immunohistochemical stains were performed on sections cut from formalin-fixed, paraffin-embedded papillary thyroid carcinoma cases with “many” MGCs. In order to distinguish the origin of MGCs, we utilized mouse

Fig. 1 Papillary thyroid carcinoma. Multinucleated giant cells are present within cystic spaces. Characteristic features include dense eosinophilic cytoplasm, well-demarcated borders, and a profusion of randomly assorted nuclei (a–b)

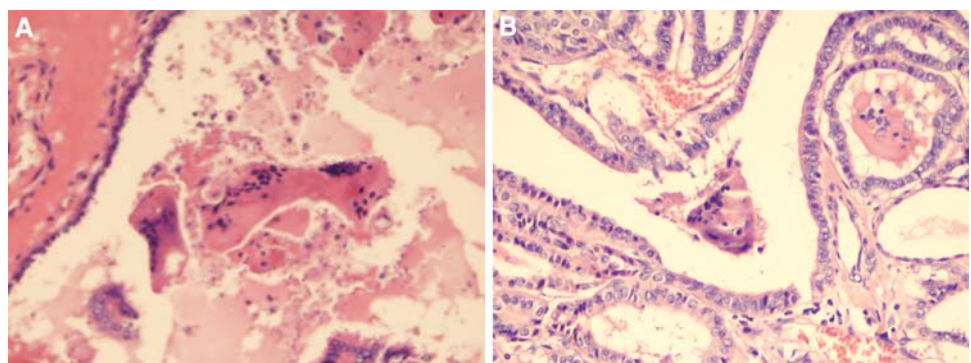
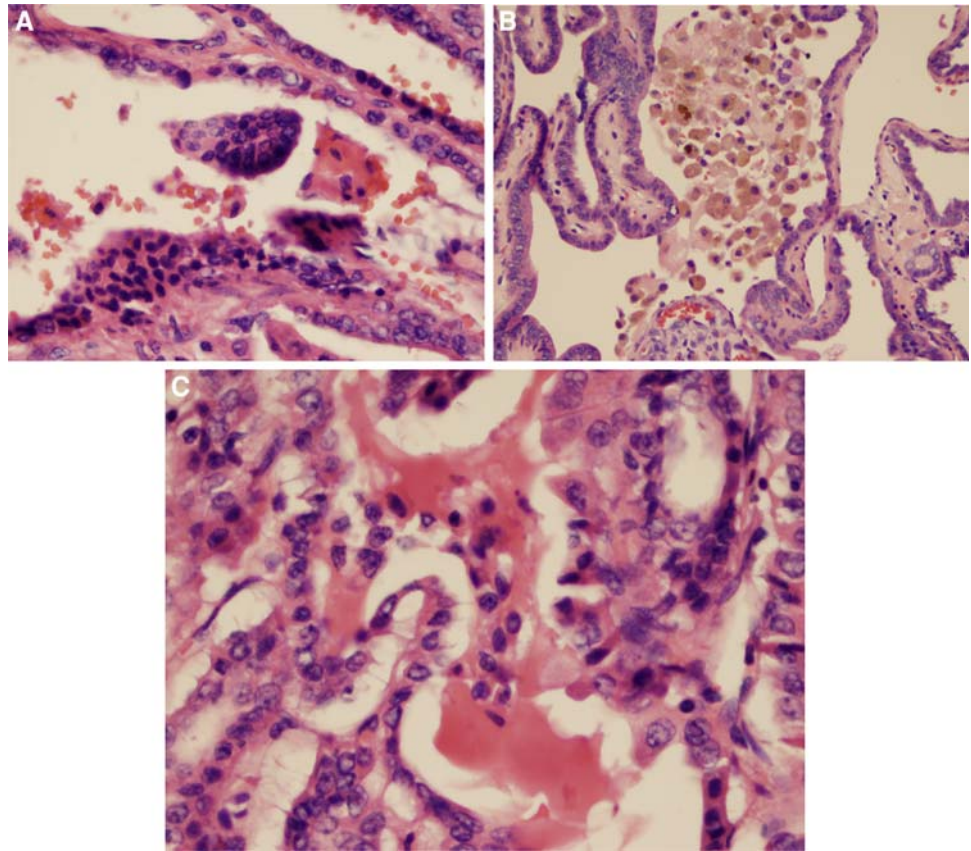


