SYSTEMATIC REVIEW AND META-ANALYSIS

Liquid biopsies in thyroid cancers: a systematic review and meta-analysis

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Abstract

Thyroid cancer (TC) represents the most common endocrine malignant tumor. Liquid biopsy has been suggested as a new and accurate biomarker in cancer. This systematic review analyzes the existing literature on circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), cell-free DNA integrity index (cfDI), and their potential as biomarkers for TC, including the subtypes: differentiated (papillary and follicular), medullary, and anaplastic. A systematic search was performed in PubMed, Embase, and Cochrane databases for published articles in English between 1 January 1970 and 6 September 2022 (PROSPERO: CRD42022358592). The literature search generated a total of 635 articles. In total, 36 articles were included (patients = 2566). Four studies reported that higher levels of CTCs were associated with metastases and worse prognosis. Nineteen studies found the presence of mutated ctDNA in TC patients. The diagnostic accuracy in detecting BRAFV600E as ctDNA was determined in 11 studies regarding papillary TC. The pooled sensitivity, specificity, and diagnostic odds ratio were estimated at 56% (95% CI 36–74), 91% (95% CI 84–95) and 12 (95% CI 4.09–33.11), respectively. Four studies concluded that the cfDI was higher in patients with TC compared to benign thyroid lesions and healthy controls. The detection of CTCs, ctDNA, and cfDI may have a potential prognostic value in TC in relation to diagnosis, disease progression, and treatment efficacy. Despite the promising potential of CTCs, ctDNA, and cfDI in TC management, limitations hinder direct comparison and generalization of findings. Standardized methodologies, larger patient cohorts, and a consensus on relevant markers are needed to validate their clinical applicability and enhance TC management.

Key Words
- thyroid cancer
- ctDNA
- CTC
- BRAFV600E

Introduction

Thyroid cancer (TC) represents the most common endocrine malignant tumor (Sung et al. 2020, ACS 2021). According to The Global Cancer Observatory database, TC accounted for 586,000 new cancer cases worldwide in 2020, corresponding to 3.0% of all cancers, making TC the ninth most common cancer (Sung et al. 2020).

The incidence of TC has increased over the years due to various factors, e.g. ionizing radiation, increased iodine intake in the population, hormonal exposure, overweight, endocrine-disrupting chemicals, increased occurrence of autoimmune thyroiditis, and due to the increased detection of papillary thyroid carcinomas (PTCs).
attributed by the increased use of ultrasonography, along with increased use of other diagnostic imaging modalities (Feldt-Rasmussen 2020, Sung et al. 2020, ACS 2021). The mortality rate of TC in the United States increased by 0.6% per year from 2009 to 2018 but has stabilized in recent years (ACS 2021). TC is divided into four different subtypes; differentiated thyroid carcinoma (DTC), medullary thyroid carcinoma (MTC), poorly differentiated thyroid carcinoma, and anaplastic thyroid carcinoma (ATC), respectively (Chmielik et al. 2018). The DTCs account for the majority of cases (Chmielik et al. 2018). They can be subdivided into PTC and follicular thyroid carcinoma (FTC), which represent 80 and 10% of all cases of TC, respectively (Haugen et al. 2016, Chmielik et al. 2018). ATC, also known as undifferentiated carcinoma, has a poor prognosis due to its aggressive tumor biology (Haugen et al. 2016).

The diagnosis of TC includes patient symptom review, clinical examination of the neck, fiber endoscopy of the vocal cords, blood samples (thyroid-stimulating hormone, thyroid peroxidase, calcitonin), imaging (ultrasound, radionuclide thyroid scan), and ultrasound-guided fine-needle aspiration with cytological examination (Gharib et al. 2016, Haugen et al. 2016, Mitchell et al. 2016). An ideal biomarker would be able to distinguish between benign and malignant nodules and monitor treatment and disease progression in postoperative surveillance. This would improve the diagnostic process, thereby avoiding unnecessary hemithyroidectomy or total thyroidectomy in patients with benign nodules and guide surgeons toward patients with malignancy.

Today the circulating biomarkers thyroglobulin (Tg) and thyroglobulin antibodies (TgAb) are used after total thyroidectomy in patients with DTC to detect residual thyroid tissue and persistent disease (Nixon et al. 2017). Between 17.7 and 25% of DTC patients have been reported with the presence of TgAb, which can interfere with Tg immunometric assay measurements, causing falsely low/undetectable Tg values, which can mask disease (Kumar et al. 1994, Spencer et al. 1998, Nixon et al. 2017). In these cases, the measurement of TgAb can be used as a surrogate for recurrence (Feldt-Rasmussen et al. 2014). In MTC, calcitonin and carcinoembryonic antigen (CEA) are monitored as biomarkers as increased calcitonin levels have a strong correlation with MTC. However, occasionally, MTCs do not secrete calcitonin (Van Veelen et al. 2009, Gambardella et al. 2019) and CEA is not a specific biomarker for MTC (Van Veelen et al. 2009).

Liquid biopsy has been suggested as a new and noninvasive way to diagnose cancer, monitor treatment response, and aid in cancer surveillance (Payne et al. 2018). Liquid biopsies consist of isolating entities released from the primary tumors such as circulating tumor cells (CTCs) or cell-free DNA (cfDNA) (Fig. 1). Several techniques have been developed to detect liquid biopsies (Palmirotta et al. 2018, Lone et al. 2022). The increased release of cfDNA in cancer patients is due to an increase in the release of circulating tumor DNA (ctDNA) (Payne et al. 2018). The challenge is to discriminate between ctDNA and DNA from normal healthy tissue. One way is to utilize somatic mutations found in cancers, e.g. the BRAF-V600E mutation (Thierry et al. 2016, Payne et al. 2018, Zhu et al. 2019). Another way is to measure the concentration of cfDNA and calculate the cfDNA integrity index (cfDI), which is the ratio of the concentration of longer DNA fragments to shorter fragments (Payne et al. 2018, Sobhani et al. 2018). Apoptosis in normal cells generates short DNA fragments of 100–200 base pairs, while necrosis, which also occurs in tumor cells, produces longer DNA fragments. The release of longer fragments by tumor cells has been reported to cause an increase in cfDI (Sobhani et al. 2018). CTCs derive from primary tumors or metastasis, entering peripheral circulation. Detection of these cells involves diverse approaches (Zhang et al. 2016, Feng et al. 2022). The most widely used method is the immunomagnetic separation technique, which utilizes antibody and surface marker interaction, allowing for either positive or negative identification of specific surface markers. Notably, the CellSearch® and AdnaTest® systems utilize this principle (Zhang et al. 2016, Feng et al. 2022).

