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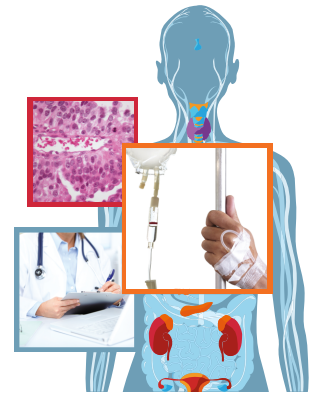

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Carcinoembryonic antigen should be concurrently checked with calcitonin to identify distant metastases in medullary thyroid cancer

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Aim: This study investigates if serum calcitonin or carcinoembryonic antigen (CEA) levels can differentiate between locoregional and metastatic medullary thyroid cancer. **Methods:** A single institution retrospective analysis was performed on 88 patients with medullary thyroid cancer between 2008 and 2014. **Results:** In M0disease, calcitonin ($p < 0.001$) and CEA ($p = 0.003$) significantly decreased postoperatively. Not only was the correlation significant between calcitonin and CEA preoperatively ($r = 0.72$; $p < 0.001$) and postoperatively ($r = 0.68$; $p < 0.001$), calcitonin could extrapolate CEA levels ($p < 0.001$). These findings were statistically insignificant in metastatic disease. **Conclusion:** Independently, calcitonin and CEA fail to differentiate between locoregional and metastatic disease. Both are essential for prognostication: loss of concordance is suspicious for metastatic disease. Hence, discordant CEA and calcitonin levels should be an indication to pursue additional imaging.

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Keywords: calcitonin • CEA • medullary thyroid cancer • metastases

Medullary thyroid cancer (MTC) is a rare thyroid malignancy that arises from the parafollicular C cells of the gland and comprises 1–5% of all thyroid cancers [1,2]. A large proportion of patients present with a solitary thyroid nodule or cervical lymphadenopathy. Common symptoms include dysphagia, coughing, hoarseness and dyspnea. Patients may also have symptoms of flushing and diarrhea associated with hypercalcitoninemia [2].

Serum calcitonin and carcinoembryonic antigen (CEA) are established tumor markers measured during both initial diagnosis for prognostication, as well as during long term monitoring for disease recurrence [3,4]. Elevated levels of calcitonin and CEA predict distant disease and decreased survival [5–7]. Between 40 and 60% of patients with MTC present with lymphadenopathy, while 10–30% present with distant metastasis [8,9]. Though prognosis for MTC is generally good, with an estimated greater than 90% cause specific survival at 20 years, the presence of metastatic disease decreases cause specific survival to only 40% at 20 years. Common sites of distant disease include lung, liver and bone [6,10]. Other factors associated with decreased survival include age, male sex, lymph node involvement, gross residual disease, large tumor size (>4 cm), extrathyroidal extension, vascular invasion, symptomatic diarrhea and failure to achieve biochemical cure [5,6,11].

Many studies have looked at a correlation between serum calcitonin and disease burden. Preoperative calcitonin levels have been shown to correlate with tumor size, presence of metastatic lymph nodes and rates of distant metastasis [12]. Normalization of calcitonin after surgical intervention is indicative of biochemical cure [9]. Rising calcitonin levels during surveillance indicates disease recurrence with subsequent decrease with reoperation [9,13]. Those with metastatic disease fail to achieve biochemical cure after primary and/or re-operative surgery [10,14]. However, if biochemical cure is not achieved, even a 50% reduction in calcitonin levels demonstrate slowing of locoregional MTC progression and decreased likelihood of developing distant disease [15]. As per National

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Comprehensive Cancer Network recommendations, once preoperative calcitonin exceeds 150 pg/ml, imaging is recommended to evaluate for distant organ involvement [3].

