Allan Vinícius Martins-de-Barros https://orcid.org/
Fábio Andrey da Costa Araújo https://orcid.org/0000-0001-5488-9333
Ana Maria Ipólito Barros https://orcid.org/0000-0001-6739-0988
Raisa Jordana Geraldine Severino Lazo https://orcid.org/0000-0003-4963-308X
Adauto Gomes Barbosa Neto https://orcid.org/0000-0002-9545-7905
Helker Albuquerque Macedo da Silva https://orcid.org/0000-0003-3549-893X
Maria Tereza Cartaxo Muniz https://orcid.org/0000-0001-9498-5223
Stefânia Jeronimo Ferreira https://orcid.org/0000-0001-6656-6435
Marianne de Vasconcelos Carvalho https://orcid.org/0000-0002-6815-5696

https://doi.org/10.5327/2525-5711.274

Original Article

Diagnostic accuracy of a mutation-specific monoclonal antibody (RM8 clone) for BRAF V600e detection by immunohistochemistry in ameloblastomas

Allan Vinícius Martins-de-Barros[1,2]
Fábio Andrey da Costa Araújo[3,4]
Ana Maria Ipólito Barros[3]
Raisa Jordana Geraldine Severino Lazo[3]
Adauto Gomes Barbosa Neto[5]
Helker Albuquerque Macedo da Silva[5]
Maria Tereza Cartaxo Muniz[5]
Stefânia Jeronimo Ferreira[1,2]
Marianne de Vasconcelos Carvalho[1,3,6]*

[1]Universidade de Pernambuco, Odontologia, Campus Arcoverde – Arcoverde (PE), Brazil.
[2]Universidade de Pernambuco, Multicampi Garanhuns, Programa de Pós-Graduação em Saúde e Desenvolvimento Socioambiental – Garanhuns (PE), Brazil.
[3]Universidade de Pernambuco, Faculdade de Odontologia, Programa de Pós-Graduação em Odontologia – Recife (PE), Brazil.
[4]Universidade de Pernambuco, Hospital Universitário Oswaldo Cruz, Departamento de Cirurgia Bucomaxilofacial – Recife (PE), Brazil.

[5]Universidade de Pernambuco, Instituto de Ciências Biológicas – Recife (PE), Brazil.
[6]Universidade de Pernambuco, Hospital Universitário Oswaldo Cruz, Centro Integrado de Anatomia Patológica – Recife (PE), Brazil.
***Corresponding to:** Email: marianne.carvalho@upe.br
Received on October 24, 2024. Accepted on February 4, 2025.

Abstract

Objective: To determine the diagnostic accuracy of IHC using anti-BRAF V600E monoclonal antibody (RM8 clone) compared to Real-Time PCR in the detection of BRAF V600E mutation in FFPE ameloblastoma samples. **Methods:** This is a diagnostic accuracy study conducted based on the STARD recommendations. The index test was the IHC with anti-BRAF V600E antibody (RM8 clone), while the reference standard was the DNA analysis through castPCR™ mutation detection assays. The target condition was the detection of BRAF V600E mutation. The FFPE ameloblastoma samples were independently submitted to index test and reference standard. Sensitivity and specificity measures, and positive and negative predictive values were calculated. **Results:** Fifty-four FFPE ameloblastoma samples were included. The sensitivity and specificity of IHC using the RM8 clone compared do Real-Time PCR for the detection of the BRAF V600E mutation in FFPE ameloblastoma samples was 1.00 (95%CI 0.90–1.00) and 0.46 (95%CI 0.21–0.73), respectively, and the diagnostic test accuracy was calculated as 85.18%. **Conclusion:** IHC using BRAF V600E-specific antibody (RM8 clone) showed extremely high sensitivity, but suboptimal specificity when compared with Real-Time PCR in the detection of BRAF V600E mutation in FPPE ameloblastoma samples.

Keywords: Ameloblastoma; Immunohistochemistry; BRAF V600E; Monoclonal Antibodies; RM8 clone.

