Diagnostic Value of Fine Needle Aspiration BRAFV600E Mutation Analysis in Papillary Thyroid Cancer: A systematic review and meta-analysis

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Diagnostic Value of Fine Needle Aspiration *BRAF*^{V600E} Mutation Analysis in Papillary Thyroid Cancer: A systematic review and meta-analysis

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Abstract

Fine needle aspiration (FNA) with cytological analysis is an initial step in diagnosing thyroid nodules that are suspicious for cancer. We systematically reviewed the test accuracy of B-type Raf kinase ($BRAF^{V600E}$) gene mutation analysis plus conventional FNA in the diagnosis of papillary thyroid cancer (PTC). We identified studies reporting $BRAF^{V600E}$ mutation analysis following FNA for evaluation of thyroid nodules through searching MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials, scanning reference lists of relevant studies, and contacting experts. Two independent reviewers screened literature results, abstracted data and appraised study quality. When appropriate, bivariate and univariate random effects meta-analyses of sensitivity and specificity were considered for all outcomes. Forty-seven studies met our inclusion criteria after screening 1560 citations and 169 full-text articles. The included studies enrolled approximately 16,170 patients with 9,924 fine needle aspiration samples evaluated for $BRAF^{V600E}$ mutation. Univariate-pooled sensitivity was 69% (95% CI; 61%, 76%) for papillary thyroid cancer. For thyroid nodules that were diagnosed cytologically as suspicious for papillary thyroid cancer, univariate-pooled sensitivity using fine needle aspiration and $BRAF^{V600E}$ results was 52% (95% CI; 39%, 64%). Despite its high specificity, our meta-analysis shows that $BRAF^{V600E}$ mutation analysis has a low sensitivity in diagnosing papillary thyroid cancer in thyroid nodules. The feasibility of this test as a single molecular tool is not well established, which indicates the need for well designed prospective clinical studies.

Introduction

Papillary thyroid cancer (PTC) represents the most common histologic type of thyroid carcinomas, accounting for approximately 85% of thyroid cancers [2,3]. PTC ranks as the eighth most common malignancy diagnosed in women and the 5-year survival rate in the US is approximately 95% [4-6].

An active mutation in the gene for the *B-type Raf kinase* (*BRAF*) has been recently described and represents the most common genetic alteration in PTC [6]. It involves a missense mutation resulting in the substitution of thymine (T) for adenine (A) at nucleotide 1799. Subsequently, valine is replaced with glutamate at residue 600, which has been found exclusively in PTC (known as *BRAF*^{V600E}) [8,9]. *BRAF* is a key component in cell proliferation, differentiation and apoptosis. Thus, *BRAF*^{V600E} mutation is associated with thyroid cancer through up regulation of cell division and proliferation leading to tumor genesis [10,11]. Furthermore, *BRAF*^{V600E} mutation was shown to be associated with extra-thyroid extension, lymph node metastasis, distant metastasis, advanced tumor-node-metastasis (TNM) stages, and cancer recurrence, which are considered to be poor prognostic factors [12-16]. Fine needle aspiration (FNA) biopsy with cytological analysis is an initial step in the diagnosis of thyroid nodules and pathology [17,18].

According to the “Bethesda System for Reporting Thyroid Cytopathology”, there are 6 general diagnostic categories for FNA cytology: I. nondiagnostic or unsatisfactory, II. benign, III. atypia of undetermined significance, IV. follicular neoplasm or suspicious for a follicular neoplasm, V. suspicious for malignancy, and VI. malignant [19]. FNA findings interpreted as categories III-V (often reported as “*indeterminate*”) occur in about 10 to 30% of FNA results and are considered a significant limitation in the investigation of thyroid nodules [17,20], making the intraoperative frozen section analysis a means of guiding for further planning [21,22]. If the accuracy of FNA can be improved by adding *BRAF*^{V600E} mutation analysis to

the conventional FNA, this may help with the investigation of these nodules.

We aimed to systematically review the test accuracy of the cytologic determination of *BRAF*^{V600E} mutational status plus conventional FNA in patients with thyroid nodules suspicious for cancer.

Materials and Methods

A systematic review protocol based on the PRISMA Statement for Protocols was used to guide the reporting of this review [23]. A draft protocol was assembled, circulated to systematic review methodologists and clinicians, and revised as necessary. It is available upon request from the authors upon request.