Similarly, studies have looked at the role of CEA in predicting disease progression and aggressiveness. Preoperative CEA levels have been determined to be more predictive of disease stage [9]. Histochemical studies on MTC tumors have demonstrated homogenous staining for calcitonin and CEA in early locoregional disease, but poor or absent staining for calcitonin and increased staining for CEA in metastatic disease of both primary and metastatic tumors. This suggests that increased CEA is a marker of disease aggressiveness [16]. Clinical studies have demonstrated abnormal CEA levels to be significantly associated with larger primary tumors, positive lymph nodes, distant metastases, decreased biochemical cure and increased mortality [17,18]. The American Thyroid Association recommends concurrent measurement of basal CEA with calcitonin, since a disproportionate elevation in CEA relative to a low or normal calcitonin level is prognostic for poorly differentiated MTC [4]. However, there is no guideline specifying at what CEA threshold or degree of disproportion between CEA and calcitonin levels is needed for additional imaging to evaluate for distant organ involvement, preoperatively or postoperatively.

For this particular study, we were interested in determining if absolute serum calcitonin or CEA levels can be utilized to predict distant metastases, either preoperatively or postoperatively and if a relationship exists between the markers, which can be utilized to accurately predict distant organ involvement.

Materials & methods

Study design & population

This is a retrospective cohort study comparing all patients diagnosed with MTC on final surgical pathology at Christian Medical College and Hospital (CMC; Vellore, India) between 2008 and 2014. Appropriate institutional review board approval was received for the patient data gathered. Factors studied include sex, age of presentation, disease presentation, site of metastases, operative intervention, tumor pathology, serum calcitonin and CEA levels, duration of follow-up and mortality. A total of 88 patients were included in the analysis, which was divided into two cohorts: those with locoregional disease (M0) and those with metastases (M1). Out of the 88 patients, 11 presented with metastatic disease. Serum calcitonin and CEA levels were collected from the patients preoperatively and postoperatively. Only the first postoperative value was used for analysis. Those with missing values were not excluded from the study. However, they were excluded from analyses requiring the missing value: nine did not have preoperative calcitonin levels, 60 did not have preoperative CEA levels, 12 did not have postoperative calcitonin levels and 40 did not have postoperative CEA levels.

Preoperative work-up, surgical intervention & histopathology

Blood samples were collected to determine preoperative serum calcitonin (normal range 0–50 pg/ml) and CEA (normal range <5 ng/ml) levels. Additional imaging was pursued with elevated calcitonin levels, typically >500 pg/ml. Preoperative work-up and operative intervention were at the discretion of the endocrine surgeon or by a multidisciplinary team. Presence of M1 disease was determined by radiologic evidence on computed tomography. Surgical specimens were examined by an endocrine pathologist at the institution for diagnosis, where tumor size, lobe involvement, focality, extrathyroidal extension and extra-capsular extension was recorded.

Statistical analysis

An independent two-tailed t-test was used to evaluate age. Pearson's χ^2 test was used to calculate significance between the gender distributions of the two cohorts. Mann–Whitney was used to compare tumor size, calcitonin and CEA levels between cohorts. Wilcoxon ranked test was used to compare paired calcitonin and CEA values. Spearman correlation was used to correlate paired calcitonin and CEA, in both preoperative and postoperative settings, for both M0 and M1 disease. Calcitonin and CEA were logistically transformed to standardize residuals before using linear regression for predictive modeling. Only patients with both calcitonin and CEA levels were included for paired analysis, correlation testing and linear regression modeling. A p-value less than 0.05 was considered significant.

Results

Demographics, follow-up & mortality

A total of 11 patients presented with metastatic MTC, out of which 82% had a solitary metastasis (as seen in Table 1). Although, more males were observed to present with metastatic disease (1:1.75 female to male ratio)

Table 1. Demographics, presentation, surgical intervention, histopathology, follow-up duration and mortality for both locoregional and distant disease.