Statement of Clinical Significance

The use of the RM8 clone for immunohistochemical detection of the BRAF V600E mutation in ameloblastomas shows high sensitivity but limited specificity, resulting in potential false positives. This impacts the diagnostic accuracy of this method, suggesting it is not yet suitable as a standalone diagnostic tool for clinical decision-making in routine practice.

INTRODUCTION

The BRAF V600E mutation is a common activating mutation that has been linked to oncogenesis because of its disruptive impacts on cell signaling through the Mitogen-Activated Protein Kinase (MAPK) pathway[1,2]. This molecular alteration was identified in about 65% of the ameloblastomas, which are a benign but locally aggressive odontogenic tumor potentially eligible to targeted therapies with BRAF inhibitors[3-5]. Therefore, the evaluation of the BRAF V600E mutation in these tumors could assume a crucial role in its management, emerging as a routine diagnostic procedure in the near future[6].

Although the standard approach for detecting the BRAF V600E mutation in solid tumors involves DNA analysis through molecular tests, These methods are costly and require complex infrastructure and specialized personnel[7]. The introduction of specific anti-BRAF V600E monoclonal antibodies has enabled its detection in formalin-fixed, paraffin-embedded (FFPE) tissue samples through immunohistochemistry (IHC), which is a more affordable alternative when compared to molecular tests[6,8].

Presently, two commercially available anti-BRAF V600E monoclonal antibodies are suitable for use in IHC: VE1 and RM8 clones. In contrast to the VE1 clone, a mouse monoclonal antibody produced with hybridoma technology, the RM8 clone is a recombinant monoclonal antibody generated through in vitro expression systems[8]. Although the exact immunogen sequences used to produce both the VE1 and RM8 clones remain proprietary information, they were designed to be BRAF V600E-specific, with no cross-reactivity with wild type BRAF.

Although IHC using the VE1 clone has shown good accuracy when compared to molecular tests[9-13], studies on the performance of the RM8 clone are scarce and data on

its sensitivity or specificity in ameloblastoma samples are not yet available. Therefore, the aim of this study was to determine the diagnostic accuracy of IHC using anti-BRAF V600E antibody (RM8 clone) compared to DNA analysis through Real-Time Polymerase Chain Reaction (PCR) in the detection of BRAF V600E mutation in ameloblastomas.

MATERIAL AND METHODS

Study design

This study was approved by the Ethics Committee of the University of Pernambuco (#4.309.512; CAAE: 35920620.7.0000.5192). This was a prospective diagnostic accuracy study, conducted according to the Standards for Reporting Diagnostic Accuracy (STARD) recommendations[14]. The index test was defined as the IHC using anti-BRAF V600E specific monoclonal antibody (RM8 clone), while the reference standard was the Competitive Allele-specific TaqMan™ Real-Time PCR Technology (castPCR™). The target condition was the detection of the BRAF V600E mutation.

Study population and sample size determination

The study population consisted of FFPE ameloblastoma specimens retrieved from the Pathology Department of a tertiary university hospital (Recife, Pernambuco, Brazil). Histological sections, 5µm in thickness from each specimen, stained with hematoxylin and eosin, were examined by two researchers experienced in Oral and Maxillofacial Pathology to confirm the diagnosis. Tumors were classified according to WHO criteria[3] and assessed for eligibility. Ameloblastomas in any clinicopathological

were eligible for inclusion. Cases lacking sufficient or suitable biological material for IHC and molecular analyses were excluded.

The sample size calculation considered the expected prevalence of the BRAF V600E mutation in ameloblastomas (65%)[4], estimated sensitivity and specificity for the index test (0.90 and 0.95, respectively, based on previous results[6] obtained for the clone VE1), a maximum accepted margin of error of 10%, and a significance level of 95%. Consequently, the sample size required to determine diagnostic accuracy (sensitivity and specificity) was set at 54 cases.

