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Diagnostic accuracy of routine calcitonin measurement for the detection of medullary thyroid carcinoma in the management of patients with nodular thyroid disease: a meta-analysis

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Abstract

Objective: The usefulness of routine calcitonin measurement for early detection of medullary thyroid carcinoma (MTC) in patients with nodular thyroid disease (NTD) has been investigated in various studies. Recently, a Cochrane review has been published on this issue, but a meta-analysis is lacking yet. Therefore, we performed this meta-analysis.

Methods: We performed an electronic search using PubMed/Medline, Embase and the Cochrane Library. Studies assessing the diagnostic accuracy of routine calcitonin measurement for detecting MTC in patients with NDT were selected. Statistics were performed by using Stata software, risk of bias was assessed using Review Manager version 5.3.

Results: Seventeen studies, involving 74,407 patients were included in the study. Meta-analysis, using the bivariate random effects model and the hierarchical summary receiver operating characteristic (HSROC) curve revealed the following pooled estimates: sensitivity 0.99 (95% CI, 0.81–1.00), specificity 0.99 (95% CI, 0.97–0.99), positive likelihood ratio (L+) 72.4 (95% CI, 32.3–162.1), and negative likelihood ratio (L–) 0.01 (95% CI, 0.00–0.23). Meta-regression analysis showed that the threshold of basal calcitonin is an independent factor, but in particular performing stimulation test is not an independent factor.

Conclusions: We showed that routine basal serum calcitonin measurement in the management of patients with thyroid nodules is valuable for the detection of MTC. However, the published cut-off values should be considered and, if applicable, the patients monitored in a wait-and-see strategy by experienced physicians to avoid overtreatment.

Key Words
- medullary thyroid carcinoma
- calcitonin
- routine calcitonin measurement
- nodular thyroid disease
- diagnostic accuracy
Introduction

Calcitonin (Ctn) is secreted by the C-cells of the thyroid (1) and is a valuable tumor marker in patients with medullary thyroid carcinoma (MTC) (2). Medullary thyroid cancer, originated from the C-cells (3), occurs rarely and corresponds to 1–3% of all histologically proven thyroid cancers in the United States, with a prevalence of 0.1–1.4% in patients with nodular thyroid disease (2, 4), appearing either sporadically or in a hereditary form as a component of the type 2 multiple endocrine neoplasia (MEN) syndromes, MEN2A and MEN2B, and the related syndrome, familial MTC (FMTC) (2). Serum Ctn levels may be increased in patients with autoimmune thyroiditis, several extrathyroidal tumors like various enteric and pulmonary neuroendocrine tumors, small cell and large cell lung cancers or prostate cancer, mastocytosis, chronic renal failure and severe pulmonary or hepatic diseases (5, 6, 7, 8, 9, 10, 11, 12).

The newest immunochemiluminometric assays (ICMAs) for measuring Ctn according to the ‘sandwich principle’ are highly sensitive and specific for mature (monomeric) form of Ctn, with largely eliminated cross-reactivity with procalcitonin or other calcitonin-related peptides (13, 14).