Categories	Metastatic (M1) (n = 11)	Locoregional (M0) (n = 77)	Significance
Gender			p = 0.42
Male	7 (63.6%)	39 (50.6%)	
Female	4 (36.4%)	38 (49.4%)	
Age	33.8 ± 9.8 years	40.9 ± 12.4 years	p = 0.07
Presentation			
Solitary nodule of thyroid	1 (9.1%)	15 (19.5%)	
Multinodular goiter	0 (0.0%)	1 (1.3%)	
Goiter with lymphadenopathy	6 (54.5%)	42 (54.5%)	
Diagnosis from histopathology	0 (0.0%)	1 (1.3%)	
Genetic screening†	0 (0.0%)	7 (9.1%)	
Nodal recurrence after previous excision	3 (27.3%)	11 (14.3%)	
Recurrent goiter after previous excision	1 (9.1%)	0 (0.0%)	
Surgery			
Total thyroidectomy	0 (0.0%)	8 (10.4%)	
Total thyroidectomy with central compartment lymphadenectomy	1 (9.1%)	8 (10.4%)	
Total thyroidectomy with central compartment lymphadenectomy and modified radical neck dissection	3 (27.3%)	39 (50.6%)	
Total thyroidectomy with central compartment lymphadenectomy, modified radical neck dissection and sternotomy for mediastinal clearance	0 (0.0%)	1 (1.3%)	
Completion thyroidectomy	0 (0.0%)	1 (1.3%)	
Completion thyroidectomy with lymphadenectomy	1 (9.1%)	1 (1.3%)	
Prophylactic thyroidectomy with central cervical lymphadenectomy	0 (0.0%)	2 (2.6%)	
Completion lymphadenectomy	0 (0.0%)	4 (5.2%)	
Tumor debulking	0 (0.0%)	1 (1.3%)	
Sternotomy for mediastinal clearance	3 (27.3%)	5 (6.5%)	
Cervical lymphadenectomy for recurrence	3 (27.3%)	7 (9.1%)	
Histopathology			
Tumor size	4.9 ± 2.4 cm	3.4 ± 2.0 cm	p = 0.09
Tumor involvement			
One lobe	1 (9.1%)	11 (14.3%)	
Both lobes	0 (0.0%)	9 (11.7%)	
Thyroid and lymph nodes	6 (54.5%)	46 (59.7%)	
Lymph nodes only	4 (36.4%)	11 (14.3%)	
Focality (within thyroid)			
Unifocal	4 (36.4%)	43 (55.8%)	
Multifocal (within one lobe)	1 (9.1%)	6 (7.8%)	
Multifocal (bilateral lobes)	2 (18.2%)	13 (16.9%)	
Not applicable	4 (36.4%)	15 (19.5%)	
Tumor extension			
Extrathyroidal	4 (36.4%)	13 (16.9%)	
Extranodal	3 (27.3%)	10 (13.0%)	
Years of follow-up			
<1 year	3 (27.3%)	18 (23.4%)	
1–3 years	2 (18.2%)	21 (27.3%)	
3–5 years	3 (27.3%)	17 (22.1%)	
>5 years	2 (18.2%)	15 (19.5%)	
Lost to follow-up	1 (9.1%)	6 (7.8%)	
Mortality			
Yes	0 (0.0%)	8 (10.4%)	
No	9 (81.8%)	56 (72.7%)	
Unknown	2 (18.2%)	13 (16.9%)	

† Medullary thyroid carcinoma diagnosed multiple endocrine neoplasia screening and genetic screening after diagnosis of pheochromocytoma.

Table 2. Unpaired calcitonin and carcinoembryonic antigen comparisons.

Serum markers	Metastatic (M1)	Locoregional (M0)	Significance
Preoperative			
Median calcitonin, IQR (pg/ml)	6220, 8454 (n = 11)	2330, 7630 (n = 68)	p = 0.60
Median CEA, IQR (ng/ml)	644, 2835 (n = 4)	72, 491 (n = 24)	p = 0.12
Postoperative			
Median calcitonin, IQR (pg/ml)	1860, 6227 (n = 8)	96, 649 (n = 68)	p = 0.001†
Median CEA, IQR (ng/ml)	308, 498 (n = 7)	37, 178 (n = 41)	p = 0.02†

†Identifies statistically significant findings.
CEA: Carcinoembryonic antigen; IQR: Interquartile range.