Fig. 2 Common histologic entities that can mimic multinucleated giant cells. Sloughed papillae, which can be distinguished by the presence of fibrovascular cores and/or nuclear features of papillary thyroid carcinoma (a). Grouped histiocytes, which can be distinguished by the lack of a well-defined communal border (b). Colloid containing degenerated nuclei, which can be distinguished by the ill-defined borders which tend to replicate the shape of the cyst as well as the hyperchromatic attenuated nuclei



monoclonal antibodies to epithelial cytokeratins (clones AE1/AE3, Dako #M3513, used at a dilution of 1:800) as well as to histiocytic markers, CD68 (clone KP1, Dako, used at a dilution of 1:1600) and CD163 (clone 10D6, Vector Laboratories #VP-C374, used at a dilution of 1:20). In order to assess the function of MGCs, we utilized mouse monoclonal antibodies to Thyroglobulin (clone DAK Tg6, Dako #M0781, used at a dilution of 1:4000) and Thyroid Transcription Factor 1 (TTF-1) (clone SPT24, Vector Laboratories #VP-T483, used at a dilution of 1:100). Our negative control consisted of IgG1 (Dako #X0931 used at a dilution of 1:50). We performed antigen retrieval in a citrate buffer at a pH of 6.1 (Dako #S1699) by incubation at 98°C for 20 min. All primary antibodies were incubated for 30 min at room temperature and were detected using the EnVision™ + Dual Link kit (Dako #K4065) with DAB staining.

MGCs displaying a granular cytoplasmic staining pattern for CD68 and/or CD163 were interpreted as being of histiocytic origin; whereas, MGCs staining positively for AEI/AEIII were interpreted as being of epithelial origin. MGCs displaying cytoplasmic positivity for thyroglobulin were interpreted as having ingested colloid; whereas, MGCs showing nuclear positivity for TTF-1 were interpreted as having ingested follicular cells.

Statistical Analysis

All statistical analysis was performed with SAS version 9 (SAS Institute Inc., Cary, NC). Descriptive statistics were used to report our findings. The prevalence of MGCs was reported as a proportion with its exact binomial confidence interval. Tumor characteristics among cases with and without MGCs were reported as frequencies for each category. Fisher's exact test was used to compare cases with and without MGCs when the variables were categorical. The Mann–Whitney test was employed when variables were ordinal, i.e., tumor size. The *t*-test was used for the age comparison.

Results

MGCs were identified in 100/172 (58.1%, 95% CI: 50.4%, 65.6%) PTC specimens; in 45 (26.2%, 95% CI: 19.8%, 33.4%), “many” MGCs were found, while in 55 (31.9%, 95% CI: 25.1%, 39.5%) MGCs were “few” (Table 1). Of the four patients in which both initial as well as subsequent lobectomy/hemilobectomy specimens were examined, all had few to no MGCs. MGCs tended to be located in cystic spaces or else within follicles, both of which are regions of

Table 1 Clinicopathologic features of papillary thyroid carcinoma cases with many versus few/no multinucleated giant cells

	Many MGCS (<i>N</i> = 45)	Few/No MGCS (<i>N</i> = 127)	Statistical significance
Average patient age at diagnosis	42.2 years (range: 20–82)	43.7 years (range: 9–82)	<i>P</i> = 0.57
Median tumor size	2.5 cm (range: 0.2–9.0)	1.8 cm (range: 0.01–5.2)	<i>P</i> = 0.003
Tumor multifocality	26 (57.8%)	51 (40.2%)	<i>P</i> = 0.06
Extrathyroidal extension of tumor	21 (46.7%)	36 (28.3%)	<i>P</i> = 0.03
Tumor recurrence	8 (17.8%)	9 (7.1%)	<i>P</i> = 0.08
Prior FNA or hemilobectomy	40 (88.9%)	99 (78.0%)	<i>P</i> = 0.62