Eligibility criteria

Studies eligible for inclusion were diagnostic randomized trials, cohort, and case control studies that were conducted in a clinical setting, reported on test accuracy, and written in English and Arabic, as these were the fluent languages of the authors. Patients were those aged 16 years or older who had thyroid nodules. The index test was FNA and $BRAF^{V600E}$ mutation analysis. $FNA-BRAF^{V600E}$ samples were not considered if they came from histological tissue, paraffin-embedded, frozen or microdissected samples. Our outcome of interest was confirmed PTC, obtained through histological assessment of tissue obtained from biopsy, surgery or autopsy (i.e. reference test).

Search

An experienced information specialist (LP) conducted all of the literature searches in consultation with the research team. The search strategy for the main electronic search (MEDLINE) is presented in the eFile 1. It was modified as necessary for EMBASE and the Cochrane Central Register of Controlled Trials and is available from the authors upon request. Medical Subject Headings and text words related to FNA and thyroid cancer were used to search MEDLINE (OVID Interface) and EMBASE (OVID Interface) from inception until November 18, 2014. The search was not limited by study design or date of dissemination. Additional articles were

identified through scanning the reference lists of included studies and screening conference symposia abstracts.

Study selection

A pilot-test was conducted prior to embarking on screening, which only occurred with high inter-rater agreement was achieved across the team. Subsequently, two reviewers independently screened all identified titles and abstracts for inclusion using a pre-defined inclusion criteria form. The full-text of potentially relevant articles were obtained and assessed in the same manner. Discrepancies were resolved by discussion or the involvement of a third reviewer.

Data collection process

A draft data extraction form was developed, piloted, and modified, as necessary. Two reviewers extracted all of the data using the standardized data extraction form, independently. Discrepancies were resolved by discussion or the involvement of a third reviewer. The data were checked a third time to ensure data integrity. When multiple study publications reported data from the same population (i.e. a companion report), the study reporting the largest sample size was considered the major publication and the other report(s) was used for supplementary data only.

Data items

The extracted data included study characteristics (e.g., study design, sample size, method of enrollment, setting, method of $BRAF^{V600E}$ mutation assessment, cytological and histologic characteristics of the samples were obtained), patient characteristics (e.g., mean age, gender), and outcome results (e.g., number of true

positive, true negative, false positive, and false negative samples required to compile a 2x2 table for the sensitivity and specificity for each study).

Methodological quality

The methodological quality of the included studies was assessed using the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy Tool, Version 1.0.0. This 20-item tool assesses the following domains: representative spectrum, acceptable reference standard, acceptable delay between tests, partial verification avoided, differential verification avoided, relevant clinical information, uninterpretable results reported, withdrawals explained, unchanged technology of the index test, clear definition of what was considered to be a “positive” result, appropriate training of test operators, treatment withheld until both the index test and the reference standard were performed, data on observer variation reported and within an acceptable range, data on instrument variation reported and within an acceptable range, pre-specification of objectives, and free from commercial funding [24]. The “established cut-off values” criterion was excluded from our assessment because none of the studies used these.

Synthesis of results

The Bethesda classification scheme was used to categorize the findings of the included studies [19]. Subsequently, sensitivity of *FNA-BRAF*^{V600E} mutation were calculated for the included studies and presented in forest plots. Bivariate meta-analysis of sensitivity and specificity was considered for all outcomes and summary receiver operating characteristic (sROC) curves were estimated, when appropriate. In situations where, specificity was either not defined (false positive and true negative values are zero) or was reported as 100% (perfect specificity) for all the studies, a univariate random effects meta-analyses were performed to provide pooled estimates

of sensitivity and their corresponding 95% confidence intervals. Statistical heterogeneity among the studies was investigated using the I^2 statistic [25,26]. All analyses were performed using the R statistical package [27].

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Results

Study selection

The literature search yielded 1560 citations (i.e., titles and abstracts; Figure 1). In total, 169 full-text articles were retrieved and examined for relevance. A total of 47 studies fulfilled the inclusion criteria and were included [6,12,20-22,28-69].

Study and patient characteristics

Around 50% of the included studies were case control studies (n=26/47), and the remaining were prospective cohort studies. 14 studies were conducted in the USA [6,12,21,31,32,42,44,45,54,56-58,65,67], 15 studies in Korea [20,22,36-39,41,49-51,59,60,62,63,68], 12 in Italy [29,33-35,40,43,46,47,55,61,64,66], two in China [48,52], and one study each were from Japan [28], Portugal [30] Germany [53] or the UK [69]. All studies were conducted between 2004 and 2014 and the number of patients ranged from 10 to 6,402 with a mean age of 47.5 (Tables 1 and 2).