Index test: immunohistochemistry with anti-BRAF V600E antibody (RM8 clone)

For immunohistochemical reactions, 3-µm-thick histological sections of each FFPE tumor sample were submitted to deparaffinization, rehydration and antigen retrieval using Trilogy solution (Cell Marque), followed by blocking of endogenous peroxidase and nonspecific binding. The primary anti-BRAF V600E specific antibody (RM8 clone, dilution 1:300. Thermo Fisher Applied Biosystems™) was incubated for 2 hours. Diaminobenzidine (DAB) was used as the chromogen for the reaction using HiDef HRP detection system (Cell Marque), and counterstaining was performed with Harris' hematoxylin. Positive (BRAF V600E ameloblastoma, previously confirmed by molecular tests) and negative (omission of the primary antibody) controls were carried out for each reaction.

The immunostaining in neoplastic cells was qualitatively and semi-quantitatively assessed by two independent examiners under a light microscope at magnifications corresponding to 100x and 400x. Ten random and representative fields were examined for each slide, and the estimated percentage of neoplastic cells with immunostaining was recorded. Reactions showing positive cytoplasmic staining in ≥10% of cells were classified as positive. Reactions with focal, scant (<10%), or absent staining were classified as negative, as previously recommended by Fregnani et al.[15]. Disagreements between the two examiners were resolved by consensus.

Reference standard: BRAF V600E detection by Real-Time polymerase chain reaction

From each case, 10µm sections of FFPE tumor tissue were processed using the MagMax™ FFPE DNA/RNA Ultra Kit (Applied Biosystems Thermo Fisher Scientific™), according to the manufacturer's instructions, to isolate genomic DNA. The double-stranded DNA concentration and purity were measured in NanoDrop Lite spectrophotometer (Thermo Scientific™) and stored at -20ºC until molecular analysis.

BRAF V600E mutation detection in DNA samples was performed in technical duplicates by Real-Time PCR using 40ng of template DNA combined with castPCR™ somatic mutation detection assays containing TaqMan™ probes specific for the c.1799T>A mutant *BRAF* allele (BRAF_476_mu) and for the reference *BRAF* gene (BRAF_rf) (Applied Biosystems Thermo Fisher Scientific™). The PCR amplification conditions followed the manufacturer's recommendations. Positive control (confirmed BRAF V600E template DNA) and No Template Control (NTC) were included in the reactions.

Amplification curves were imported to Mutation Detector™ software (Thermo Fisher Life Technologies), where the mutational status of the *BRAF* gene in each sample was analyzed. To ensure the blinding, the examiners assessed the results of the reference standard (Real-Time PCR) without knowledge of the results of the index test (IHC), and vice versa.

Additional molecular analysis: *BRAF V600E* detection by Sanger Sequencing

In instances of discordance between the index test and the reference standard, samples were submitted to Sanger Sequencing to confirm the mutational status. For this purpose, the exon 15 of *BRAF* gene was amplified in conventional PCR using DreamTaq Green PCR Master Mix (2X) (Applied Biosystems Thermo Fisher

Scientific™) and the following primers: 5'-CTCTTCATAATGCTTGCTCTGATAGG-3' (forward) and 5'-AGTTGAGACCTTCAATGACTTTCTAGT-3' (reverse). The amplification protocol consisted of initial denaturation at 95°C for 1 minute, 40 cycles of denaturation at 95°C for 30 seconds, hybridization at 59°C for 30 seconds and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 3 minutes. The amplicons were visualized through agarose gel electrophoresis and treated with ExoSAP-IT™ PCR Product Cleanup (Applied Biosystems Thermo Fisher Scientific™) before sequencing.

The sequencing reaction was carried out with the above-described primers and Big Dye Terminator kit (Applied Biosystems Thermo Fisher Scientific™). Each DNA sequence was determined by capillary electrophoresis with the ABI PRISM® 3500 Genetic Analyzer (Applied Biosystems). The data was analyzed using the software BioEdit Sequence Alignment Editor version 5.0.9, and the sequence of nitrogenous bases of the *BRAF* gene amplicon was identified.