Alternatively, other methods isolate CTCs based on distinct physical properties, such as size, density, and electric charge. Microfluidic systems, for instance, capture CTCs based on size, while the Oncoquick® system and DEPArray™ system utilize density and electric charge, respectively (Zhang et al. 2016, Feng et al. 2022).

This systematic review analyzes the existing literature on CTCs, ctDNA, and cfDI and their potential as biomarkers for TC in relation to diagnosis, disease progression, and treatment efficacy.

Materials and methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews
Eligibility criteria

Articles were excluded if they did not include patients with TC, did not specify subtype of TC, included fewer than ten patients, were published before 1970, and if the study was a review and/or meta-analysis.

Studies describing the use of ctDNA, CTCs, and cfDI for diagnosis, prognosis, or surveillance in TC were included. The following data were extracted from the included studies: publication year, type of TC, country, number of patients, number of controls, assay type, type of alteration investigated, media investigated, sampling time, percentage of ctDNA-positive patients, concordance of ctDNA mutation in relation to tumor mutation, and cfDI range.

Systematic literature search strategy

A preliminary search of the Cochrane Library was performed to identify a pre-existing review on the subject, but none was found. One author (WAZ) systemically searched the PubMed, Embase, and Cochrane databases. The search was last updated on 6 September 2022. Data extraction and assessment were independently made by one author (WAZ), and in case of doubt of inclusion, the articles were discussed with another author (KKJ).

In PubMed, the search consisted of the keywords related to cfDNA and CTCs alterations (ctDNA, cfDNA, CTC, circulating epithelial cell, BRAFV600E, rearranged during transfection protooncogene (RET), DNA methylation), the media (plasma, serum, liquid biopsy), and thyroid cancer (thyroid tumor, thyroid tumor, thyroid carcinoma, TC, PTC, FTC, MTC, and ATC). In Embase and Cochrane, the search terms included ctDNA, cfDNA, CTC, thyroid tumor, thyroid tumour, thyroid carcinoma, TC, PTC, FTC, and ATC.
Statistical analysis

Statistical analysis was performed using R studio, version 1.3.1. For each study, we tabulated the number of true positives (BRAFV600E detected in ctDNA and in tumor tissue), false positives (BRAFV600E detected in ctDNA but not detected in tumor tissue), false negatives (BRAFV600E detected in tumor tissue but not detected in ctDNA), and true negatives (BRAFV600E not detected in both ctDNA and tumor tissue). A meta-analysis was constructed to calculate diagnostic accuracy including sensitivity, specificity, diagnostic odds ratio, area under the curve (AUC), and corresponding 95% confidence intervals (95% CI). Forest plots were constructed in R studio depicting sensitivity and specificity. We generated a summary receiver operating characteristic (sROC) curve and calculated the AUC. In R studio, the ‘meta’ and ‘mada’ packages were used to conduct the meta-analysis.

Results

The literature search in PubMed and Embase generated a total of 635 articles. Of these, 584 articles were excluded based on title and abstract. Fifty-one articles were assessed for eligibility, which resulted in the inclusion of 35 articles. One additional study was identified through reference lists (Fig. 2) (Tseng et al. 2017). The 36 included articles were published between 2010 and 2022 and included a total of 2566 patients (range: 28–234 patients). Of these, 1826 had confirmed DTC, 230 had MTC, and 164 had ATC. For the detection of CTCs, the majority of the included studies used immune-based detection, whereby antibodies selectively bind to cell surface markers such as epithelial cell adhesion molecule (EpCAM), thyroid-stimulating hormone receptor (TSHR), and/or pondoplanin (PDPN). In addition, the CTCs needed to be negative for CD45, a differential marker for white blood cells. Once the antibody was bound to the surface marker, it was then visualized by immunofluorescence microscopy (Habli et al. 2020). The studies regarding cfDNA used different methods for detecting genetic alterations, including polymerase chain reaction (PCR), real-time PCR, digital PCR, droplet digital PCR (ddPCR), and next-generation sequencing (NGS) (Elazezy & Joosse 2018). The general principle behind all these methods is to isolate and quantify DNA fragments that are released by tumor cells into the bloodstream. For the detection of

Figure 2

PRISMA flowchart of article selection process. Flowchart. An overview of the selection process of the study. A total of 36 studies were included in the study. n = number of articles. Modified version of the PRISMA 2009 flow diagram (Moher et al. 2009). CTC, circulating tumor cells; ctDNA, circulating tumor DNA; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analyses; TC, thyroid cancer.
Table 1  The ten included studies involving CTCs in TC.

