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Medullary Thyroid Cancer That Stains Negative For CA 19-9 Has Decreased Metastatic Potential

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Abstract

Objective—Presently, no clinical tools are available to diagnose the metastatic potential of medullary thyroid cancer (MTC) at disease presentation. Surveillance with calcitonin and carcinoembryonic antigen is currently recommended for observation and diagnosis of metastatic disease after initial treatment of MTC. Recently, CA 19-9 staining has been associated with aggressive forms of MTC and metastatic spread. This pilot study explores if positive CA19-9 staining of MTC tissue is associated with its metastatic potential.

Methods—Sixteen cases of MTC were identified and the specimens immunostained for CA 19-9 and other MTC tumor markers. Clinical information about patients' MTC was collected through a retrospective chart review.

Results—63% of the specimens stained positive for CA19-9. The median size of positively staining specimens was 2.6 cm (interquartile range 1.2 cm–3.2 cm) compared to 0.7cm (0.5 cm–1.2 cm) in negatively staining MTC specimens ($p=0.04$). All specimens from patients diagnosed with stage IV MTC, stained positive for CA19-9 compared to only 40% of cases that were classified as stage I–III ($p=0.03$). Furthermore, 100% of the primary specimens that were documented to have metastatic spread, stained positive for CA19-9. The sensitivity for ruling out stage IV MTC based on negative staining for CA 19-9 was 100%.

Conclusion—Based on these results we conclude that negative staining of MTC for CA19-9 may be associated with its decreased metastatic potential.

Keywords

medullary thyroid cancer; CA19-9; MTC

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Introduction

Calcitonin (Ct) and carcinoembryonic antigen (CEA) are markers routinely used for diagnosis and surveillance of medullary thyroid cancer (MTC)(1, 2). CA19-9, a well-studied marker of pancreatic and gastrointestinal malignancies, was also detected in approximately 6 percent of pathologic specimens of MTC, although it has not been associated with clinically relevant parameters (3). A case report published by our group in 2011 was the first to link positive staining of MTC tissue for CA19-9 to aggressive metastatic spread of the disease (4). Since then, a similar case was described, in which rapidly metastasizing MTC stained positive for CA19-9 (5).

At present, it is often difficult to detect or predict distant metastatic spread of MTC at the time of initial diagnosis by utilizing the currently available clinical tools. Thus, clinicians often rely on post-operative monitoring of Ct and CEA levels to detect persistent or recurrent disease. However, the previously reported association between CA19-9 and metastatic spread of MTC raises the possibility that this marker may have a novel application in predicting metastatic potential of MTC, possibly even at the time of initial diagnosis. Therefore, we conducted a pilot study to explore if positive CA19-9 staining of MTC tissue predicts its metastatic potential.

Materials and Methods

Material Retrieval

We conducted an observational retrospective pilot study using the specimens and medical records of individuals who had a diagnosis of MTC and whose specimens were available for pathological evaluation within the Montefiore Medical Centers, Albert Einstein College of Medicine, Bronx, NY. Individuals with a pathological diagnosis of MTC between January 1, 1997 and May 31, 2011 were identified using Clinical Looking Glass, a search engine linked to the Montefiore electronic clinical records and a Montefiore pathology database. Additional cases that occurred during the above specified time frame were identified via collaboration with Montefiore affiliated endocrinologists, otolaryngologists, and endocrine surgeons.

Immunohistochemistry

The pathology department retrieved archived formalin-fixed paraffin embedded tissue blocks, hematoxylin and eosin-stained slides and immunohistochemically (IHC) stained slides for each subject. On review no discrepancies in histopathologic diagnosis or interpretation of IHC staining were identified when compared to the original pathology report. All previous IHC stained slides were included for analysis. For subjects whose complete set of IHC stained slides was unavailable and for the IHC analysis required for this study, additional sections of formalin-fixed paraffin embedded tissue were cut and prepared for IHC staining. We utilized the following antibodies: calcitonin (Dako Denmark), CEA polyclonal (Dako Denmark), CA 19-9 (Dako North America, Inc.), CA 125 (Leica), chromogranin (Dako Denmark) and synaptophysin (Dako Denmark). The slides for IHC analysis were stained with an automatic slide stainer (Dako LV-1 Autostainer/Dako universal staining system) for