The routine measurement of serum Ctn in patients with nodular thyroid disease may be a suitable method to identify MTC, often in an early stage, with a positive impact on prognosis (15). Even though its cost effectiveness has been shown (4), the recommendations for the routine measurement of Ctn are not uniform. It was advised by the European Consensus published in 2006 (16). However, the ATA and AACE/ACE/AME guidelines do not advocate for or against the routine measurement of serum Ctn (17, 18) or limit the Ctn measurement to patients who are submitted for surgery (17). In a recent systematic review (including trials published until 2013) Verbeek et al. showed the high sensitivity and specificity
| First author year | Country | Study design | Basal Ctn threshold, pg/mL | Stimulation test (Pentagastrin) if basal Ctn is elevated | Stimulated Ctn threshold, pg/mL | Pat. total, n | Studies with basal calcitonin threshold and stimulated Ctn threshold | Studies with basal calcitonin threshold and stimulated Ctn threshold | Nodular thyroid disease; status 6 | Calcitonin assay |
|-------------------|---------|-------------|-----------------------------|----------------------------------------------------------|-------------------------------|--------------|-------------------------------------------------|-------------------------------------------------|------------------------------------|------------------|
| Rieu 1995 (34)    | F       | PCo         | RIA: 35 IRMA: 10 (m/f)      | Yes                                                      | 100 (m/f)                     | 469          | 4 0 0 465                                       | 4 0 0 465                                       | Uni-nodular, multi-nodular         | IRMA 5          |
| Oezgen 1999 (35)  | TR      | Pco         | 30 (m/f)                    | No                                                       | –                             | 773          | 4 0 0 769                                       | 3 0 0 583 1 0 0 186                  | Uni-nodular, multi-nodular         | IRMA 5          |
| Hahm 2001 (36)    | KR      | Co          | 10 (m/f)                    | Yes                                                      | 100 (m/f)                     | 1448         | 10 46 0 1392                                    | 10 2 0 1436                                    | Uni-nodular, multi-nodular         | RIA 5 §          |
| Hatzl-Griesenhofer 2002 (37) | A | RCo | 4.6 (f), 11.5 (m) | Yes (not, if bCtn is >80 pg/mL) | 100 (m/f)                     | 3899         | 12 218 0 3669                                   | 12 23 0 3796                                   | Nodular diffuse-nodular            | ICMA 5 §§        |
| Elisei 2004 (15)  | I       | Co          | 20 (m/f)                    | Yes (in n=44)                                            | 60 (m/f)                      | 10864        | 44 3 0 10817                                   | 44 0 0 10818                                   | Uni-nodular, multi-nodular nodules | ICMA 5 §§        |
| Karanikas 2004 (38) | A      | Co          | 10 (m/f)                    | Yes                                                      | 100 (m/f)                     | 195          | 1 12 0 182                                   | 1 1 0 193                                    | Uni-nodular, multi-nodular         | ICMA 5 §§        |
| Vierhapper 2005 (39) | A      | Co          | 10 (m/f)                    | Yes                                                      | 100 (m/f)                     | 10157*        | 33 474 3 9647                                   | 31 72 3 10025                                  | Uni-nodular, multi-nodular nodules | ICMA 5 §§        |
| Papi 2006 (40)    | I       | Co          | 5 (m/f)                     | Yes (not, if bCtn is ≥100 pg/mL)                         | 100 (m/f)                     | 1425         | 9 14 0 1402                                   | 9 1 0 1415                                   | Nodular multi-nodular              | ICMA 5 §§        |
| Costante 2007 (41) | I      | Co          | 10 gray zone 10–20 (m/f)    | Yes (not, if bCtn is ≥100 pg/mL)                         | 100 (m/f)                     | 5817         | 15 267 0 5535                                   | 15 11 0 5791                                   | Nodular multi-nodular              | ICMA 5 §§        |
| Rink 2009 (42)    | D       | Co          | 10 (m/f)                    | Yes                                                      | 80 (m) (50 (f)                 | 21928        | 28 857 0 21043                                  | 11 51 0 21199                                  | Uni-nodular, multi-nodular         | ICMA 5 §§        |
| Hasselgren 2010 (43) | DK     | RCo         | RIA: 100 (m/f) ICMA: 10.5 (m), 7.3 (f) | No                                                       | -                             | 702          | 6 33 0 663                                   |                                      | Uni-nodular, multi-nodular         | ICMA 5 §§        |
| Herrmann 2010 (44) | D      | RCo         | 10 (m/f)                    | Yes (not, if bCtn is ≥100 pg/mL)                         | 100 (m/f)                     | 1007         | 2 15 0 990                                   | 2 3 0 1002                                   | Uni-nodular, multi-nodular nodules | ICMA 5 §§        |
| Grani 2012 (45)   | I       | CsRo        | 10 (m/f)                    | No                                                       | -                             | 1073         | 2 39 0 1032                                   |                                      | Uni-nodular, multi-nodular nodules | ICMA 5 §§        |
| Schneider 2012 (46) | D      | Co          | 13 (m/f)                    | Yes (not, if bCtn is ≥100 pg/mL, n = 14)                | 100 (m/f)                     | 11270        | 10 22 2 11236                                  | 9 8 2 11238                                   | Uni-nodular, multi-nodular         | ICMA 5 §§        |

(Continued)
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Table 1

| First author | Year | Country | Study design | Basal Ctn threshold (pg/mL) | Stimulated Ctn threshold (pg/mL) | Pat. total, n | Study with basal Ctn threshold and stimulated Ctn threshold | Females | Males | TP | TN | FP | FN |
|--------------|------|---------|-------------|-----------------------------|-------------------------------|--------------|----------------------------------------------------------|---------|-------|----|----|----|----|
| Giovanella    | 2013 | CH      | PCO         | 10 (m/f)                    | Yes                           | 1236         | TP: 10, FP: 2, TN: 1222, FN: 2                           | TP: 2   | TN: 1220 | 640 | 0  | 2   | 4  |
| Silvestre     | 2017 | TR      | TR          | 10 (m/f)                    | Yes, only if basal Ctn is elevated | 1000         | TP: 10, FP: 2, TN: 1222, FN: 2                           | TP: 2   | TN: 1220 | 640 | 0  | 2   | 4  |
| Turk 2017     | 10   | TR      | TR          | 10 (m/f)                    | Yes, only if basal Ctn is elevated | 1000         | TP: 10, FP: 2, TN: 1222, FN: 2                           | TP: 2   | TN: 1220 | 640 | 0  | 2   | 4  |

Note: The meta-analysis was performed according to the Synthesizing Evidence from Diagnostic Accuracy Tests (SEDATE) guideline on reporting a diagnostic test accuracy meta-analysis; an updated Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guideline (20, 21) (the checklist is provided as Supplementary Table 1, see section on supplementary materials given at the end of this article). A predefined study protocol was created but not registered. Ethical approval or informed consent was not required for this meta-analysis.