Methodological quality assessment results

At least 70% (>29/47) of the included studies adequately addressed 12 of the quality assessment items (Supplementary Table). In contrast, only one study clearly reported an acceptable delay between tests, 3 studies conducted the reference standard without knowledge of the index test (this was scored as no in 30 studies and unclear in the rest), 3 studies conducted the index test without knowledge of the reference standard (this was scored as no in 27 studies and unclear in the rest), 12 studies described withdrawals (this was scored as no in 25 and unclear in the rest), 4 studies withheld treatment until the reference standard/index test were assessed (this was scored as no in 20 studies and unclear in the rest), 4 studies reported data within an acceptable range for observer variation (this was scored as no in the rest of the

studies), and 46 of the studies did not report data on instrument variation within an acceptable range (this was scored as unclear in one study).

Meta-analysis results

Indeterminate group (Bethesda categories III-V)

Seven studies reported this outcome. Among them, 1 reported zero false positive and true negative values, and hence specificity could not be estimated [32]. For the remaining 6 studies, specificity is reported as 100%, consequently pooling the estimates was not necessary. We performed a univariate random effects meta-analysis to pool sensitivity of $BRAF^{V600E}$ mutation across the 7 studies that reported the outcome. The pooled sensitivity estimate was 30% (95% confidence interval [CI]; 11%, 50%), ($I^2=79.54\%$). The forest plot for sensitivity is presented in figures 2.

Atypia of undetermined significance

Four studies reported this outcome, among them specificity and sensitivity could not be estimated for 3 studies due to data ambiguity. We, therefore, performed a univariate random effects meta-analysis to pool sensitivity estimates from one study that examined $BRAF^{V600E}$ mutation among the atypia of undetermined significance group as diagnosed by the reference standard [44]. The pooled sensitivity was 31%, (95% CI; 6%, 56%).

Suspicious for malignancy

Nine studies reported this outcome. Among them, 4 reported zero false positive and true negative values, and hence specificity could not be estimated [40,44,55,58]. For the remaining 5 studies, specificity is reported as 100%, consequently pooling the estimates was not necessary. We performed a univariate

random effects meta-analysis to pool sensitivity of $BRAF^{V600E}$ mutation across the nine studies that reported the outcome. The pooled sensitivity estimate was 52% (95% CI; 39%, 64%), ($I^2=77.42\%$). The forest plot of sensitivity is presented in figure 3.

Malignancy - PTC

Twenty-seven studies reported this outcome in which 19 studies showed high heterogeneity. Specificity can only be estimated for one study [50], since both true negative and false positive values are zero for all the remaining studies, consequently, specificity could not be estimated (Figure 4). For Kwak et al 2010, there were only 3 true negatives and all of them were detected indicating 100% specificity [50].

Therefore, we performed a univariate random effect meta-analysis to pool sensitivity estimates across the 19 studies. The pooled sensitivity estimate of $BRAF^{V600E}$ mutation in identifying patients with malignant PTC was 69% (95% CI; 61%, 76%), ($I^2=90.37\%$). The forest plot of sensitivity is presented in figure 4.

RET/PTC rearrangement

We further extracted data from the included studies related to another common mutation in PTC (*RET/PTC* rearrangement) for comparison purposes. Only six studies examined this mutation along with $BRAF^{V600E}$ mutation [29,30,33,35,46,53]. The coupled forest plot for sensitivity/specificity and the sROC curve are presented in figures 5a and 5b, respectively.

Discussion

Our meta-analysis shows that $BRAF^{V600E}$ mutation analysis of FNA cytology has only 30% sensitivity for PTC in those cases that were reported as indeterminate. Similarly, among suspicious for malignancy FNA cytology group, $BRAF^{V600E}$ mutation analysis has a sensitivity of 52%. Moreover, the cytological determination of $BRAF^{V600E}$ mutational status in PTC cytology group was correlated with the final pathologic diagnosis with only 69% sensitivity. Our collective results suggest that the proportion of patients with PTC that are correctly identified using $FNA-BRAF^{V600E}$ is low, although specificity overall is shown to be very high. These results are consistent with similar previous meta-analysis results by Jia et al [70]. However, our review is more comprehensive with 8 times the number of included studies and provides an up-to-date assessment of this issue. Moreover, we stratified our findings according to Bethesda System for Reporting Thyroid Cytopathology to give a more accurate estimate on the test sensitivity and specificity among different cytologic findings [70].