than locoregional disease (1:1 female to male ratio), it was not statistically significant (p = 0.42). M1 patients were observably younger (33.8 + 9.8) than M0 patients (41.0 + 12.3 years), but the difference was not statistically significant (p = 0.07). Most patients had a solitary metastasis (81.8%) to bone (n = 2), lung (n = 2) and liver (n = 5); the remaining two patients had metastases to multiple sites (18.2%). By the end of the study, only one patient in the M1 cohort did not follow-up, while six patients from the M0 cohort were lost to follow-up. The remaining patients were followed between 3 and 96 months. Out of the ten M1 patients who were followed, nine were known to be alive by the end of the study in 2015. The mortality of the final patient was unknown.

Presentation, surgical intervention & histopathology

Over half of the patients had metastatic disease on initial presentations (63.6%): one patient had a solitary thyroid nodule (9.1%), while the other six patients had goiter with lymphadenopathy (54.5%). Over half of the patients in both the M1 and M0 cohorts initially presented with goiter and lymphadenopathy (54.5% for both). Those who presented via genetic screening consisted of seven individuals: two with pheochromocytomas and five after MEN screening. Surgical intervention and histopathology are as detailed in Table 1. More than a third of the M1 cohort had unifocal disease (36.4%), while less than a third had multifocal disease (27.3%). In contrast, more than half of the M0 cohort had unifocal disease (55.8%), while approximately a quarter had had multifocal disease (24.7%). Primary tumors ranged between 0.3 and 9 cm, averaging 4.9 cm in the M1 cohort and 3.4 cm in the M0 cohort. Out of the M1 patients, four had extrathyroidal extension: one presented with a solitary thyroid nodule, whereas the other three had goiters with lymphadenopathy. Out of these three patients, two had multiple foci of disease. Out of the four patients with histopathology demonstrating lymph node only involvement, only one had extranodal extension, while the other two patients presented with goiter and lymphadenopathy.

Serum markers

As seen in Table 2, there was no significant difference between preoperative calcitonin (p = 0.60) and CEA (p = 0.12) levels between the two cohorts, but there was a statistically significant difference between the postoperative levels (p = 0.001 for calcitonin, p = 0.02 for CEA). Interestingly, both patients with solitary metastatic disease to bone, had preoperative calcitonin levels of 34.5 and 263 pg/ml, one of which is lower than the recommended level for additional imaging (150 pg/ml). Both of these levels are lower than the median value of 644 pg/ml in the M1 group.

Preoperative and postoperative serum calcitonin and CEA levels were compared in patients with both markers (see Table 3). There were no significant differences between preoperative and postoperative calcitonin levels in the M1 cohort (p = 0.33), but there was a significant decrease in the M0 cohort ($p < 0.001$). Similarly, CEA did not significantly change in the M0 (p = 0.11), but significantly declined in the M0 group (p = 0.003). As seen in Figure 1, calcitonin and CEA levels do not significantly correlate in M1 disease preoperatively (p = 0.67) or postoperatively (p = 0.54). However, the correlation was significant in the M0 cohort, both preoperatively and postoperatively ($p < 0.001$ for both; see Figure 2). As seen in Table 4, both models demonstrated that serum calcitonin level does predict serum CEA level ($p < 0.001$ for both), however the predictive value is only moderate: the calcitonin level explains 54% of the variance in the CEA level preoperatively and 50% postoperatively.

Table 3. Paired calcitonin and carcinoembryonic antigen comparisons.

Serum markers	Preoperative value	Postoperative value	Significance
Median calcitonin (pg/ml)			
Metastatic (M1) (n = 8)	7090	1860	p = 0.33
Locoregional (M0) (n = 59)	1806	199	p < 0.001†
Median CEA (ng/ml)			
Metastatic (M1) (n = 3)	466	387	p = 0.11
Locoregional (M0) (n = 16)	39	10	p = 0.003†

†Identifies statistically significant findings.
CEA: Carcinoembryonic antigen.