colloid deposition. The cases with “many” MGCs consisted of a mixture of papillary (*n* = 39) and follicular variant of papillary (*n* = 6) histology. In cases with few or no MGCs, the most common histologic pattern was also papillary (*n* = 86), followed by follicular variant (*n* = 37). Other variants included oncocyctic (*n* = 2), diffuse sclerosing (*n* = 1), and columnar (*n* = 1). Overall, follicular variants of papillary thyroid carcinoma were far more likely to show few or no MGCs than many: 37/43 (86.0%, 95% CI: 72%, 95%) vs 6/43 (14.0%, 95% CI: 5%, 28%). The average age of patients with numerous as opposed to rare/none MGCs was 42.2 (range 20–82) vs 43.7 (range 9–82) years. This difference was not statistically significant [*P* = 0.57]. The mean sizes of PTC in cases with many as opposed to rare/no MGCs was 2.50 cm (range 0.2–9.0 cm) vs 1.76 (range 0.01–5.2 cm) [*P* = 0.0025]. The cases of PTC with many MGCs had higher multifocality (26/45 vs 51/127 [*P* = 0.055]), extrathyroidal extension (21/45 vs 36/127 [*P* = 0.028]), and recurrence (8/45 vs 9/127 [*P* = 0.076]), than did cases with rare or no MGCs. The majority of patients both with and without numerous

MGCs had previous histories of FNA or hemilobectomy: 40/45 and 99/127 respectively [*P* = 0.073]. Immunohistochemistry performed on cases of PTC with numerous MGCs revealed the majority of MGCs to be positive for CD68 (45/45), CD163 (44/45), thyroglobulin (34/45) and negative for AEI/AEIII (44/45) and TTF-1 (44/45) (Fig. 3).

Discussion

The results of this study indicate that MGCs can be found in at least half of PTC cases (100/172; 58.1%), and that they are most likely of histiocytic rather than epithelial origin. The majority of MGCs demonstrated a granular cytoplasmic staining pattern for both histiocytic markers CD68 (45/45) and CD163 (44/45) and were negative for epithelial marker AEI/AEIII (44/45). This finding agrees with those of previous studies. Guiter et al. examined 76 cases of PTC, and found MGCs in histologic sections in 46% (35/76). A panel of immunohistochemical stains was performed on ten of the cases: MGCs were uniformly