30 minutes at room temperature followed by the secondary antibody. 3,3'-Diaminobenzidine was used as the chromogen. The slides were counterstained with hematoxylin. Appropriate negative controls were included. Two pathologists, a resident and a senior pathologist, independently evaluated all IHC stained slides using simple light microscopy. Any discrepancies were reviewed together and a final determination was made based on consensus. Each of the six IHC stained slides per subject was categorized by the percentage of tumor cells staining. We utilized the following four categories: no staining (0% of cells), individual cells (<5% of cells), focal/scattered (5–49% of cells), and diffuse (>50% of cells). Two endocrinologists reviewed the subjects' medical charts and collected pertinent clinical information. The endocrinologists were blinded to the results of the IHC staining for CA 19-9 at the time of the chart review. The study was approved by the Albert Einstein College of Medicine Institutional Review Board.

Statistical Analysis

Statistical analysis was performed using STATA software, version 12 (StataCorp LP, College Station, TX). Normality was assessed by inspection of empirical distributions. Group characteristics were compared using bivariate statistics, with non-parametric tests applied when appropriate. Fisher's exact test was employed to assess the association between CA19-9 staining and MTC characteristics. IHC staining patterns were dichotomized into a group with absent staining (CA19-9 Negative) and a group with identifiable staining (CA19-9 Positive). IHC staining for CA 19-9 was considered positive if the staining included either of the following patterns: individual cells, focal/scattered or diffuse staining. A computed p-value ≤ 0.05 was considered to be statistically significant.

Results

Sixteen cases were identified with pathological specimens available for analysis. Clinical and pathologic characteristics of the cases are detailed in the Table. Nine of the cases were female and seven had hereditary forms of MTC. Of the identified cases, 12 were primary thyroid specimens, 3 were metastatic lesions and 1 was a locally recurrent invasive MTC lesion.

All of the specimens stained positive for calcitonin, CEA, and chromogranin, confirming the diagnosis of MTC. Ninety-four percent of specimens stained positive for synaptophysin. All of the specimens stained negative for CA 125. Of all the specimens, 62.5% stained positive for CA 19-9. Specimens that stained positive were defined by having individual cells, focal/scattered and diffuse staining patterns for CA 19-9 (see Figure). The primary thyroid MTC lesions that stained positive for CA19-9 were larger than the lesions that stained negative for CA19-9, median tumor size (interquartile range) 2.6 cm (1.2 cm–3.2 cm) vs. 0.7cm (0.5 cm–1.2 cm), respectively (p=0.04).

All cases that were classified as stage IV, based on the presence of distant metastases or extension beyond the thyroid capsule and invasion of soft tissues, stained positive for CA 19-9 compared to only 40% of cases that were classified as stage I-III (p=0.03). Furthermore, 100% of the primary specimens that were documented to have metastatic spread, stained positive for CA 19-9. On the other hand, none of the six cases that stained

negative for CA 19-9 were identified to have metastatic disease. Specimens that were associated with later stages of MTC were more likely to have a greater percentage of cells that stained positive for CA 19-9. For example, 5/6 (83.3%) of Stage IV cases demonstrated focal/scattered or diffuse staining. On the other hand, 1/5 (20%) of Stage I cases demonstrated focal/scattered staining and none demonstrated diffuse staining.

Positive staining for CA 19-9 was not associated with the presence of lymphatic invasion ($p>0.99$) or with inherited forms of MTC ($p>0.99$). Based on the results of this pilot study, negative staining for CA19-9 was determined to be 100% sensitive for ruling out stage IV MTC and metastatic disease.

Discussion

In this pilot study, we identified CA 19-9 as a marker associated with metastatic potential of MTC. None of the tumors that stained negative for CA 19-9 progressed to metastatic disease during the study period. On the other hand, all of the specimens that came from metastatic lesions or primary lesions with known metastases stained positive for CA 19-9. Another characteristic that was associated with positive CA 19-9 staining was larger size of the primary MTC lesions, suggesting that as the tumor grows it may acquire metastatic potential. CA 19-9 staining was not associated with lymphatic invasion, thus implying that it may play a role as a contributor to hematogeneous dissemination of MTC.

Positive staining for CA19-9 was previously reported in some MTCs (3); however, this pathological finding was not related to clinical findings. Our group first reported a case of very aggressive MTC that presented with elevated CA 19-9 levels in serum and diffuse metastases that stained positive for CA 19-9(4). Subsequently, a similar case was described in Italy (5). These reports and the results of our pilot study suggest that CA19-9 may be a marker for metastatic potential of MTC. Based on our pilot study, individuals whose MTC tumors stain negative for CA 19-9 may have a better prognosis and lower likelihood for developing recurrent or metastatic disease.