Several studies have shown that $BRAF^{V600E}$ mutation is an indicator of poor disease prognosis since it's associated with advanced disease stage, and cancer recurrence [71]. More recently, the presence of $BRAF^{V600E}$ mutation was significantly associated with increased cancer-related mortality among patients with PTC [72]. Therefore, early recognition and preoperative determination of $BRAF^{V600E}$ mutation is thought to be of great value when determining the management and operative strategy. For example, recent studies suggest a prophylactic central compartment lymph node dissection done simultaneously with total thyroidectomy for patients with positive $BRAF^{V600E}$ mutation [73,74]. However, our data shows that $BRAF^{V600E}$ screening is unlikely to alter the initial management strategy considering the significantly low sensitivity of this test which cannot consistently rule out PTC when

tests are negative. Therefore, the use of $BRAF^{V600E}$ as a single molecular test is not clinically practical. Moreover, our data confirms an important limitation highlighted by Xing et al, in which $BRAF^{V600E}$ mutation will be of limited value with non-PTC samples, since it is mostly associated with PTC [75].

Since it was first introduced, FNA cytology provided great advantages over surgical evaluation and true cut biopsy. It provided simple and quick method for evaluating thyroid nodules, and was well tolerated pain by most patients. However, like any other diagnostic technique, FNA is not without its disadvantages. For example, FNA findings interpreted as non-diagnostic in about 10 to 30% of FNA results. This important limitation led researchers to work on more accurate and less invasive way to diagnose suspicious thyroid nodules including the $BRAF^{V600E}$ mutational analysis, which is generally (as per our meta-analysis) insufficient when it is done alone for diagnostic purpose. Moreover, additional markers have been examined to improve the sensitivity of this test. For example, miRInformTM (Asuragen), which is a panel that consists of seven validated molecular markers “mutations” (*KRAS*, *BRAF*, *HRAS*, *NRAS*, *RET/PTC 1*, *RET/PTC3* and *PAX8/PPAR γ*) [76]. As per our results, very few of the included studies accounted for other mutations (e.g. only 6 studies reported the *RET/PTC* rearrangement) [28,29,32,34,45,52]. Thus, we cannot comment on the utility of combining $BRAF^{V600E}$ mutation with other mutations. However, upon further analysis, *RET/PTC* rearrangement showed slightly higher sensitivity as compared to $BRAF^{V600E}$ mutation [29,30,33,35,46,53]. Furthermore, the clinical applications of these panels have not been established. Therefore, implementing these panels with different mutations may not be feasible when individual variations between institutions and endocrine pathologists are considered.

The revised American Thyroid Association (ATA) management guidelines for patients with thyroid nodules and differentiated thyroid cancer strongly recommends doing a lobectomy or total thyroidectomy for suspicious/indeterminate thyroid nodules depending on the lesion's size and other risk factors [17]. According to the ATA, the use of molecular markers including *BRAF*^{V600E} might be considered depending on the clinico-pathological information and physician's judgment [77].

The large number of included studies and patients analyzed with worldwide geographic variation represents a major strength of our systematic review. However, there are some limitations. More than 50% of the included studies were conducted in three countries (USA, Italy, Korea). Therefore, our conclusion may not be generalizable to all patients with thyroid nodules globally. Moreover, data were pooled across studies from different institutions and countries where variation in the technical accuracy may alter the overall sensitivity and specificity. However, most studies used the same tests to examine the presence of *BRAF*^{V600E} mutation (e.g. PCR, DNA sequencing). All studies were conducted recently and none of our analyses showed any statistical heterogeneity.

The methodological quality of the included studies was comparable. The majority of the included studies used a sampling strategy that is likely representative of the patients who will receive the test in practice. All included studies used acceptable reference standard, which will likely classify the target condition correctly, and reported relevant clinical information, where the same clinical data available when test results were interpreted as would be available when the test is used in practice. The fact that reference standard results were interpreted without knowledge of the results of the index test in only 3 studies, and the index test results were interpreted without knowledge of the results of the reference standard results in 4

studies are an important limitation worth mentioning. Failure to assess the index and reference test results blindly can lead to more optimistic conclusions. This limitation can only be avoided by using blind assessment of the results in well-designed prospective clinical studies and ideally randomized clinical trials. Moreover, half of the included studies were case control studies which can falsely overestimate the accuracy of a diagnostic test [78]. Finally, many of the studies did not adequately report the results in a clear manner or were ambiguous; as such, we were unable to include these in our analysis.