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Type of TC</th>
<th>Country</th>
<th>Groups: patients (P) and controls (C)</th>
<th>Assay type</th>
<th>Type of alteration</th>
<th>Timepoint of sampling</th>
<th>Results</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tseng et al. (2017)</td>
<td>DTC</td>
<td>Taiwan</td>
<td>$P_{DTC} = 129$</td>
<td>PowerMag after immunofluorescence</td>
<td>Expression of EpCAM, TSHR, and PDPN</td>
<td>One sample; sampling point 6 weeks after surgery or RIT</td>
<td>CTC levels in patients with incomplete vs excellent response to therapy EpCAM (cells/mL): 124 vs 14 TSHR (cells/mL): 105 vs 17 PDPN (cells/mL): 34 vs 6 $P &lt; 0.0001$</td>
<td>Blood</td>
</tr>
<tr>
<td>Ehlers et al. (2018)</td>
<td>PTC, MTC, FTC</td>
<td>Germany</td>
<td>$P_{PTC} = 33, P_{FTC} = 20, P_{MTC} = 14, C = 15$</td>
<td>Immunofluorescence</td>
<td>EpCAM and CD45</td>
<td>One sample; after surgery and RIT</td>
<td>CTC levels in TC patients compared to normal controls: $2.4 \pm 3.1$ CTCs vs $0.2 \pm 0.4$ CTCs $P &lt; 0.0001$ No significant differences were seen when comparing TC subtypes</td>
<td>Blood</td>
</tr>
<tr>
<td>Qiu et al. (2018)</td>
<td>DTC</td>
<td>China</td>
<td>$P_{DTC} = 72$, of these 42 with dm and 30 without dm C = 30</td>
<td>NE–iFISH</td>
<td>Expression of EpCAM and CD45</td>
<td>One sample; after surgery, timepoint not specified</td>
<td>CTCs ≥ 5 in 7.5 mL blood are correlated with dm in DTC CTCs ≥ 7 may predict dm in DTC patients with poor response to RIT and associated with worse prognosis</td>
<td>Blood</td>
</tr>
<tr>
<td>Lin et al. (2018)</td>
<td>PTC</td>
<td>Taiwan</td>
<td>$P_{PTC} = 119, P_{FTC} = 5$</td>
<td>PowerMag after immunofluorescence</td>
<td>Expression of EpCAM, TSHR, and PDPN</td>
<td>One sample; sampling point 4–6 weeks after surgery or RIT</td>
<td>CTC levels in patients with evidence of TC resistance to therapy EpCAM (cells/mL): 87 vs 19 TSHR (cells/mL): 101 vs 25 PDPN (cells/mL): 36 vs 8 $P &lt; 0.001$</td>
<td>Blood</td>
</tr>
<tr>
<td>Schmidt et al. (2021)</td>
<td>DTC</td>
<td>Germany</td>
<td>$P_{DTC} = 55$</td>
<td>Immunofluorescence</td>
<td>Expression of EpCAM</td>
<td>Two sample points; before RIT and 6 weeks after</td>
<td>CTC levels in patients before RIT and 6 weeks after $0.27 \pm 0.47$ vs $0.05 \pm 0.15$, $P = 0.0215$</td>
<td>Blood</td>
</tr>
<tr>
<td>Winkens et al. (2014)</td>
<td>DTC</td>
<td>Germany</td>
<td>$P_{DTC} = 28$, of these, 13 patients received first RIT 4–6 weeks after surgery. The remaining 15 patients had evidence/persistence of DTC tissue</td>
<td>Immunofluorescence</td>
<td>Expression of EpCAM</td>
<td>Four sample points; before RIT, second day post RIT, 14 days post RIT, and 90 days post RIT</td>
<td>CTC levels in patients receiving first RIT (CEC/mL): before RIT: 13,386 2 days after RIT: 13,322 14 days after RIT: 14,622 90 days after RIT: 20,583 CTC levels in patients with persistence of DTC receiving RIT (CEC/mL): before RIT: 16,027 2 days after RIT: 10,299 14 days after RIT: 4068 90 days after RIT: 10,141</td>
<td>Blood</td>
</tr>
</tbody>
</table>

(Continued)
### Table 1

The table represents the characteristics of the included studies investigating CTCs in TC.

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Type of TC</th>
<th>Country</th>
<th>Groups: patients (P) and controls (C)</th>
<th>Assay type</th>
<th>Type of alteration</th>
<th>Timepoint of sampling</th>
<th>Results</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zheng et al. (2019)</td>
<td>DTC</td>
<td>China</td>
<td>$P_{PDC} = 234$</td>
<td>CanPatrol™ after immunofluorescence</td>
<td>Expression of NIS and EMT phenotypes of CTC</td>
<td>Two sample points; after surgery and after RIT</td>
<td>Response to RIT based on NIS expression. 78.9% of patients with high expression of NIS showed ER to RIT compared to 53.9% of patients with low NIS expression ($P = 0.047$)</td>
<td>Blood</td>
</tr>
<tr>
<td>Xu et al. (2016)</td>
<td>DTC and MTC</td>
<td>USA</td>
<td>$P_{MTC} = 18 P_{DTC}$ with $d_m = 14$, $C = 10$</td>
<td>Veridex™ Cellsearch</td>
<td>Expression of EpCAM and CD45</td>
<td>Patients with metastatic disease, after surgery, systematic therapy, tyrosine kinase inhibitors and/or RIT</td>
<td>Six MTC patients with $d_m$ who had $\geq 5$ CTCs/7.5 mL at the time of sampling died during follow-up, compared with 8 of 12 MTC patients with dm (67%) with &lt;5 CTCs/7.5 mL ($P = 0.245$)</td>
<td>Blood</td>
</tr>
<tr>
<td>Lin et al. (2016)</td>
<td>PTC</td>
<td>Taiwan</td>
<td>$P_{PTC} = 48$, of these 22 with $d_m$, $C = 17$</td>
<td>PowerMag after immunofluorescence</td>
<td>EpCAM and TSHR</td>
<td>One sample; after surgery and RIT</td>
<td>CTC levels comparison in controls, PTC patients without $d_m$ and PTC patients with $d_m$, respectively EpCAM (cells/mL): 6, 12, and 91 $P &lt; 0.0001$ TSHR (cells/mL): 9, 16, and 100 $P &lt; 0.0001$</td>
<td>Blood</td>
</tr>
<tr>
<td>Li et al. (2018)</td>
<td>PTC</td>
<td>Taiwan</td>
<td>$P_{PTC} = 25$, all patients were positive for TgAb and undetectable levels of Tg</td>
<td>PowerMag after immunofluorescence</td>
<td>EpCAM and TSHR</td>
<td>One sample; after surgery and RIT. Sample point not specified</td>
<td>Higher levels of CTCs in $d_m$. Patients were classified into two groups: recurrence group ($n = 7$) and remission group ($n = 18$). CTC levels comparison in recurrence and remission group, respectively EpCAM (cells/mL): 73 and 11 $P &lt; 0.0001$ TSHR (cells/mL): 54 and 5 $P &lt; 0.0001$</td>
<td>Blood</td>
</tr>
</tbody>
</table>

CD45, cluster of differentiation 45; CTC, circulating tumor cell; dm, distant metastasis; DTC, differentiated thyroid carcinoma; EMT, epithelial-mesenchymal transition; EpCAM, epithelial cell adhesion molecule; ER, excellent response; IR, incomplete response; MTC, medullary thyroid carcinoma; NE-iFISH, negative enrichment immunofluorescence and in situ chromosomal hybridization; NIS, sodium/iodide symporter; PDPN, pondoplanin; PTC, papillary thyroid carcinoma; RIT, radioiodine therapy; TC, thyroid cancer; TSHR, thyroid-stimulating hormone receptor.