Statistical and diagnostic accuracy analysis

The database was built on the software Microsoft 365 Excel®. For diagnostic accuracy analysis, a 2 x 2 contingency table (true positive, false positive, false negative, and true negative) was generated based on the agreement between the results of diagnostic tests for detecting the BRAF V600E mutation in the ameloblastoma samples using IHC (index test) and Real-Time PCR (reference standard). Sensitivity and specificity measures as well as positive and negative predictive values, with their respective 95% confidence intervals were calculated.

RESULTS

Demographic and clinicopathological characteristics of the sample

The flow diagram of the cases included in the study is shown in Figure 1.

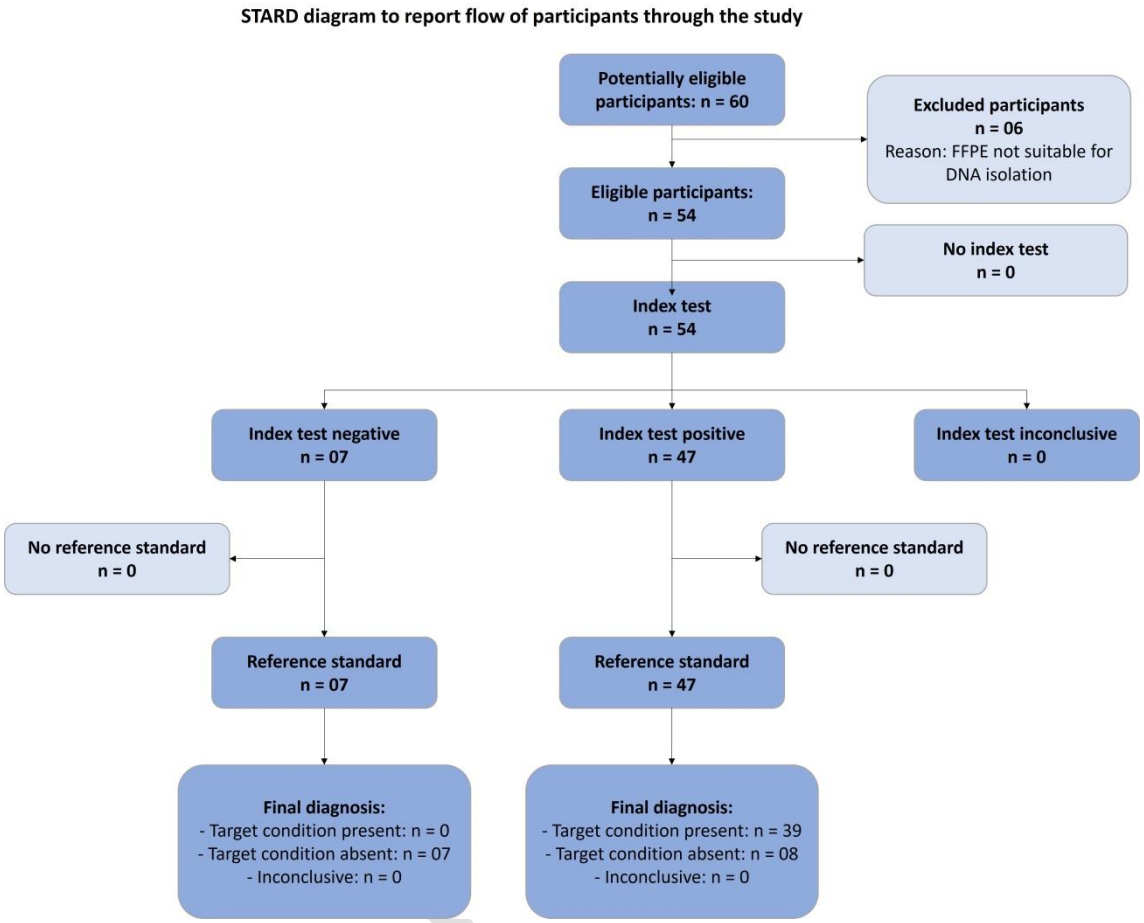

Figure 1. STARD flow diagram of the participants of the study.