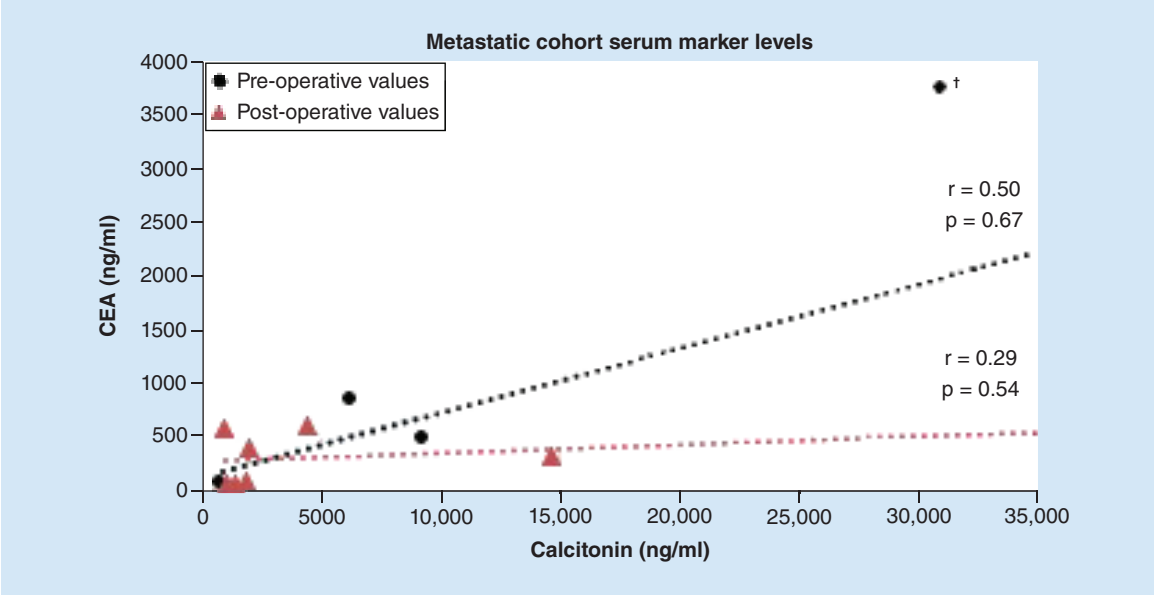

Figure 1. Paired preoperative (black) and postoperative (red) Spearman correlation of serum calcitonin and carcinoembryonic antigen in metastatic disease is shown. †Outlier not included in correlation calculation. Both lines had weak r-values and no statistical significance.

Table 4. Linear regression analysis for predicting preoperative and postoperative carcinoembryonic antigen levels based off measured calcitonin levels in nonmetastatic model.

Variable	Constant ± SE	B ± SE	R^2	t-score	p-value	95% CI on B
Preoperative calcitonin†	-0.73 ± 0.54	0.85 ± 0.17	0.54	5.12	<0.001	0.51–1.19
Postoperative calcitonin‡	0.02 ± 0.25	0.64 ± 0.11	0.50	6.01	<0.001	0.42–0.85

†Log_{10} (predicted preoperative CEA) = $-0.73 + 0.85\text{log}_{10}$ (preoperative calcitonin).
‡Log_{10} (predicted postoperative CEA) = $0.02 + 0.64\text{log}_{10}$ (postoperative calcitonin).
CEA: Carcinoembryonic antigen; SE: Standard of error.

Discussion

MTC is a rare thyroid malignancy associated with a poor survival in contrast to more common types of differentiated thyroid cancer. Consistent with existing population statistics, our M1 cohort comprised approximately 12.5% of the total patients surveyed, with common metastatic sites being liver, lung and bone.