In summary, we found no strong evidence to support the implementation of *BRAF*^{V600E} mutation analysis plus FNA as a single screening test for patients with suspicious and indeterminate thyroid nodules. This conclusion was exclusively based on the literature, which may differ from clinical practice. Preoperative risk stratification should be considered the main guidance for the best management plan in individual patients. Other diagnostic tests should be the focus of further clinical studies.

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Study concept and design: Drs. Fnais, Tricco and Straus.

Acquisition of data: Drs Fnais and Tricco, and Ms. Soobiah and Perrier.

Analysis and interpretation of data: Drs Fnais and Tricco, and Ms. Soobiah.

Drafting of the manuscript: Drs Fnais, Tricco and Al-Qahtani, and Ms. Soobiah.

Critical revision of the manuscript for important intellectual content: Drs Fnais, Tricco, Straus, Hamid and Al-Qahtani, and Ms. Soobiah.

Statistical analysis: Dr. Jemila S. Hamid.

Administrative, technical, or material support: Drs. Fnais and Tricco, and Ms. Perrier.

Study supervision: Drs Fnais, Tricco and Straus.

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Figure 1: Study flow-chart

Figure 1: Flowchart of the literature search and study selection process in a 2012 systematic review of the literature on the diagnostic value of *FNA-BRAF^{V600E}* mutation analysis in papillary thyroid cancer.

Figure 2: Summary measures for sensitivity of *BRAF*^{V600E} mutation analysis in indeterminate group

Figure 3: Summary measures for sensitivity of $BRAF^{V600E}$ mutation analysis in suspicious for malignancy group

Figure 4: Summary measures for sensitivity of *BRAF*^{V600E} mutation analysis in PTC group

Figure 5: Summary measures for sensitivity and specificity of $BRAF^{V600E}$ mutation analysis in RET/PTC rearrangement

A. Coupled forest; for studies with one or more zero counts, 0.5 is added to the data.

B. Summary ROC curve obtained from bivariate meta-analysis.

Table 1: Study Characteristics

	First Author	Country; Sitting	Study Design	Mean Age (SD)
1.	Cohen et al 2004 [21]	USA; John Hopkins Medical Institution	Case control	NR
2.	Hayashida et al 2004 [28]	Japan; Nagasaki University Hospital in Japan	Cohort	53
3.	Salvatore et al 2004 [29]	Italy; Pathology Dept, University of Naples and University of Pisa	Case control	42 (16)
4.	Xing et al 2004 [6]	USA; John Hopkins Hospital (Baltimore, MD, USA)	Cohort	NR
5.	Domingues et al 2005 [30]	Portugal; Instituto Portugues Oncologia	Cohort	NR
6.	Chung et al 2006 [20]	Korea; Research Institute and Hospital National Cancer Center, Seoul National University Hospital	Cohort	46.4
7.	Jin et al 2006 [31]	USA; Mayo Clinic, Rochester, MD, USA	Case control	52
8.	Rowe et al 2006 [32]	USA; University of Utah Department of Surgery	Case control	NR
9.	Pizzolanti et al 2007 [33]	Italy; Faculty of Medicine, University of Palermo	Cohort	49.6 (14.29)
10.	Sapio et al 2007 [34]	Italy; Azienda Osepdaliera Universitaria Federico II, Naples; Ospedale Mauriziano, Turin	Case control	NR
11.	Sapio et al 2007 [35]	Italy; Azienda Osepdaliera Universitaria Federico II, Naples; Ospedale Mauriziano, Turin	Case control	NR
12.	Kim et al 2008 [36]	Korea; Konkuk University Hospital	Case control	NR
13.	Bentz et al 2009 [12]	USA; University of Utah Department of Surgery	Case control	40.9 (2.5)
14.	Suk Jo et al 2009 [37]	Korea; Center for Endocrine Surgery, Chungham National University Hospital	Cohort	NR
15.	Kim et al 2009 [38]	Korea; Konkuk University Hospital	Case control	NR
16.	Kwak et al 2009 [39]	Korea; Severance Hospital, Korea	Case control	45.2