Patients and methods

The meta-analysis was performed according to the Synthesizing Evidence from Diagnostic Accuracy Tests (SEDATE) guideline on reporting a diagnostic test accuracy meta-analysis; an updated Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guideline (20, 21) (the checklist is provided as Supplementary Table 1, see section on supplementary materials given at the end of this article). A predefined study protocol was created but not registered. Ethical approval or informed consent was not required for this meta-analysis.

Data search and study selection

We searched the electronic databases of PubMed/MEDLINE, EMBASE and Cochrane Library systematically (updated on December 10, 2020) with the search strategies given in Supplementary Table 2; without language and time restriction in any of these databases. Furthermore, references of retrieved studies were searched for eligible studies. Electronic archives of medical societies (Endocrine Society (https://www.endocrine.org/meetings/endo-annual-meetings; accessed December 18, 2020) and Deutsche Gesellschaft für Nuklearmedizin e.V. (https://www.nuklearmedizin.de/jahrestagungen/abstr-online2020/abstract_search.php?navId=227; accessed December 18, 2020) were also searched. Studies meeting the following inclusion criteria were included: routine calcitonin measurement in serum (with or without a pentagastrin or calcium stimulation test) was performed routinely in all included patients with nodular thyroid disease, diagnosed by palpation or ultrasonography. Exclusion criteria were: data for 2 × 2 table not provided; preoperative measurement of calcitonin in serum; calcitonin measurement as screening test, as screening is epidemiologically defined as testing in healthy people of Ctn testing, still questioning the value of its routine use due to the low prevalence of MTC and to the risk of overdiagnosis (19). They included the trials published until 2013, searched the electronic databases last at June 6, 2018, but not assessed the potentially relevant studies identified in their last search for inclusion. A meta-analysis on this subject has not been published until now. Therefore, we performed this meta-analysis to elucidate the diagnostic accuracy of routine serum calcitonin measurement for detection of MTC in the management of patients with nodular thyroid disease.
(people with thyroid nodules are not healthy); inclusion of patients with familial history of medullary thyroid carcinoma (MTC); incomplete surgery (e.g. lobectomy); case reports or case series; case-control study; duplication of a study (in case of duplication, inclusion of the study with the longest follow-up); only meeting communication, not published as full-text article.

**Data extraction and quality assessment**

Two authors (I V and R G) independently reviewed all eligible articles and extracted the relevant data. In case of disagreement, after consultation with a third author (M W) regarding the eligibility, consensus was found. We used the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool (22, 23) in Review Manager (RevMan) version 5.3. (Nordic Cochrane Center), which assesses the quality of the included studies in terms of biases affecting their applicability in four domains: index test, reference standard, patient selection and flow and timing. The two authors evaluated each of the items. **Index test** was defined as measurement of basal Ctn in serum (and additionally Ctn measurement in serum after stimulation, if needed). There were various assays for this test, which has to be addressed. Histological observation was considered as the **reference standard**

![Figure 2](https://ec.bioscientifica.com)

**Figure 2**

(A) Risk of bias and applicability concerns graph on each domain presented as percentages across all included studies. (B) Risk of bias and applicability concerns summary for each included study. \( n = 17 \) trials.
Diagnostic accuracy of calcitonin measurement

Statistical analysis

For the meta-analysis of diagnostic test accuracy studies, the hierarchical summary receiver operating characteristic (HSROC) and bivariate methods are the most appropriate methodological approaches (https://eunetheta.eu/wp-content/uploads/2018/01/2014-05-19_meta-a_diagn_draft-gl_2nd_revision_clear_0.pdf, accessed December 18, 2020). Therefore, we performed a meta-analysis using the hierarchical logistic regression modeling to determine summary estimates of the sensitivity, specificity, diagnostic odds ratio, and likelihood ratios by the bivariate random effects model (24) for calculating summary estimates of sensitivity and specificity, and the HSROC curve for modeling the parameters for the ROC curves (25, 26, 27) using Stata, version 11 (Stata Corp, College Station, Texas) with the metandi, metandiplot, and midas commands (28). Evaluation of funnel plot asymmetry, meta-regression analysis and the funnel plots were performed using Stata, version 11 (Stata Corp) with the midas command (28). Publication bias (bias across studies) was assessed by the Deeks’ funnel plot asymmetry test (28, 29) using Stata, version 11 (Stata Corp, College Station, Texas) with the midas command; \( P < 0.1 \) indicated publication bias. For cells containing zero 0.5 was used as the continuity correction (this is default for the metandi command in Stata), as suggested in the literature (30). Positive likelihood ratios of greater than 2.0 or negative likelihood ratios less than 0.5 with 95% CIs not including 1.0 were considered statistically significant (31, 32). The primary endpoint was defined as the sensitivity and specificity. Predefined secondary endpoints were: positive predictive value (\( PPV = TP / (TP + FP) \)), whereas \( TP = \) true positives, \( FP = \) false positives, negative predictive value (\( NPV = TN / (FN + TN) \)), whereas \( TN = \) true negatives, \( FN = \) false negatives, positive likelihood ratio (\( L^+ = (sensitivity / (1 − specificity)) \)) and negative likelihood ratio (\( L^- = (1 − sensitivity) / specificity \)). The TN (true negative) cases were calculated using the formula \( TN = \) total number of patients − (FP + TP + FN). In studies in which Ctn-negative cases were further clarified, the (very rare) cases of proven MTC were included in the meta-analysis as FN. In studies in which the follow-up of Ctn-negative patients was not reported, the FN rate in the meta-analysis was set to zero, analogous to the procedure of Verbeek et al. 2020 (19).