CA 19-9, is a carbohydrate Lewis blood group antigen, which is secreted by or expressed on the surface of cancer cells. It can be detected by monoclonal antibodies in the serum or tissue. Despite a limited understanding of the function of CA 19-9 in MTC, this tumor marker has been thoroughly investigated in pancreatic malignancies. In studies of patients with pancreatic cancer, higher preoperative CA 19-9 levels have been correlated with greater disease burden and shorter survival (6, 7). Post pancreatic resection CA 19-9 concentration has also been found to be a predictor of survival (8, 9).

In vivo and in-vitro studies have demonstrated that CA 19-9 may play an active role in metastatic spread of cancer (10, 11). Lewis blood antigens, including the epitope CA 19-9, bind to selectins located on platelets, leukocytes, and endothelial surfaces (12, 13). It is proposed that this interaction traps the cancer cell in the aggregate of platelets and leukocytes on the endothelial surface, allowing it to migrate into the extravascular space and establish metastatic foci (12, 14). Thus, this may represent a model for metastatic spread of MTC expressing CA 19-9.

Development of cancer therapies targeting carbohydrate tumor antigens is currently in progress. Conventional monoclonal antibodies, monoclonal antibodies bound to radioactive isotopes and therapeutic nanoparticles, as well as, vaccines targeting CA19-9 are undergoing investigation (11, 15, 16). Thus, improved understanding and characterization of the role of CA 19-9 in MTC may make it amenable to novel therapies in the future. Furthermore, several cases in the literature, including ours, have identified individuals with MTC whose levels of calcitonin decline or remain stable, despite progression of disease (4, 5, 17). CA 19-9 may be a useful tumor marker to follow these patients, if it is confirmed to be positive in that subgroup.

Our pilot study is the first to associate CA 19-9 with metastatic potential in MTC. We showed that positive staining of MTC for CA 19-9 is associated with metastatic spread of disease and that negative CA 19-9 staining is linked to less aggressive MTC without metastatic potential. Even though these findings are based on a small sample size, they may have important diagnostic and therapeutic implications for patient with MTC and should be confirmed with a larger future study.

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References

1. Barbet J, Campion L, Kraeber-Bodere F, Chatal JF. Prognostic impact of serum calcitonin and carcinoembryonic antigen doubling-times in patients with medullary thyroid carcinoma. *J Clin Endocrinol Metab.* 2005; 90(11):6077–84. [PubMed: 16091497]
2. Laure Giraudet A, Al Ghulzan A, Auperin A, et al. Progression of medullary thyroid carcinoma: assessment with calcitonin and carcinoembryonic antigen doubling times. *Eur J Endocrinol.* 2008; 158(2):239–46. [PubMed: 18230832]
3. Larena A, Vierbuchen M, Fischer R. Blood group antigen expression in malignant tumors of the thyroid: a parallel between medullary and nonmedullary carcinomas. *Langenbecks Arch Chir.* 1995; 380(5):269–72. [PubMed: 7500798]
4. Milman S, Whitney KD, Fleischer N. Metastatic medullary thyroid cancer presenting with elevated levels of CA 19-9 and CA 125. *Thyroid.* 2011; 21(8):913–6. [PubMed: 21751886]
5. Elisei R, Lorusso L, Romei C, et al. Medullary thyroid cancer secreting carbohydrate antigen 19-9 (Ca 19-9): a fatal case report. *J Clin Endocrinol Metab.* 2013; 98(9):3550–4. [PubMed: 23861463]
6. Maithel SK, Maloney S, Winston C, et al. Preoperative CA 19-9 and the yield of staging laparoscopy in patients with radiographically resectable pancreatic adenocarcinoma. *Ann Surg Oncol.* 2008; 15(12):3512–20. [PubMed: 18781364]
7. Safi F, Schlosser W, Falkenreck S, Beger HG. Prognostic value of CA 19-9 serum course in pancreatic cancer. *Hepatogastroenterology.* 1998; 45(19):253–9. [PubMed: 9496523]
8. Berger AC, Garcia M Jr, Hoffman JP, et al. Postresection CA 19-9 predicts overall survival in patients with pancreatic cancer treated with adjuvant chemoradiation: a prospective validation by RTOG 9704. *J Clin Oncol.* 2008; 26(36):5918–22. [PubMed: 19029412]
9. Montgomery RC, Hoffman JP, Riley LB, Rogatko A, Ridge JA, Eisenberg BL. Prediction of recurrence and survival by post-resection CA 19-9 values in patients with adenocarcinoma of the pancreas. *Ann Surg Oncol.* 1997; 4(7):551–6. [PubMed: 9367020]