17.	Marchetti et al 2009 [40]	Italy; Section of Cytopathology, Division of Surgical, Molecular and Ultrastructural pathology	Case control	38.5
18.	Moon et al 2009 [41]	Korea; Severance Hospital, Korea	Case control	45.7
19.	Xing et al 2009 [42]	USA; John Hopkins Hospital (Baltimore, MD, USA)	Case-series	(median) 44.5
20.	Zatelli et al 2009 [43]	Italy; Section of Endocrinology, University of Ferrara	Cohort	50.7 (0.7)
21.	Yip et al 2009 [44]	USA; University of Pittsburg Medical Center	Case control	NR
22.	Nikiforov et al 2009 [45]	USA; University of Colorado Denver school of Medicine, University of Cincinnati Medical Centre	Cohort	NR
23.	Cantara et al 2010 [46]	Italy; Department of Surgery, University of Sienna	Cohort	51.2 (13.8)
24.	Girlando et al 2010 [47]	Italy; North Italian population	Cohort	NR
25.	Guo et al 2010 [48]	China; First Affiliated Hospital of Xi'an Jiaotong University	Cohort	NR
26.	Hwang et al 2010 [49]	Korea; Samsung Medical Centre, Sungkyurkwan University school of Medicine	Cohort	47
27.	Kwak et al 2010 [50]	Korea; Severance Hospital, Korea	Case control	45.5
28.	Lee et al 2010 [51]	Korea; Konkuk University Hospital	Cohort	48.3 (13.7)
29.	Lin et al 2010 [52]	China; First Affiliated Hospital of Wenzhou Medical College	Cohort	49.2
30.	Musholt et al 2010 [53]	Germany; Mainz University (nuclear medicine, endocrinology, pediatric endocrinology, endocrine surgery), outpatient clinics in Mainz, and Augsburg	Cohort	NR
31.	Ohori et al 2010 [54]	USA; University of Pittsburg Medical Center	Case control	NR
32.	Proietti et al 2010 [55]	Italy; University Hospital of Pisa	Case control	44.7
33.	Adeniran et al 2011 [56]	USA; NR (clinical)	Cohort	52.6 (15.6)
34.	Adeniran et al 2011 [57]	USA; Department of Pathology, Diagnostic Radiology and Surgery, Yale University	Case control	49.4 (only those with BRAF mutation)
35.	Colanta et al 2011 [58]	USA; Memorial Sloan-Kettering Cancer Center	Case control	46

36.	Kim et al 2011 [59]	Korea; Konkuk University Hospital	Cohort	NR
37.	Moon et al 2011 [60]	Korea; Yonsei University College of Medicine	Case control	49.4
38.	Pelizzo et al 2011 [61]	Italy; University Hospital of Padova	Cohort	47.8
39.	Chang et al 2012 [22]	Korea; University of Guro Hospital	Case control	NR
40.	Kang et al 2012 [62]	Korea; Department of Pathology, Samsung Medical Center	Case control	47
41.	Moon et al 2012 [63]	Korea; Konkuk University Medical Center	Case control	50
42.	Rossi et al 2012 [64]	Italy; University of Ferrara, and Azienda Ospedaliero Universitaria di Ferrara	Cohort	52
43.	Ohori et al 2013 [65]	USA; University of Pittsburgh Medical Center-Presbyterian	Case control	Age range (33.8 - 47.9)
44.	Rossi et al 2013 [66]	Italy; Agostino Gemelli School of Medicine, Catholic University of the Sacred Heart	Cohort	47 (median)
45.	Aragon et al 2014 [67]	USA; The Johns Hopkins University School of Medicine	Case control	46.7 (13.5)
46.	Hyeon et al 2014 [68]	Korea; Department of Pathology, Samsung Medical Center	Case control	49.7
47.	Poller et al 2014 [69]	UK; Queen Alexandra Hospital	Case control	NR

Abbreviation: NR, Not reported.