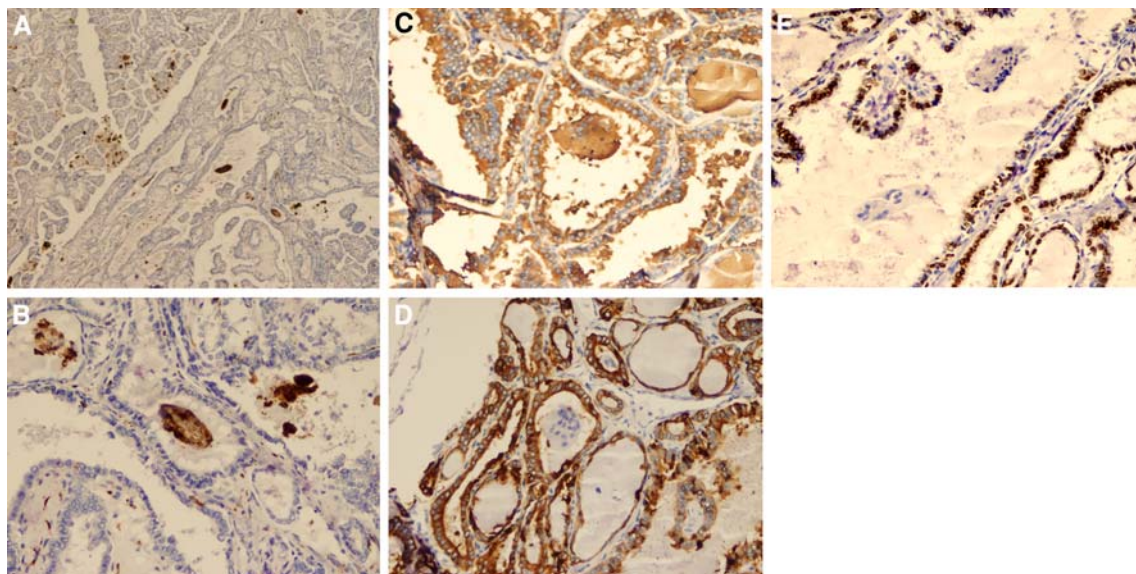


Fig. 3 Multinucleated giant cells showed a cytoplasmic staining pattern for CD68 (a), CD163 (b), and Thyroglobulin (c) in the majority of cases. They showed a lack of cytoplasmic and nuclear positivity for AEI/AEIII (d) and TTF-1 (e) respectively

negative for AEI/AEIII cytokeratins, and positive for lysozyme, KP1/CD68, and alpha-1-antichymotrypsin—indicating a histiocytic rather than epithelial origin. Tabbara et al. compared ten cases of PTC, eight cases of follicular variant of PTC, and 11 cases of follicular adenomas. MGCs were present in 100% of cases of PTC, 63% of follicular variant cases, and 0% of follicular adenomas. Immunohistochemistry was performed on 15 cases with MGCs; however, not all immunostained sections ultimately contained MGCs. Of those sections which did, all MGCs were negative for epithelial markers EMA and AEI/AEIII, and the majority were positive for one or more histiocytic markers.

Unlike Tabbara et al., our results indicate that MGCs in PTC function primarily as colloidophages. The majority demonstrated cytoplasmic positivity for thyroglobulin (34/45; 75.6%), and the dense, glossy, eosinophilic appearance of the cytoplasm was reminiscent of colloid. Also, MGCs tended to be located either within cystically dilated spaces or else within follicles, both of which are regions of colloid deposition. It seems likely that the chemically altered colloid produced in PTC plays a role in generating the MGC reaction. There appears to be no linkage between the injury induced by previous FNA and the appearance of MGCs, as the majority of patients both with and without “many” MGCs had histories of prior FNA and/or hemilobectomy. There also appears to be no evidence to support the theory that MGCs act as a non-specific immune response, ingesting malignant follicle cells. Thyroid follicular cells normally express TTF-1 in an intranuclear staining pattern. In only one case in our series did MGCs show intranuclear positivity for TTF-1, suggesting follicular cell phagocytosis.

We found that there was a definite prognostic significance to having “many” MGCs in PTC. Those cases of PTC with many MGCs were statistically more likely to have extra-thyroidal extension ($P = 0.028$) and greater tumor size ($P = 0.0025$) at surgical resection than those with few/no MGCs. We speculate that this may be attributable to the fact that larger tumors more frequently have cystically dilated spaces. The resulting larger amount of colloid deposition could stimulate greater than average MGC colloidophagy. Interestingly, cases of the follicular variant of PTC, which tend to be composed of small to moderate-sized follicles rather than cystically dilated spaces, had a far lower likelihood of having “many” MGCs than did cases of non-variant PTC. While not statistically significant, we did see a trend in which cases with “many” MGCs showed a greater

probability of multifocality and recurrence than cases with few/no MGCs ($P = 0.055$ and $P = 0.076$ respectively). We found no clinicopathologic correlation between average patient age at diagnosis and the presence of MGCs.

To our knowledge, this study represents the most comprehensive analysis of MGCs in PTC to date. Although MGCs have long been known as associated features of PTC, their significance was uncertain. Our results, however, suggest that the finding of multiple MGCs may be an important prognostic indicator and that cases of PTC should therefore be carefully screened for their presence. In cases of multiple MGCs, a more aggressive surgical approach and/or more careful post-surgical follow-up may be merited.

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