The table represents the characteristics of the included studies investigating CTCs in TC.
mutation found in ctDNA the primers are allele-specific (Elazezy & Joosse 2018).

**Circulating tumor cells**

Ten studies evaluated the detection of CTCs in patients with DTC (Table 1), and one study also assessed CTCs in patients with MTC (patients = 814, controls = 72). The studies were conducted at varying disease course timepoints.

**Diagnosing TC**

Two studies found a significant increase in CTCs in DTC patients compared to healthy controls (Ehlers et al. 2018, Qiu et al. 2018). Qiu et al. (2018) reported the detection of CTCs in 62 out of 72 subjects (86%) (Qiu et al. 2018). Ehlers et al. (2018) further discovered that the number of CTCs correlated significantly with tumor stage at initial diagnosis (Ehlers et al. 2018).

**Monitoring treatment response and prognosis**

Five studies investigated the use of CTCs in monitoring treatment response and prognosis of patients with DTC. (Winkens et al. 2014, Tseng et al. 2017, Lin et al. 2018, Zheng et al. 2019, Schmidt et al. 2021). Two of these studies analyzed the expression of EpCAM, TSHR, and/or PDPN in CTCs and concluded that the level of these markers was statistically higher in patients with an incomplete response to treatment, evaluated by biochemical and/or structural evidence of persistent TC (Tseng et al. 2017, Lin et al. 2018). Schmidt et al. (2021) demonstrated a significant reduction in CTCs 6 weeks after radiiodine therapy (RIT) as adjuvant therapy contrary to Winkens et al. (2014) who found no recognizable correlation in the levels of CTCs before and after RIT (2 days, 14 days, and 90 days post to RIT) (Winkens et al. 2014). One study by Zheng et al. (2019) detected CTCs based on the presence of sodium/iodide symporter (NIS) and epithelial–mesenchymal transition markers and reported that patients with a high NIS expression at baseline were more likely to show excellent response to first RIT compared to those with a low NIS expression at baseline, which was independent of the status of Tg and TgAb (Zheng et al. 2019).

**CTC levels in metastasis**

Four studies determined that higher levels of CTC were associated with metastasis and worse prognosis in TC patients (Lin et al. 2016, Xu et al. 2016, Lin et al. 2018, Qiu et al. 2018). Qiu et al. (2018) determined that the presence of ≥5 CTCs was significantly associated with distant metastasis, and CTCs ≥ 7 were related to poor response to RIT in DTC patients, evaluated according to the 2015 American Thyroid Association guideline (Qiu et al. 2018). In addition, two studies reported the detection of CTCs in three patients with distant metastasis, but undetectable levels of Tg and no evidence of TgAb (Lin et al. 2016, Ehlers et al. 2018). Li et al. (2018) included 25 PTC patients with positive serum TgAb and undetectable serum Tg and revealed that the median numbers of CTCs were significantly increased in the recurrence group compared to the remission group (EpCAM-CTCs 72.5 vs 10.8 and TSHR- CTCs 54 vs 5.3) (Li et al. 2018).

Xu et al. (2016) reported that the presence of ≥5 CTCs in patients with metastatic MTC was associated with worse overall survival (Xu et al. 2016).

Due to great variability in method and aim among the included studies addressing CTC, it was not possible to conduct a meta-analysis on CTCs.

**ctDNA and cfDI**

Twenty-two studies measured mutation detection in ctDNA (patients = 1384, controls = 165), while five studies calculated the cfDI (patients = 549, controls = 208) (Tables 2 and 3). One article dealt with both identifying ctDNA and calculating the cfDI (Zane et al. 2013). The studies were conducted at varying disease course timepoints.

**ctDNA in PTC, MTC, and ATC**

Table 2 The 22 studies involving the detection of ctDNA in TC.