Table 2: Patient Characteristics

	First Author	#Patients enrolled, analyzed	Method of BRAF ^{V600E} mutation assessment	Tested with BRAF ^{V600E}	Tested with Reference
1.	Cohen et al 2004 [21]	92, NR	PCR and DNA sequencing	186	92
2.	Hayashida et al 2004 [28]	NR	PCR and DNA sequencing	58	NR
3.	Salvatore et al 2004 [29]	96, 96	PCR and DNA sequencing	96	96
4.	Xing et al 2004 [6]	45, 40	PCR and DNA sequencing	45	45
5.	Domingues et al 2005 [30]	52, 59	PCR and DNA sequencing	59	24
6.	Chung et al 2006 [20]	NR	PCR and DNA sequencing	NR	NR
7.	Jin et al 2006 [31]	71, 71	PCR and DNA sequencing	71	71
8.	Rowe et al 2006 [32]	24, 19	PCR and DNA sequencing	24	19
9.	Pizzolanti et al 2007 [33]	156, 49	PCR	156	49
10.	Sapio et al 2007 [34]	144, 82	PCR and DNA sequencing	144	82
11.	Sapio et al 2007 [35]	131, 80	PCR	131	80
12.	Kim et al 2008 [36]	103, 103	PCR and DNA sequencing	103	103
13.	Bentz et al 2009 [12]	45, 45	PCR	42	45
14.	Suk Jo et al 2009 [37]	101, 101	PCR and DNA sequencing	101	55
15.	Kim et al 2009 [38]	101, 101	PCR and DNA sequencing	101	101
16.	Kwak et al 2009 [39]	630, 339	PCR and DNA sequencing	339	662
17.	Marchetti et al 2009 [40]	111, 111	PCR and DNA sequencing	111	111
18.	Moon et al 2009 [41]	91, 91	PCR and DNA sequencing	91	91
19.	Xing et al 2009 [42]	190, 190	PCR and DNA sequencing	190	190
20.	Zatelli et al 2009 [43]	374, 166	PCR and DNA sequencing	374	166

21.	Yip et al 2009 [44]	332, 206	PCR	332	332
22.	Nikiforov et al 2009 [45]	328, 69	PCR and DNA sequencing	328	69
23.	Cantara et al 2010 [46]	174, 174	PCR	235	174
24.	Girlando et al 2010 [47]	NR	PCR and DNA sequencing	91	64
25.	Guo et al 2010 [48]	10, 10	NR	10	10
26.	Hwang et al 2010 [49]	135, 135	PCR and DNA sequencing	135	135
27.	Kwak et al 2010 [50]	130, 118	PCR and DNA sequencing	130	130
28.	Lee et al 2010 [51]	85, 52	DPO system, and DNA sequencing	85	85
29.	Lin et al 2010 [52]	61, 20	PCR	61	61
30.	Musholt et al 2010 [53]	290, 83	PCR and DNA sequencing	290	93
31.	Ohori et al 2010 [54]	NR	PCR	NR	NR
32.	Proietti et al 2010 [55]	187, 87	PCR and DNA sequencing	187	187
33.	Adeniran et al 2011 [56]	68, 146	PCR	146	68
34.	Adeniran et al 2011 [57]	81, 81	PCR and DNA sequencing	84	84
35.	Colanta et al 2011 [58]	NR, 52	PCR and DNA sequencing	52	52
36.	Kim et al 2011 [59]	865, 194	PCR	849	NR
37.	Moon et al 2011 [60]	295, 102	PCR	295	102
38.	Pelizzo et al 2011 [61]	270, 270	PCR and DNA sequencing	270	270
39.	Chang et al 2012 [22]	191, 191	PCR and DNA sequencing	191	191
40.	Kang et al 2012 [62]	1060, 1060	PCR and DNA sequencing	1060	313

41.	Moon et al 2012 [63]	524, 553	PCR and DNA sequencing	553	375
42.	Rossi et al 2012 [64]	1856, 1856	PCR and DNA sequencing	1856	325
43.	Ohuri et al 2013 [65]	131, 131	PCR and DNA sequencing	131	119
44.	Rossi et al 2013 [66]	230; NR	PCR and DNA sequencing	73	50
45.	Aragon et al 2014 [67]	87; 87	NR	29	29
46.	Hyeon et al 2014 [68]	NR; 6402	PCR and DNA sequencing	Unclear	Unclear
47.	Poller et al 2014 [69]	NR, 207	PCR and DNA sequencing	29	207

Abbreviation: NR, Not reported.

Highlights

- FNA with cytological analysis is an initial step in diagnosing thyroid nodules that are suspicious for cancer.
- New genetic markers including *BRAF*^{V600E} gene mutation analysis are available that may increase the accuracy of FNA for diagnosing thyroid cancer.
- The results of our study indicate a low sensitivity of *BRAF*^{V600E} mutational analysis in diagnosing PTC.
- The feasibility of this test as a single molecular tool is not well established, which indicates the need for well-designed prospective clinical studies.