Sensitivity analyses were performed by excluding studies that are considered outliers in a statistical sense and by restricting the meta-analysis to subgroups (33). Following subgroup analyses for the primary endpoint were predefined: assay for Ctn measurement (immunochemiluminometric assays (ICMA) vs other assays), threshold for basal calcitonin (≥10 pg/mL vs between 4.6 and 100 pg/mL), using of (pentagastrin or calcium) stimulation test (stimulation test performed vs not performed); gender (females vs males); country of origin (Europe, Asia, others); if applicable. For exploring heterogeneity, a meta-regression analysis (https://methods.cochrane.org/sdt/handbook-dta-reviews, accessed December 18, 2020) with following predefined study-level covariates (potential confounders) was intended: (1) assay for calcitonin measurement (ICMA (yes) vs other assays (no)), (2) threshold for basal calcitonin (≥10 pg/mL (yes) vs other thresholds (no)), (3) using of (pentagastrin or calcium) stimulation test (stimulation test performed (yes) vs not performed (no)); (4) gender (females vs males).
Results

Study selection and characteristics

The literature search identified 1382 records with potentially relevant studies. As shown in Fig. 1, 17 studies met the inclusion and exclusion criteria and were included in the meta-analysis. The included studies had a total of 74,407 patients. None of the trials was a case-control study. The detailed characteristics of the included studies are given in Table 1 ((15, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49)). According to the QUADAS-2 tool (22, 23), the methodological quality of the included trials was acceptable (Fig. 2).

Risk of bias and publication bias

The Deeks’ funnel plot asymmetry test suggested no significant evidence for publication bias (Fig. 3).

Meta-analysis

In our meta-analysis, we included 17 trials with in total 74,407 patients with nodular thyroid disease; 203 patients had medullary thyroid carcinoma, with a prevalence between 0.11% (in Schneider et al. 2012) (46) and 0.85% (in Hasselgren et al. 2010) (43). Regarding all included studies (n = 17) the summary estimates of sensitivity and specificity for the threshold between 4.6 and 100 pg/mL of basal calcitonin measurement was 0.99 (95% CI, 0.81–1.00) and 0.99 (95% CI, 0.97–0.99), respectively (Fig. 4); the pooled estimates of L+ and L− were 72.4 (95% CI, 32.3–162.1) and 0.01 (95% CI, 0.00–0.23), respectively (Supplementary Table 3). Post-test probabilities are shown in Supplementary Fig. 1. The hierarchical SROC (HSROC) curve for all included studies is depicted in Fig. 5, where the study No 7 by Vierhapper et al. (39) is an outlier (Supplementary Fig. 2).

![Coupled forest plot illustrating sensitivity and specificity for all included studies (with a basal calcitonin threshold between 4.6 and 100 pg/mL), n = 17. Pooled sensitivity: 0.99 (95% CI, 0.81–1.00), pooled specificity: 0.99 (95% CI, 0.97–0.99), pooled L+: 72.4 (95% CI, 32.3–162.1), pooled L−: 0.01 (95% CI, 0.00–0.23).](https://doi.org/10.1530/EC-21-0030)
In the sensitivity analysis, exploring the possible reasons of between-study heterogeneity, after omitting the mentioned outlier trial (39), the summary estimates remained without significant changes; the summary estimates of sensitivity and specificity were 1.00 (95% CI, 0.37–1.00) and 0.99 (95% CI, 0.97–0.99), respectively (Supplementary Fig. 3); the pooled estimates of L+ and L− were 690 (95% CI, 314.1–1515.6) and 0.01 (95% CI, 0.00–0.25), respectively (Supplementary Table 4). The hierarchical SROC (HSROC) curve for this analysis is depicted in Supplementary Fig. 4. Omitting the study by Karanakis et al. (38) which is not an outlier in the HSROC curve, showed no significant influence on the estimates, too (Supplementary Table 5). The hierarchical SROC (HSROC) curve for this subgroup is depicted in Supplementary Fig. 6. Omitting the study by Vierhapper et al. (39) which is still an outlier.