<table>
<thead>
<tr>
<th>Study reference</th>
<th>Type of TC</th>
<th>Genes</th>
<th>Country</th>
<th>Groups: patients (P) and controls (C)</th>
<th>Assay type</th>
<th>Number of sample points and treatment status in patients</th>
<th>Media</th>
<th>ctDNA-positive patients (%)</th>
<th>Concordance of ctDNA mutation with tumor mutation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zane et al. (2013)</td>
<td>PTC, MTC, ATC, and FA</td>
<td>BRAFV600E, SLC5A8, SLC26A4</td>
<td>Italy</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 86, P&lt;sub&gt;MTC&lt;/sub&gt; = 58, P&lt;sub&gt;ATC&lt;/sub&gt; = 9, P&lt;sub&gt;FA&lt;/sub&gt; = 23, C = 19</td>
<td>qPCR</td>
<td>One sample; presurgical patients</td>
<td>Plasma</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pupilli et al. (2013)</td>
<td>PTC and NTD</td>
<td>BRAFV600E</td>
<td>Italy</td>
<td>P&lt;sub&gt;PTS&lt;/sub&gt; = 103, 38 of these with PTC, C = 49</td>
<td>qPCR</td>
<td>Two sample points; presurgical and postsurgical patients (±RIT)</td>
<td>Plasma</td>
<td>PTC&lt;sub&gt;B&lt;/sub&gt; = 23.7 ± 5.4, PTC&lt;sub&gt;C&lt;/sub&gt; = 6.5 ± 3.7, BL = 9.9 ± 3.2, C = 1.7 ± 0.3</td>
<td>92</td>
</tr>
<tr>
<td>Chuang et al. (2010)</td>
<td>PTC</td>
<td>BRAFV600E</td>
<td>USA</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 14, P&lt;sub&gt;PTC&lt;/sub&gt; = 4, P&lt;sub&gt;BL&lt;/sub&gt; = 9, P&lt;sub&gt;Symptom&lt;/sub&gt; = 1</td>
<td>qPCR</td>
<td>One sample; presurgical patients</td>
<td>Plasma</td>
<td>21</td>
<td>60</td>
</tr>
<tr>
<td>Khatami et al. (2020)</td>
<td>PTC</td>
<td>BRAFV600E, RASSF1, SCL5A8</td>
<td>Iran</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 57, C = 45</td>
<td>qPCR</td>
<td>One sample; presurgical patients</td>
<td>Plasma</td>
<td>57.9</td>
<td>84.6</td>
</tr>
<tr>
<td>Kim et al. (2015)</td>
<td>PTC</td>
<td>BRAFV600E</td>
<td>Korea</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 72</td>
<td>qPCR</td>
<td>One sample; presurgical patients</td>
<td>Plasma</td>
<td>4.1</td>
<td>6.1</td>
</tr>
<tr>
<td>Lubitz et al. (2016)</td>
<td>PTC</td>
<td>BRAFV600E</td>
<td>USA</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 48</td>
<td>qPCR</td>
<td>One sample; presurgical or before BRAFi</td>
<td>Blood</td>
<td>-</td>
<td>71</td>
</tr>
<tr>
<td>Almubarak et al. (2019)</td>
<td>PTC</td>
<td>BRAFV600E</td>
<td>Saudi Arabia</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 38</td>
<td>3D dPCR</td>
<td>One sample; presurgical patients</td>
<td>Blood</td>
<td>37</td>
<td>86</td>
</tr>
<tr>
<td>Patel et al. (2021)</td>
<td>PTC, nPTC, FTC</td>
<td>BRAFV600E</td>
<td>Canada</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 45, P&lt;sub&gt;nPTC&lt;/sub&gt; = 23, P&lt;sub&gt;PTC&lt;/sub&gt; = 3, P&lt;sub&gt;BL&lt;/sub&gt; = 38</td>
<td>qPCR</td>
<td>Two sample points; presurgical and postsurgical patients</td>
<td>Plasma</td>
<td>BT = 13.8, AT = 1.13</td>
<td>40</td>
</tr>
<tr>
<td>Jensen et al. (2020)</td>
<td>PTC</td>
<td>BRAFV600E</td>
<td>USA</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 57</td>
<td>dPCR and COLD PCR</td>
<td>One sample; presurgical patients and before RIT</td>
<td>Plasma</td>
<td>dPCR = 14, dPCR-COLD PCR = 42.1</td>
<td>14</td>
</tr>
<tr>
<td>Sato et al. (2021)</td>
<td>PTC</td>
<td>BRAFV600E</td>
<td>Japan</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 22</td>
<td>ddPCR</td>
<td>Two sample points; presurgical and postsurgical patients</td>
<td>Plasma</td>
<td>BT = 73</td>
<td>31</td>
</tr>
<tr>
<td>Li et al. (2019)</td>
<td>PTC</td>
<td>BRAFV600E</td>
<td>China</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 59</td>
<td>3D dPCR</td>
<td>One sample; presurgical patients</td>
<td>Plasma</td>
<td>27</td>
<td>61.5</td>
</tr>
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</table>

(Continued)
<table>
<thead>
<tr>
<th>Study reference</th>
<th>Type of TC</th>
<th>Genes</th>
<th>Country</th>
<th>Groups: patients (P) and controls (C)</th>
<th>Assay type</th>
<th>Number of sample points and treatment status in patients</th>
<th>Media</th>
<th>ctDNA-positive patients (%)</th>
<th>Concordance of ctDNA mutation with tumor mutation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gouda et al. (2022)</td>
<td>PTC</td>
<td>BRAFV600E</td>
<td>USA</td>
<td>$P_{PTC} = 33$</td>
<td>qPCR</td>
<td>One sample; both presurgical and postsurgical patients</td>
<td>Plasma</td>
<td>33</td>
<td>52</td>
</tr>
<tr>
<td>Condello et al. (2018)</td>
<td>PTC</td>
<td>BRAFV600E</td>
<td>Italy</td>
<td>$P_{PTC} = 59$; of these, 13 with dm</td>
<td>qPCR and dPCR</td>
<td>One sample; presurgical patients</td>
<td>Plasma</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kwak et al. (2013)</td>
<td>PTC</td>
<td>BRAFV600E</td>
<td>Korea</td>
<td>$P_{PTC} = 94$</td>
<td>qPCR</td>
<td>One sample; presurgical patients</td>
<td>Serum</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Allin et al. (2018)</td>
<td>DTC, MTC, PDTC, and ATC</td>
<td>Multigene panel (including BRAFV600ENRAS, RET, and more)</td>
<td>UK</td>
<td>$P_{DTC} = 32$, $P_{MTC} = 15$, $P_{PDTC} = 3$, $P_{ATC} = 1$</td>
<td>ddPCR</td>
<td>Multiple sample points; postsurgical patients, samples were gathered at study entry and every third month</td>
<td>Plasma</td>
<td>PTC = 73, FTC = 79, MTC = 85, ATC = 100, PDTC = 100</td>
<td>-</td>
</tr>
<tr>
<td>Lan et al. (2020)</td>
<td>PTC</td>
<td>Multigene panel (including BRAFV600ENRAS, RET, and more)</td>
<td>China</td>
<td>$P_{PTC} = 66$; of these, 20 with dm</td>
<td>GeneseeqPrime</td>
<td>One sample; presurgical and before RIT/chemotherapy</td>
<td>Plasma</td>
<td>PTC$<em>{DM} = 60$, PTC$</em>{ND} = 20$, Overall = 39</td>
<td>-</td>
</tr>
<tr>
<td>Lubitz et al. (2018)</td>
<td>PTC</td>
<td>BRAFV600E</td>
<td>USA</td>
<td>$P_{PTC} = 54$, $C = 27$</td>
<td>qPCR</td>
<td>Two sample points; in presurgical patients and postsurgical patients</td>
<td>Blood</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cote et al. (2017)</td>
<td>MTC</td>
<td>RET M918T</td>
<td>USA</td>
<td>$P_{MTC} = 75$, $C = 25$</td>
<td>ddPCR</td>
<td>Multiple sample points; first sample was collected in both pre- and post-treated patients. Patients were prospectively followed and had plasma drawn during scheduled appointments for evaluating levels of RET M918T</td>
<td>Plasma</td>
<td>21</td>
<td>32</td>
</tr>
<tr>
<td>Ciampi et al. (2022)</td>
<td>MTC</td>
<td>Multigene panel (including RET, KRAS, and more)</td>
<td>Italy</td>
<td>$P_{MTC} = 29$</td>
<td>NGS</td>
<td>Two sample points; in presurgical patients and postsurgical patients</td>
<td>Plasma</td>
<td>RET M918T = 10, KRAS = 2.9</td>
<td>43</td>
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(Continued)
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<tr>
<th>Study reference</th>
<th>Type of TC</th>
<th>Genes</th>
<th>Country</th>
<th>Groups: patients (P) and controls (C)</th>
<th>Assay type</th>
<th>Number of sample points and treatment status in patients</th>
<th>Media</th>
<th>ctDNA-positive patients (%)</th>
<th>Concordance of ctDNA mutation with tumor mutation (%)</th>
</tr>
</thead>
</table>
| Sandulache et al. (2017) | ATC        | Multigene panel (including BRAFV600E, TP53, PIK3CA, and more) | USA      | P
ATC = 23                              | NGS              | One sample point; included three patient groups: (i) patients with dm and/or LG without treatment. (ii) patients treated for LG or dm, with minimal residual disease. (iii) patients without active disease. | Plasma      | -                          | 72 (treatment naïve, BRAF 7/7, TP53 4/6, PIK3CA 3/5, NRAS 3/3) |
| Iyer et al. (2018)     | ATC        | BRAFV600E                                  | USA      | P
ATC = 44                              | NGS and ddPCR       | Multiple sample points; first sample was collected in pretreated patients. Sixteen patients were prospectively followed during treatment and had plasma drawn during scheduled appointments | Plasma      | -                          | NGS = 91, ddPCR = 93                                      |
| Qin et al. (2021)      | ATC        | Multigene panel (including BRAFV600E, TP53, PIK3CA, and more) | USA      | P
ATC = 87                              | NGS              | Multiple sample points; Twenty-eight treatment-naive patients had samples collected. Thirty-two patients received BRAFi treatment and were prospectively followed during treatment and had plasma drawn during scheduled appointments | Plasma      | -                          | Treatment-naive patients: BRAFV600E = 92.9, TP53 = 82.1, PIK3CA = 92.9 |