10. Kawarada Y, Ishikura H, Kishimoto T, Kato H, Yano T, Yoshiki T. The role of sialylated Lewis antigens on hematogenous metastases of human pancreas carcinoma cell lines in vivo. *Pathol Res Pract.* 2000; 196(4):259–63. [PubMed: 10782470]
11. Ugorski M, Laskowska A. Sialyl Lewis(a): a tumor-associated carbohydrate antigen involved in adhesion and metastatic potential of cancer cells. *Acta Biochim Pol.* 2002; 49(2):303–11. [PubMed: 12362971]
12. Drake PM, Cho W, Li B, et al. Sweetening the pot: adding glycosylation to the biomarker discovery equation. *Clin Chem.* 2010; 56(2):223–36. [PubMed: 19959616]
13. Thomas SN, Zhu F, Schnaar RL, Alves CS, Konstantopoulos K. Carcinoembryonic antigen and CD44 variant isoforms cooperate to mediate colon carcinoma cell adhesion to E- and L-selectin in shear flow. *J Biol Chem.* 2008; 283(23):15647–55. [PubMed: 18375392]
14. Sanders DS, Kerr MA. Lewis blood group and CEA related antigens; coexpressed cell-cell adhesion molecules with roles in the biological progression and dissemination of tumours. *Mol Pathol.* 1999; 52(4):174–8. [PubMed: 10694936]
15. Girgis MD, Federman N, Rochefort MM, et al. An engineered anti-CA19-9 cys-diabody for positron emission tomography imaging of pancreatic cancer and targeting of polymerized liposomal nanoparticles. *J Surg Res.* 2013; 185(1):45–55. [PubMed: 23827791]
16. Heimbürg-Molinaro J, Lum M, Vijay G, Jain M, Almogren A, Rittenhouse-Olson K. Cancer vaccines and carbohydrate epitopes. *Vaccine.* 2011; 29(48):8802–26. [PubMed: 21964054]
17. Mendelsohn G, Wells SA Jr, Baylin SB. Relationship of tissue carcinoembryonic antigen and calcitonin to tumor virulence in medullary thyroid carcinoma. An immunohistochemical study in early, localized, and virulent disseminated stages of disease. *Cancer.* 1984; 54(4):657–62. [PubMed: 6378353]

Figure.
Medullary thyroid cancer cell immunostaining patterns for CA 19-9 at 100× power (A and B) and 200× power (C and D) with light microscopy. **A.** No staining. **B.** Individual cell staining. **C.** Focal/scattered cell staining. **D.** Diffuse cell staining.

Table

Clinical and pathologic characteristics.

Characteristic	CA 19-9 Negative (n=6)	CA 19-9 Positive (n=10)	p-value
Female, n	4	5	0.63
Male, n	2	5	
Sporadic, n	3	6	>0.99
Inherited, n	3	4	
Primary Unifocal, n	5	3	0.55
Primary Multifocal, n	1	3	
Metastatic lesion, n	0	3	n/a
Local Recurrence, n	0	1	n/a
Primary lesion with known distant metastases, n	0	2	n/a
Age (yr) at diagnosis for sporadic cases, median (IQR)	50 (48–58)	63 (60–75)	0.12
Age (yr) at diagnosis for inherited cases, median (IQR)	9 (8–30)	32.5 (15.5–51)	0.16
Primary tumor size ^a (cm), median (IQR)	0.7 (0.5–1.2)	2.6 (1.2–3.2)	0.04
Stage, n			
Stage I	3	2	0.03 ^b
Stage II	1	1	
Stage III	2	1	
Stage IV	0	6	
Lymphatic invasion present ^c , n	2	3	>0.99
Lymphatic invasion absent ^c , n	4	3	

IQR: interquartile range

^a n=12, includes only intrathyroidal lesions, excludes metastatic and recurrent lesions^b p-value for comparison between stage IV and all other stages combined^c n=12, includes intrathyroidal lesions only