In subgroup analyses, summary estimates of sensitivity and specificity in the subgroup (n= 12 trials) with a combined basal and stimulated calcitonin measurement with a threshold between 4.6 and 35 pg/mL of basal calcitonin, and with a threshold between 50 and 100 pg/mL of stimulated calcitonin were 0.99 (95% CI, 0.79–0.10) and 1.00 (95% CI, 1.00–1.00), respectively (Supplementary Fig. 7); the pooled estimates of L+ and L− were 690 (95% CI, 314.1–1515.6) and 0.01 (95% CI, 0.00–0.25), respectively (Supplementary Table 7). The hierarchical SROC (HSROC) curve for this subgroup is depicted in Supplementary Fig. 8. In this subgroup the specificity (1.00 (95% CI, 1.00–1.00) vs 0.97 (95% CI, 0.96–0.98) vs 0.99 (95% CI, 0.97–0.99)) and the L+ (690.0 (95% CI, 314.1–1515.6) vs 32.6 (95% CI, 23.6–44.3) vs 72.4 (95% CI, 32.3–162.1) were superior to the findings for the basal calcitonin measurement with a threshold of ≥ 10 pg/mL and between 4.6 and 100 pg/mL, respectively. In the subgroup with combined basal and stimulated calcitonin measurement the post-predictive probability was higher (99% vs 95% as shown in the Supplementary Figs 1 and 9) than in all included studies with basal calcitonin measurement with a threshold between 4.6 and100 pg/mL; favoring the combined basal and stimulated calcitonin measurement.

The meta-regression analysis showed, that the covariate ‘threshold for basal calcitonin’ (≥ 10 pg/mL vs other thresholds), but not the covariate ‘performing stimulation (pentagastrin) test’ (stimulation test performed vs not performed) is an independent influencing factor (Fig. 8 and Supplementary Table 8). The latter finding is in contrast to the subgroup analysis, as shown previously.

Subgroup analysis for the influence of gender was intended, but have been not performed due to the small number of studies (n= 6) (35, 37, 39, 40, 44, 47) with gender-specific 2 × 2 table data. The covariate gender was therefore excluded from the intended meta-regression analysis.

Discussion

In this study, we performed a meta-analysis for the diagnostic accuracy of routine serum calcitonin measurement for the detection of medullary thyroid carcinoma in patients with nodular thyroid disease.
Concerning all included studies, with a threshold between 4.6 and 100 pg/mL of basal calcitonin measurement, the summary estimates of sensitivity and specificity for detecting of MTC were 0.99 (95% CI, 0.81–1.00) and 0.99 (95% CI, 0.97–0.99), respectively; the pooled estimates of L+ and L− were 72.4 (95% CI, 32.3–162.1) and 0.01 (95% CI, 0.00–0.23), respectively. There was some degree of between-study heterogeneity, but no indication for publication bias. Sensitivity analysis showed no influence of particular studies on the summary estimates.

Our results indicate that both, basal calcitonin measurement as well as stimulated calcitonin measurement can cover almost 100% patients with MTC. However, particularly in iodine-replete countries where solitary MTC develops against a backdrop of normal thyroid tissue, due to the low prevalence of MTC in patients with nodular thyroid disease, the false positive rate might be high, with the risk of an unnecessary thyroidectomy, with possible risk of operative complications and the necessity for life-long levothyroxine supplementation. In iodine-deficient countries, bilateral goiter is common, require total thyroidectomy regardless of the level of serum calcitonin. In the latter scenario, the level of calcitonin may guide the extent of node dissection at the time of thyroidectomy, as advocated in the 2015 revised ATA guidelines on MTC (2).

Studies evaluating cut-off levels for routine calcitonin measurement in patients with nodular thyroid disease concerning the recommendations of thyroidectomy due to suspicion for MTC revealed gender-specific cut-off values for basal calcitonin of >30 pg/mL for females and >60 pg/mL for males, which were not inferior to pentagastrin stimulated calcitonin levels (50, 51, 52). Almost 100% of patients with preoperative basal calcitonin values >100 pg/mL had MTC, whereas with basal calcitonin levels between 10 and 20 pg/mL only 5% of the participants had MTC (50). Due to the availability of innovative ICMAs (53), the non-availability of...
pentagastrin and the efforts for the calcium stimulation test, the routine measurement of basal calcitonin has become focus of interest (54). Patients with preoperative basal calcitonin levels < 100 pg/mL may be cured in almost 100% of cases (55). Based on the available literature, some authors suggested a wait-and-see approach for patients with basal calcitonin levels < 30 pg/mL in females and < 60 pg/mL, with consequent recommendation for operative intervention in case of increasing calcitonin levels. Contrarily, females with calcitonin levels ≥30 pg/mL and males with calcitonin levels ≥ 60 pg/mL can be monitored or surgery considered, whereas surgery is recommended in patients with basal calcitonin values > 100 pg/mL (10, 54, 56). Recently, Niederle et al. reported, that calcitonin measurement after calcium stimulation did not improve the preoperative diagnostic; this is important and is in line with the findings in our meta-regression analysis. Niederle et al. additionally suggested that basal calcitonin levels >43 and >100 pg/mL for males and of >23 and 85 pg/mL for females are relevant for advising patients and planning the extent of surgery (57).