The table represents the characteristics of the included studies investigating ctDNA in TC. 
ATC, anaplastic thyroid carcinoma; BL, benign lesions; BT, before treatment; COLD PCR, co-amplification at lower denaturation temperature-based PCR; ctDNA, circulating tumor DNA; ddPCR, droplet digital PCR; dPCR, digital PCR; DTC, differentiated thyroid carcinoma; FA, follicular adenoma; MTC, medullary thyroid carcinoma; NGS, next-generation sequencing; nPTC, nonclassical PTC; NTD, nodular thyroid lesions; PCR, polymerase chain reaction; PDTC, poorly differentiated thyroid carcinoma; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; PTC, papillary thyroid carcinoma; qPCR, real-time PCR; RET, rearranged during transfection protooncogene; SLC26A4, solute carrier family 26 member 4; SLC5A8, solute carrier family 5 member 8; TC, thyroid cancer; TP53, tumor protein p53.

Considerable variability in sensitivity and specificity estimates were observed ranging from 6% (95% CI 1–17) to 92% (95% CI 62-99) in sensitivity (Fig. 3), and from 50% (95% CI 2–98) to 98% (95% CI 74–99) in specificity (Fig. 4). The meta-analysis revealed a pooled sensitivity and specificity at 56% (95% CI 36–74) and 91% (95% CI 84–95), respectively. The diagnostic odds ratio was calculated to 12 (95% CI 4.1–33.1) and the sROC curve (Fig. 5) revealed an AUC of 0.77 (95% CI 0.68–0.87).

Five of the studies concluded that the detection of ctDNA was higher in PTC patients with progression or distant metastasis (Allin et al. 2018, Almubarak et al. 2019, Khatami et al. 2020, Lan et al. 2020, Sato et al. 2021).

Three studies investigated mutations found in plasma as ctDNA in patients with MTC (Cote et al. 2017, Allin et al. 2018, Ciampi et al. 2022). Cote et al. (2017) and Ciampi et al. (2022) detected RET M918T as ctDNA in 32 and 43% of patients with advanced MTC, respectively, harboring the mutation in tumor tissue (Cote et al. 2017, Ciampi et al. 2022). Allin et al. (2018) examined several mutations in ctDNA and detected ctDNA in 85% of patients with MTC. The majority of the mutations were detected in the RET gene (Allin et al. 2018).

In three studies concerning patients with ATC, the concordance between BRAFV600E in ctDNA and tumor tissue varied from 91 to 100%, in treatment-naive patients (Sandulache et al. 2017, Iyer et al. 2018, Qin et al. 2021). The most commonly mutated genes in ATCs were reported to be TP53, BRAFV600E, and PIK3CA, which were correlated to worse overall survival (Sandulache et al. 2017, Qin et al. 2021). In addition, Iyer et al. (2018) reported that serial monitoring of ctDNA levels, as a biomarker of response to treatment in relation to imaging, was concordant with tumor shrinkage in 16 patients (out of 17 patients, 94%) and tumor growth in 7 patients (out of 15 patients, 47%) (Iyer et al. 2018).

Role of ctDNA in monitoring treatment response and disease progression

Three studies in PTC patients reported a reduction in BRAFV600E ctDNA after surgery in PTC patients (Pupilli et al. 2013, Lubitz et al. 2018, Patel et al. 2021) and in two studies after targeted therapy dabrafenib (BRAF inhibitor) and trametinib (mitogen-activated enzyme kinase inhibitor) (Patel et al. 2021) and sorafenib (protein kinase inhibitor) (Allin et al. 2018). Two studies showed cases where ctDNA was detected in three patients with PTC; however, Tg levels were undetected (Allin et al. 2018, Lubitz et al. 2018). Allin et al. (2018) also reported earlier changes in ctDNA in DTC patients with targeted therapy for progressive disease than Tg. In addition, ctDNA revealed an earlier increase compared to calcitonin and CEA in three patients with MTC who had evidence of progressive disease (Allin et al. 2018).