Preoperative serum markers have always held a prognostic role. Previous studies revealed that a higher preoperative calcitonin level decreased chances of biologic cure, though calcitonin level variation did not specifically correlate with regional lymph node involvement or overall stage of disease [19]. Several larger studies have similarly looked

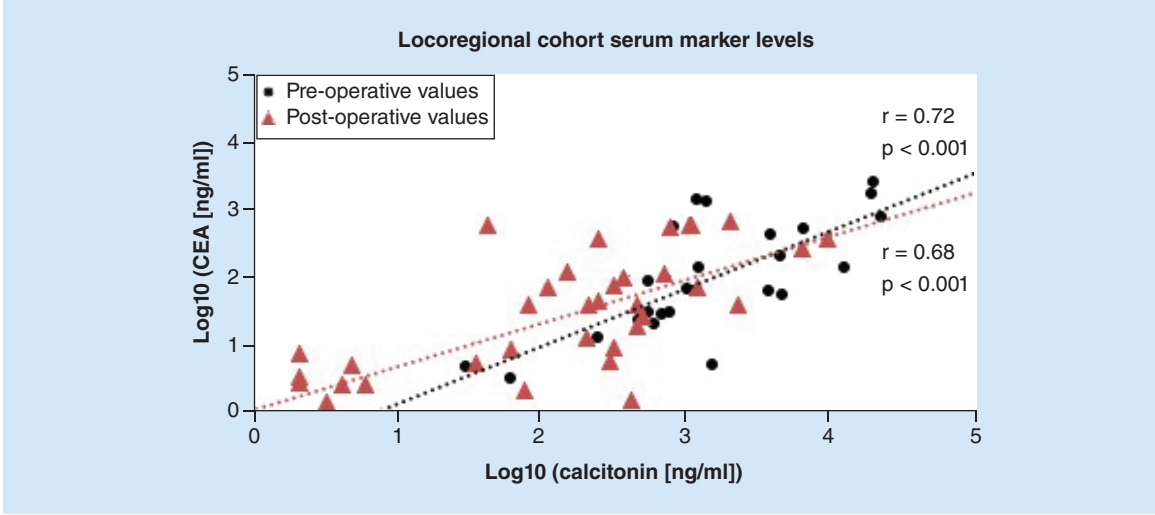

Figure 2. Paired preoperative (black) and postoperative (red) linear regression between log_{10}-transformed serum calcitonin and carcinoembryonic antigen nonmetastatic disease. Both preoperative ($r = 0.72$) and postoperative ($r = 0.68$) lines showed moderate to strong correlations between serum calcitonin and carcinoembryonic antigen (both $p < 0.001$) as calculated with Spearman correlation. Both regression models were similarly significant ($p < 0.001$).

at CEA. Turkdogan *et al.* noted a greater than 25-fold increase in serum CEA levels between stage III and stage IVC (average CEA of 15.7 and 405.9 ng/ml, respectively). In between stage IVA and stage IVC, there was a greater than fivefold difference (average CEA of 74.7 and 405.9 ng/ml respectively); between stage IVB and stage IVC, there was a greater than threefold difference (average CEA of 128.2 and 405.9 ng/ml, respectively) [18]. Machens *et al.* performed a multivariate analysis in 2007 on preoperative CEA, which determined that a CEA level >30.0 ng/ml had a higher likelihood of central neck and ipsilateral lateral neck lymph node metastases, and a CEA level >100.0 ng/ml signifies a higher likelihood of contralateral lateral neck lymph node metastases and distant metastases [17]. Patients rarely present with MTC in the absence of calcitonin and/or CEA elevation. Gambardella *et al.* performed a systematic review of these patients with nonsecretory MTC. These patients had an average calcitonin of 8.66 pg/ml (ranges from 0.8 to 38 pg/ml) and an average CEA of 7.22 ng/ml (ranges from 0.5 to 56.7 ng/ml), which included those who had lymphadenopathy and/or metastatic disease [20]. Hence, absolute values of serum calcitonin and CEA are limited in their ability to differentiate between metastatic and locoregional disease.