There are several limitations in our study: for example, (a) The validity of the evidence is limited, as in almost all of the included studies no adequate reference standard for verification of FN and TN cases is available: ideally, this would be a histological exclusion of MTC in a representative number patients with calcitonin levels below the cut-off value after processing the entire resected tissue after total thyroidectomy using thin-layer technology. Only in a few studies the histological findings of some patients who underwent thyroid surgery despite calcitonin values below the cutoff were reported. For example, Schneider et al. (46) reported two patients with incidentally diagnosed MTC in patients with false-negative basal calcitonin levels. These few MTC cases in patients who were not detected with pathological screening Ctn values were assessed as FN in our meta-analysis. Because of the extreme rarity of such cases, it seemed legitimate for the meta-analysis to set the FN rate to zero in studies in which false-negative cases were not reported. The diagnostic accuracy can be affected even

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Figure 7
Hierarchical summary receiver-operating characteristics (SROC) plot for the subgroup of studies with a threshold of basal calcitonin measurement ≥10 pg/mL; n = 9 trials.

Figure 8
Meta-regression analysis in all included studies (n = 17) for the following covariates: (1) basal calcitonin threshold (>10 pg/mL (yes) vs other basal thresholds (no)), and (2) performing of stimulation test (performed (yes) vs not performed (no)) for the basal calcitonin measurement (with a threshold between 4.6 and 100 pg/mL), indicating that the covariate ‘basal calcitonin threshold’, but not ‘performing of stimulation test’ is significantly influencing the sensitivity as well as specificity, as an independent influencing factor.
when a small number of patients with very rare diseases, like the MTC, is missed. Additionally, this may lead to a high risk of bias with regard to flow and timing in the assessment of methodological quality. (b) Because of the small number of trials only few subgroup analyses could be performed. (c) There is somewhat between-study heterogeneity, in particular due to the different cut-off levels of basal calcitonin measurement and using of different assays for calcitonin measurements. (d) Included studies using disparate provocative agents for pentagastrin test were not evaluated separately but lumped together in the meta-analysis.

In conclusion, our results indicate that both basal and combined basal and stimulated calcitonin testing have a high sensitivity and specificity. We showed that routine calcitonin measurement in serum in the management of patients with thyroid nodules is valuable for the detection of medullary thyroid cancer. However, the published cut-off values should be considered and, if applicable the patients monitored, in a wait-and-see strategy, in experienced hands to avoid overtreatment.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/EC-21-0030.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References
1 Raue F, Zink A & Scherubl H. Regulation of calcitonin secretion and calcitonin gene expression. In Recent Results in Cancer Research. Medullary Thyroid Carcinoma, pp. 1–18. Ed F Raue. Berlin: Springer, 1992.
2 Wells Jr SA, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, Lee N, Machens A, Moyle JF, Pacini F, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid 2015 25 567–610. (https://doi.org/10.1089/thy.2014.0335)
3 Williams ED. Histogenesis of medullary carcinoma of the thyroid. Journal of Clinical Pathology 1966 19 114–118. (https://doi.org/10.1136/jcp.19.2.114)
4 Cheung K, Roman SA, Wang TS, Walker HD & Sosa JA. Calcitonin measurement in the evaluation of thyroid nodules in the United States: a cost-effectiveness and decision analysis. Journal of Clinical Endocrinology and Metabolism 2008 93 2173–2180. (https://doi.org/10.1210/jc.2007-2496)
5 Borchhardt KA, Hori WH & Sunder-Plassmann G. Reversibility of ‘secondary hypercalcitoninemia’ after kidney transplantation. American Journal of Transplantation 2005 5 1757–1763. (https://doi.org/10.1111/j.1600-6143.2005.00908.x)
6 Bevilacqua M, Dominguez LJ, Righini V, Valdés V, Vago T, Leopaldi E, Baldi G, Barrella M & Barbagallo M. Dissimilar PTH, gastrin, and calcitonin responses to oral calcium and peptones in hypocalciuric hypercalcemia, primary hyperparathyroidism, and normal subjects: a useful tool for differential diagnosis. Journal of Bone and Mineral Research 2006 21 406–412. (https://doi.org/10.1359/jbmr.2005.081210)
7 Schuetz M, Duan H, Wahl K, Pirich C, Antoni A, Kommata S, Kletter K, Dudczak R, Karanakis G & Willheim M. T lymphocyte cytokine production patterns in Hashimoto patients with elevated calcitonin levels and their relationship to tumor initiation. Anticancer Research 2006 26 4591–4596.
8 Pratz KW, Ma C, Aubry MC, Vritska TJ & Erlichman C. Large cell carcinoma with calcitonin and vasoactive intestinal polypeptide-associated Verner-Morrison syndrome. Mayo Clinic Proceedings 2005 80 116–120. (https://doi.org/10.1016/S0025-6196(11)62968-6)
9 Sim SJ, Glassman AB, Ro JY, Lee JF, Logothetis CJ & Liu FJ. Serum calcitonin in small cell carcinoma of the prostate. Annals of Clinical and Laboratory Science 1996 26 487–495.
10 Machens A, Haedecke J, Holzhausen HJ, Thomusch O, Schneyer U & Dralle H. Differential diagnosis of calcitonin-secreting neuroendocrine carcinoma of the forhead by pentagastrin stimulation. Langenbeck's Archives of Surgery 2000 385 398–401. (https://doi.org/10.1007/s004230000169)
11 Yocum MW, Butterfield JH & Gharib H. Increased plasma calcitonin levels in systemic mast cell disease. Mayo Clinic Proceedings 1994 69 987–990. (https://doi.org/10.1016/S0025-6196(12)61825-4)
12 Toledo SP, Lorenco DM, Jr, Santos MA, Tavares MR, Toledo RA & Correia-Deur JE. Hypercalcitoninemia is not pathognomonic of medullary thyroid carcinoma. Clinics 2009 64 699–706. (https://doi.org/10.1590/S1807-59322009000700015)
13 Becker KL, Nylen ES, White JC, Muller B & Snider Jr RH. Clinical review 167: procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. Journal of Clinical Endocrinology and Metabolism 2004 89 1512–1525. (https://doi.org/10.1210/jc.2002-021444)
14 Whang KT, Steinwald PM, White JC, Nylen ES, Snider RH, Simon GL, Goldberg RL & Becker KL. Serum calcitonin precursors in sepsis and systemic inflammation. Journal of Clinical Endocrinology and Metabolism 1998 83 3296–3301. (https://doi.org/10.1210/jcem.83.9.5129)
15 Elisei R, Bottici V, Luchetti F, Di Coscio G, Romei C, Grasso L, Miccoli P, Iaconci P, Basolo F, Pincherla A, et al. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. Journal of Clinical Endocrinology and Metabolism 2004 89 163–168. (https://doi.org/10.1210/jc.2003-030550)
16 Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W & European Thyroid Cancer Taskforce. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. European Journal of Endocrinology 2006 154 787–803. (https://doi.org/10.1530/eje.1.02158)
17 Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, et al. American Thyroid Association Management Guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association Guidelines task force on thyroid nodules and differentiated thyroid cancer: the American Thyroid Association Guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid 2016 26 1–133. (https://doi.org/10.1089/thy.2015.0020)
18 Gharib H, Papini E, Garber JR, Duick DS, Harrell RM, Hegedus L, Paschke R, Valcavi R, Vitti P & Nodules AAAT. FoT American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi Medical
Guidelines for clinical practice for the diagnosis and management of thyroid nodules – 2016 update. Endocrine Practice 2016 22 622–639.