Cell-free DNA integrity index

Five studies evaluated whether cfDI could serve as a diagnostic tool in differentiating benign from malignant lesions in patients with thyroid nodules (Zane et al. 2013, Salvianti et al. 2017, Thakur et al. 2019, Higazi et al. 2021, Klimaite et al. 2022). Four of the studies reported that the cfDI was significantly higher in patients with TC compared to patients with benign nodules and healthy subjects (Table 3) (Zane et al. 2013, Salvianti et al. 2017, Higazi et al. 2021, Klimaite et al. 2022). Thakur et al. (2019) revealed no significant difference in cfDI between individuals with benign and malignant lesions (Thakur et al. 2019).

Discussion

Thirty-four studies examined the use of CTCs, ctDNA, and cfDI as liquid biopsy and their ability to assess tumor presence, disease progression, and monitoring treatment efficacy in TC patients.

The presence of CTCs in the blood was found to correlate with disease stage, tumor size, and metastasis, suggesting that CTCs could be utilized to monitor tumor progression and treatment response and assess the risk of metastasis in TC patients. In breast cancer, CTCs are considered efficient as prognostic biomarkers (Zhao et al. 2011, Yan et al. 2017, Pang et al. 2021) and included in the American Joint Committee on Cancer Staging Manual (Edge 2017). For TC, this is not
<table>
<thead>
<tr>
<th>Study reference</th>
<th>Type of thyroid lesion</th>
<th>Genes</th>
<th>Country</th>
<th>Groups: patients (P) and controls (C)</th>
<th>Assay type</th>
<th>Number of sample points and treatment status in patients</th>
<th>Media</th>
<th>Integrity index (range)</th>
</tr>
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<tbody>
<tr>
<td>Zane et al. (2013)</td>
<td>PTC, MTC, ATC and FA</td>
<td>ALU-83 ALU-244</td>
<td>Italy</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 86, P&lt;sub&gt;MTC&lt;/sub&gt; = 58, P&lt;sub&gt;ATC&lt;/sub&gt; = 9, P&lt;sub&gt;FA&lt;/sub&gt; = 23, C = 19</td>
<td>qPCR</td>
<td>One sample; presurgical patients</td>
<td>Plasma</td>
<td>PTC = 0.29 (0.11–0.96), MTC = 0.35 (0.13–0.88), ATC = 0.68 (0.32–0.98), FA = 0.23 (0.12–0.68)</td>
</tr>
<tr>
<td>Thakur et al. (2019)</td>
<td>NTD and PTC</td>
<td>ALU-115 ALU-247 APP-67 APP-180</td>
<td>USA</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 38, P&lt;sub&gt;BL&lt;/sub&gt; = 29, P&lt;sub&gt;THY4+THY5&lt;/sub&gt; = 28, P&lt;sub&gt;THY2&lt;/sub&gt; = 44, P&lt;sub&gt;THY3&lt;/sub&gt; = 25, C = 49</td>
<td>qPCR</td>
<td>One sample; presurgical patients</td>
<td>Plasma</td>
<td>PLT = 0.45 (0.36–0.57), Thy4+Thy5 = 1.02 (0.22–2.02), Thy 3 = 0.83 (0.01–2.21), Thy 2 = 0.67 (0.22–1.24)</td>
</tr>
<tr>
<td>Salvianti et al. (2017)</td>
<td>DTC and NTD</td>
<td>APP-67 APP-180</td>
<td>Italy</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 86, P&lt;sub&gt;MTC&lt;/sub&gt; = 58, P&lt;sub&gt;ATC&lt;/sub&gt; = 9, P&lt;sub&gt;FA&lt;/sub&gt; = 23, C = 19</td>
<td>qPCR</td>
<td>Two sample points; presurgical and postsurgical patients (±RIT)</td>
<td>Plasma</td>
<td>Thy4+Thy5 = 1.02 (0.22–2.02), Thy 3 = 0.83 (0.01–2.21), Thy 2 = 0.67 (0.22–1.24)</td>
</tr>
<tr>
<td>Higazi et al. (2021)</td>
<td>NTD, PTC, MTC and ATD</td>
<td>ALU-83 ALU-244</td>
<td>Egypt</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 18, P&lt;sub&gt;MTC&lt;/sub&gt; = 21, P&lt;sub&gt;ATC&lt;/sub&gt; = 25, P&lt;sub&gt;BL&lt;/sub&gt; = 25, C = 25</td>
<td>qPCR</td>
<td>One sample; presurgical patients</td>
<td>Plasma</td>
<td>PTC: 0.4 (0.3–0.5), FT: 0.4 (0.36–0.52), MTC: 0.6 (0.5–0.7), BL: 0.15 (0.07–0.2), ATD: 0.1 (0.01–0.13)</td>
</tr>
<tr>
<td>Klimaite et al. (2022)</td>
<td>NTD and PTC</td>
<td>β-actin-99 β-actin-394</td>
<td>Lithuania</td>
<td>P&lt;sub&gt;PTC&lt;/sub&gt; = 68, P&lt;sub&gt;MTC&lt;/sub&gt; = 31, P&lt;sub&gt;ATC&lt;/sub&gt; = 25, P&lt;sub&gt;BL&lt;/sub&gt; = 31, C = 86</td>
<td>qPCR</td>
<td>Two sample points; presurgical and postsurgical patients</td>
<td>Plasma</td>
<td>Controls: 0.2 (0.13–0.27), Pre-operative PTC = 0.48 (0.06–1.33), Post-operative PTC = 0.35, NTD = 0.36, Controls = 0.13</td>
</tr>
</tbody>
</table>

The table represents the characteristics of the included studies determining the cfDI in TC. ALU, *Arthrobacter luteus*; APP, amyloid precursor protein; ATC, anaplastic thyroid carcinoma; ATD, autoimmune thyroid disease; BL, benign lesions; cfDI, cell-free DNA integrity; DTC, differentiated thyroid carcinoma; FA, follicular adenoma; MTC, medullary thyroid carcinoma; NTD, nodular thyroid lesions; PCR, polymerase chain reaction; PTC, papillary thyroid carcinoma; qPCR, real-time PCR; RET, rearranged during transfection protooncogene; THY 2, nonneoplastic; THY 3, follicular lesions; THY 4, suspicious of malignancy; THY 5, diagnostic of malignancy.
the case yet. Determining the best method for detecting CTCs from the included studies is not possible, due to the limited number of studies and the wide variety of techniques employed for CTC isolation within these studies. The best detection rates were obtained by utilizing TSHR and EpCAM as markers for CTCs in TC; therefore, future studies should focus on these markers.