In our study, though there was an observable difference in median calcitonin and CEA levels between the M1 and M0 cohorts, there was not a significant difference in preoperative serum marker levels (see Table 2). Consistent with existing literature, we noted a significant drop in postoperative serum calcitonin and CEA in the M0 cohort (see Table 3). While the calcitonin level decreased, it did not completely normalize. This likely reflects residual disease burden after surgical resection within our studied populations [21–23]. Given the similar change seen with CEA, this may be a basis of considering whether a similar phenomenon of 'biochemical cure' could be observed with CEA normalization. Should a residually elevated CEA level remain, it could indicate persistent disease burden. This likely explains why those with metastatic disease would not see the statistically significant drop in either calcitonin or CEA level after initial surgical intervention or neck re-operation [6,8–10]. This residual elevation in serum marker levels likely explains the difference observed between the M1 and M0 cohorts in postoperative serum marker levels (see Table 2) [13,14].

Although we did not find a statistical difference in preoperative calcitonin or CEA between M0 and M1 disease, our observations and the evidence in literature argues that having a known CEA assists with identification of distant disease. Neither preoperative calcitonin nor CEA level differed between the M1 and M0 cohorts, which indicate that neither marker is superior in determining who would benefit from additional imaging. While our predictive model would generate an expected CEA level based on the measured calcitonin, a value greatly outside the prediction would be an indication to pursue additional imaging in either the preoperative or postoperative setting. Given the statistically insignificant correlation between serum calcitonin and CEA levels in those with

metastatic disease, it would suggest that changes of any single marker may not convey the whole clinical picture. Hence, we believe that it would behoove the patient to have both serum markers to assess burden of disease. Given the relationship seen between calcitonin and CEA, physicians would better capture patients whose serum markers deviate from the expected pattern and be sensitized to the likelihood of metastases. However, it is unclear if this model would assist in detecting nonsecretory MTC.

Thus, CEA should be used in conjunction with serum calcitonin level in both the preoperative and postoperative setting, to determine when further imaging is necessary for work-up of metastatic disease. While we may not have detected a significant difference in preoperative serum marker levels between metastatic and nonmetastatic patient cohorts, this is likely due to our study being underpowered by the limited size of our cohorts. As such, we cannot extrapolate what absolute value of calcitonin or CEA would be the ideal threshold to pursue further work-up for metastatic disease. Though data exist to suggest values of calcitonin and CEA indicative of advanced disease, evidence remains limited on the sensitivity and specificity of these serum level cutoffs to differentiate between aggressive locoregional and metastatic disease [17–19].

We anticipate that over the next few years, further inquiry into the relationship between serum calcitonin and CEA levels will better characterize the trend between the two values across different stages. This would ideally improve the screening process by accurately targeting high-risk patients preoperatively and postoperatively for metastatic disease, disease advancement and/or disease recurrence. Simultaneously, the field will likely evolve to incorporate additional screening markers, including repurposing existing tumor markers or identifying novel ones. Thus far, Alencar *et al.* evaluated Ca19-9 as a prognostic marker, while multiple groups have identified promising serum miRNA's for MTC diagnosis and prognosis [24–27]. As the field advances, we anticipate the development of a screening panel or algorithm with greater sensitivity and specificity for metastatic disease to assist preoperative work-up.

Summary points

- Neither calcitonin nor carcinoembryonic antigen (CEA) alone was able to distinguish those with metastatic disease from those without, hence CEA is noninferior to calcitonin.
- Those with metastatic disease failed to experience a significant decrease in either calcitonin or CEA level, suggesting that residual levels likely reflect distant disease burden. This suggests that changes in carcinoembryonic CEA can similarly reflect a 'biochemical cure'.
- While serum calcitonin correlates and to a degree, predicts what serum CEA would be, it cannot substitute a known level.
- Given the prognostic value seen with having both calcitonin and CEA levels, there would be greater accuracy in identifying patients with alarming changes in serum marker levels more likely to indicate metastatic disease.
- CEA ought to be checked concurrently with calcitonin in both the preoperative and postoperative settings in order to guide additional imaging.

Author contributions

RZ Pywell, H Chen and D Abraham conceived the presented idea. H Chen and D Abraham supervised the project. AJ Cherian compiled the patients and obtained all the raw data. RZ Pywell and M Enman performed the analytic calculations. RZ Pywell wrote the manuscript with support from H Chen and D Abraham. All authors discussed the results and contributed to the final manuscript.

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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