19 Verbeek HH, de Groot JWB, Sluiter WJ, Muller Koboldt AC, van den Hevel ER, Phukker JT & Links TP. Calcitonin testing for detection of medullary thyroid cancer in people with thyroid nodules. Cochrane Database of Systematic Reviews 2020 3 CD010159. ([https://doi.org/10.1002/14651858.CD010159.pub2](https://doi.org/10.1002/14651858.CD010159.pub2))

20 Moher D, Liberati A, Tetzlaff J, Altman DG & PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Medicine* 2009 6 e1000097. ([https://doi.org/10.1371/journal.pmed.1000097](https://doi.org/10.1371/journal.pmed.1000097))

21 Sotiriadis A, Papaefthorou SI & Martins WP. Synthesizing evidence from diagnostic accuracy tests: the SEDATE guideline. *Ultrasound in Obstetrics and Gynecology* 2016 47 386–395. ([https://doi.org/10.1002/uog.15762](https://doi.org/10.1002/uog.15762))

22 Whiting PF, Rutjes AW, Reitsma JB, Bossuyt PM & Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Medical Research Methodology 2003 3 25. ([https://doi.org/10.1186/1471-2288-3-25](https://doi.org/10.1186/1471-2288-3-25))

23 Whiting PF, Rutjes AW, Woodgate ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM & QUADAS-2 Group. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Annals of Internal Medicine* 2011 155 529–536. ([https://doi.org/10.7326/0003-4819-155-8-201110180-00009](https://doi.org/10.7326/0003-4819-155-8-201110180-00009))

24 Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM & Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. *Journal of Clinical Epidemiology* 2005 58 982–990. ([https://doi.org/10.1016/j.jclinepi.2005.02.022](https://doi.org/10.1016/j.jclinepi.2005.02.022))

25 Rutter CM & Gatsonis CA. A hierarchical regression approach to meta-analysis of diagnostic test accuracy evaluations. *Statistics in Medicine* 2001 20 2865–2884. ([https://doi.org/10.1002/sim.942](https://doi.org/10.1002/sim.942))

26 Harbord RM, Deeks JJ, Egger M, Whiting P & Sterne JA. A unification of models for meta-analysis of diagnostic accuracy studies. *Biostatistics* 2007 8 239–251. ([https://doi.org/10.1093/biostatistics/kx004](https://doi.org/10.1093/biostatistics/kx004))

27 Gatsonis C & Paiwal P. Meta-analysis of diagnostic and screening test accuracy evaluations: methodologic primer. *American Journal of Roentgenology* 2006 187 271–281. ([https://doi.org/10.2214/AJR.06.0226](https://doi.org/10.2214/AJR.06.0226))

28 Deeks JJ, Macaskill P & Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. *Journal of Clinical Epidemiology* 2005 58 882–893. ([https://doi.org/10.1016/j.jclinepi.2005.01.016](https://doi.org/10.1016/j.jclinepi.2005.01.016))