Detecting BRAFV600E as ctDNA in PTC patients showed a moderate sensitivity (56%; 95% CI 36–74), and high specificity of (91%; 95% CI 84–95). However, significant heterogeneity ($t^2=83\%$, $P<0.05$) affected result accuracy (Higgins et al. 2003). The diagnostic odds ratio (12) and AUC (0.77) indicated poor diagnostic accuracy (Swets 1988, Glas et al. 2003). While the BRAFV600E is the most commonly mutated gene in PTC (rate of 29–83%) (Trovisco et al. 2006), considering other mutated genes in future studies is essential, and expanded genetic mutation panels in ctDNA are recommended (Agrawal et al. 2014). The level of ctDNA correlated with advanced disease stages, metastasis, and treatment response, suggesting ctDNA’s potential for noninvasive disease tracking.

For the detection of ctDNA in MTC patients, the results varied considerably. Both the use of a single gene variant (RET M918T) and single timepoint analysis in the study by Cote et al. (2017) is likely to have been responsible for their lower detection rate, compared to the study by Allin et al. (2018). Both the study by Allin et al. (2018) and Ciampi et al. (2022) investigated multiple genes. The lower detection rate of ctDNA in the study by Ciampi et al. (2022) might be attributed to other factors, such as differences in methodology, patient selection, and disease characteristics. Further research is necessary for the utilization of ctDNA in MTC patients.

The highest level of concordance between ctDNA and tumor tissue was demonstrated in the context of ATC. Iyer et al. (2018) demonstrated that the level of BRAFV600E ctDNA correlated with tumor response, where no definitive biomarker exists. Target therapy with dabrafenib and trametinib has shown promising results in BRAFV600E patients with ATC (Subbiah et al. 2018), and this treatment in combination is approved by the Food and Drug Administration (FDA) (FDA 2018). Studies concerning the same treatment in patients with BRAFV600E-positive melanoma showed that it was possible to monitor treatment response by measuring BRAFV600E ctDNA (Schreuer et al. 2016). Hence, this
data might imply that monitoring BRAFV600E ctDNA in ATC could be a valuable tool to determine treatment efficacy and resistance to therapy.

The cfDI was significantly higher in TC patients compared to patients with benign nodules in the majority of the studies evaluated. This is consistent with previous studies regarding breast, colorectal, and prostate cancer (Stötzer et al. 2014, El-Gayar et al. 2016, Fawzy et al. 2016).

For the detection of ctDNA, the highest detection rate was observed for ddPCR and NGS (Table 2), and the literature reports that these methods have the lowest limit of detection (Siravegna et al. 2017). Future research should prioritize these methods for ctDNA detection and focus on the use of ctDNA in monitoring treatment response, progression, and advanced cases of TC with undetectable levels of conventional biomarkers.

Although promising, the clinical utility of CTCs, ctDNA, and cfDI in TC management is hindered by limitations that prevent direct comparisons and generalization of findings.

In the case of CTCs, variations in detection cut-off values, cancer status, previous therapeutic interventions, and detection methods across studies, along with the nonspecific markers used for CTC detection, such as EpCAM, PDPN, and TSHR, affect the reliability of results (Zhang et al. 2009, Williams 2011, Tsai et al. 2020). Despite this, CTCs may still prove helpful in monitoring disease status and recurrence in DTC patients, especially after lobectomy, in conjunction with Tg and TgAb.

For ctDNA, variations in analytical methods, highly selected patients, disease activity at blood sampling time, and sample sizes contribute to considerable variation in study results. Additionally, tumor location, size, and vascularity affect ctDNA shedding rates into circulation, leading to differences in ctDNA levels among patients (Elazezy & Joosse 2018).

The variability in gene markers, segment repeats, and TC subtypes used in cfDI studies also limits comparison. Standardized methodologies and a consensus on relevant markers are necessary to ensure an accurate assessment of cfDI’s clinical significance.

CTCs, ctDNA, and cfDI hold promise for enhancing the management of TC, but the available evidence regarding their use is still limited. To overcome limitations and validate their clinical applicability, standardized methodologies and larger patient cohorts are required.

**Conclusion**

The findings of this systematic review confirmed that the detection of CTCs, ctDNA, and cfDI may have a prognostic value in TC in relation to monitoring diagnosis, disease progression, and treatment efficacy. The utilization of CTCs, ctDNA, and cfDI may be particularly useful when conventional markers are insufficient, e.g. in dedifferentiated disease or when Tg is undetected. Despite the promising potential of CTCs, ctDNA, and cfDI in TC management, limitations hinder direct comparison and generalization of findings. Standardized methodologies, larger patient cohorts, and a consensus on relevant markers are needed to validate their clinical applicability and enhance TC management.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this systematic review.

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References


Gouda MA, Ong E, Huang HJ, McPhaul LW, Yoon S, Janku F & Giamouakis AG 2022 Ultraselective detection of BRAF V600E mutations in circulating tumor DNA of patients with metastatic thyroid cancer. Endocrine 76 491–494. (https://doi.org/10.1007/s12020-022-03004-z)


Khatami F, Larijani B, Heshmat R, Nasiri S, Haddadi-Aghdam M, Teimoori-Toolabi I & Tanvarag SM 2020 Hypermethylated RASSF1 and SLCSA8 promoters alongside BRAFV600E mutation as biomarkers for papillary


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