29 van Enst WA, Ochodo E, Scholten RJ, Hooft L & Leeflang MM. Investigation of publication bias in meta-analyses of diagnostic test accuracy: a meta-epidemiological study. *BMC Medical Research Methodology* 2014 14 70. ([https://doi.org/10.1186/1471-2288-14-70](https://doi.org/10.1186/1471-2288-14-70))

30 Sweeting MJ, Sutton AJ & Lambert PC. What to add to nothing? Use and avoidance of continuity corrections in meta-analysis of sparse data. *Statistics in Medicine* 2004 23 1531–1575. ([https://doi.org/10.1002/sim.1761](https://doi.org/10.1002/sim.1761))

31 Wilson MC, Henderson MC & Smetana GW. Chapter 5: Evidence-based clinical decision making. In *The Patient History; An Evidence-based Approach to Differential Diagnosis*, 2nd ed. Eds MC Wilson, MC Henderson & GW Smetana. McGraw-Hill, 2012.

32 McGee S. Simplifying likelihood ratios. *Journal of General Internal Medicine* 2002 17 646–649. ([https://doi.org/10.1046/j.1525-1497.2002.10730.x](https://doi.org/10.1046/j.1525-1497.2002.10730.x))

33 Harbord RM & Whiting P. metandi: meta-analysis of diagnostic accuracy using hierarchical logistic regression. In *Meta-Analysis in Stata: An Updated Collection from the Stata Journal*, 2nd ed., pp. 211–229. Eds TM Palmer & JAC Sterne. Texas, USA: Stata Press, 2016.
47 Giovanella L, Verburg FA, Imperiali M, Valabrega S, Trimboli P & Ceriani L. Comparison of serum calcitonin and procalcitonin in detecting medullary thyroid carcinoma among patients with thyroid nodules. Clinical Chemistry and Laboratory Medicine 2013 51 1477–1481. (https://doi.org/10.1515/cclm-2012-0610)

48 Turk Y, Makay O, Ozdemir M, Ertunc G, Demir B, Icoz G, Akyildiz M & Yilmaz M. Routine calcitonin measurement in nodular thyroid disease management: is it worthwhile? Annals of Surgical Treatment and Research 2017 92 173–178. (https://doi.org/10.4174/astr.2017.92.4.173)

49 Silvestre C, Sampaio Matias J, Proenca H & Bugalho MJ. Calcitonin screening in nodular thyroid disease: is there a definitive answer? European Thyroid Journal 2019 8 79–82. (https://doi.org/10.1159/000494834)

50 Mian C, Perrino M, Colombo C, Cavedon E, Pennelli G, Ferrero S, De Leo S, Sarais C, Cacciato C, Manfredi GI, et al. Refining calcium test for the diagnosis of medullary thyroid cancer: cutoffs, procedures, and safety. Journal of Clinical Endocrinology and Metabolism 2014 99 1656–1664. (https://doi.org/10.1210/jc.2013-4088)

51 Allelein S, Ehlers M, Morneau C, Schwartz K, Goretzki PE, Seppel T, Feldkamp J, Krieg A, Knoebel A, et al. Measurement of basal serum calcitonin for the diagnosis of medullary thyroid cancer. Hormone and Metabolic Research 2018 50 23–28. (https://doi.org/10.1055/s-0043-122237)

52 Rosario PW & Calsolari MR. Usefulness of serum calcitonin in patients without a suspicious history of medullary thyroid carcinoma and with thyroid nodules without an indication for fine-needle aspiration or with benign cytology. Hormone and Metabolic Research 2016 48 372–276. (https://doi.org/10.1055/s-0042-107246)

53 Kratzsch J, Petzold A, Raue F, Reinhardt W, Brocker-Preuss M, Gorges R, Mann K, Karges W, Morgenthaler N, Luster M, et al. Basal and stimulated calcitonin and procalcitonin by various assays in patients with and without medullary thyroid cancer. Clinical Chemistry 2011 57 467–474. (https://doi.org/10.1373/clinchem.2010.151688)

54 Frank-Raue K, Schott M, Raue F & im Namen der Sektion Schilddrüse der DGE. Recommendation for calcitonin screening in nodular goiter. Deutsche Medizinische Wochenschrift 2018 143 1065–1069. (https://doi.org/10.1055/a-0585-8097)

55 Machens A & Dralle H. Surgical cure rates of sporadic medullary thyroid cancer in the era of calcitonin screening. European Journal of Endocrinology 2016 175 219–228. (https://doi.org/10.1530/EJE-16-0325)

56 Raue F & Frank-Raue K. Medullary thyroid carcinoma and multiple endocrine neoplasia type 2. Deutsche Medizinische Wochenschrift 2020 145 1245–1251. (https://doi.org/10.1055/a-1005-8798)

57 Niederle MB, Scheuba C, Riss P, Selberherr A, Koperek O & Niederle B. Early diagnosis of medullary thyroid cancer: are calcitonin stimulation tests still indicated in the era of highly sensitive calcitonin immunoassays? Thyroid 2020 30 974–984. (https://doi.org/10.1089/thy.2019.0785)

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