Fifty-four FFPE ameloblastoma samples were included. The age at diagnosis varied from 11 to 74 years, with a mean age of 34.48 ± 16.43 years, and the male:female ratio was 1.25:1. Detailed baseline demographic and clinicopathological characteristics of the sample are displayed in Table 1.

Table 1. Demographic and clinicopathological characteristics of the sample.

Age at diagnosis	**Years**	
Age range	11–74	
Mean±SD	34.48±16.43	
	n	**%**
Sex		
Women	24	44.4
Men	30	55.6
Skin color		

Brown	39	72.2
White	09	16.7
Black	06	11.1
Anatomic location of the tumor		
Mandible	53	98.1
Maxilla	01	1.9
Clinicopathological variant		
Ameloblastoma, conventional	52	96.2
Follicular	20	37.0
Plexiform	16	29.6
Acanthomatous	08	14.8
Basal cell	07	12.9
Desmoplastic	01	1.9
Ameloblastoma, unicystic	01	1.9
Ameloblastoma, adenoid	01	1.9

SD: standard deviation.

Descriptive analysis of the BRAF V600E immunostaining pattern

Positive BRAF V600E immunostaining was identified in 47 out of 54 samples. The immunoexpression pattern was mainly cytoplasmic, distributed throughout both peripheral palisade ameloblast-like and central stellate reticulum-like areas of neoplastic odontogenic epithelium. No stromal immunostaining was observed. Figure 2 illustrates the BRAF V600E immunostaining pattern in different samples.

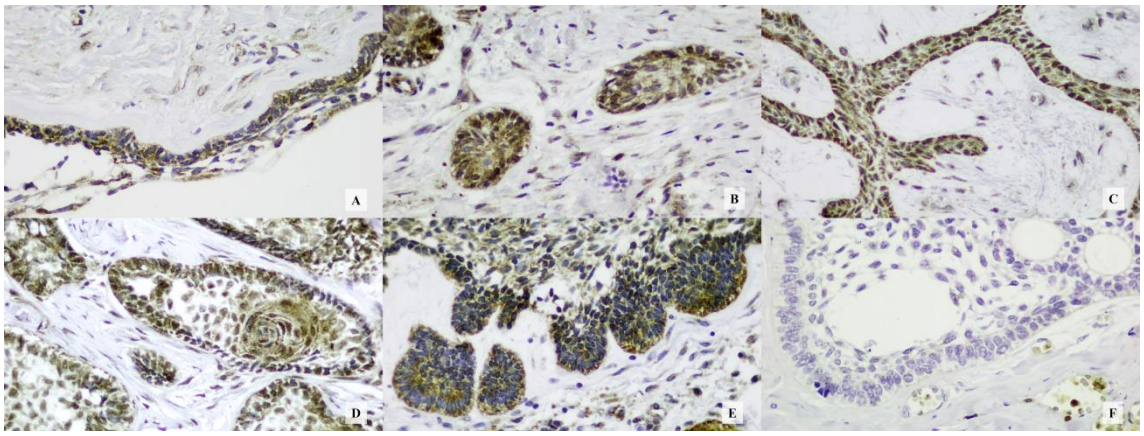

Figure 2. BRAF V600E immunostaining pattern in different ameloblastoma samples. **A**, Positive immunostaining in unicystic ameloblastoma (400x). **B**, Positive immunostaining in follicular conventional ameloblastoma. **C**, Positive immunostaining in plexiform conventional ameloblastoma. **D**, Positive immunostaining in acanthomatous conventional ameloblastoma. **E**, Positive immunostaining in basal cell conventional ameloblastoma. **F**, Negative immunostaining in follicular conventional ameloblastoma.

Background and non-specific staining were observed in wild-type BRAF normal structures adjacent to the tumor site, such as oral mucosa epithelium, salivary gland ducts, and red blood cells, irrespective of tumor mutational status, suggesting the presence of some IHC cross-reactions with tissue immunogens other than specific-

BRAF V600E (Figure 3).

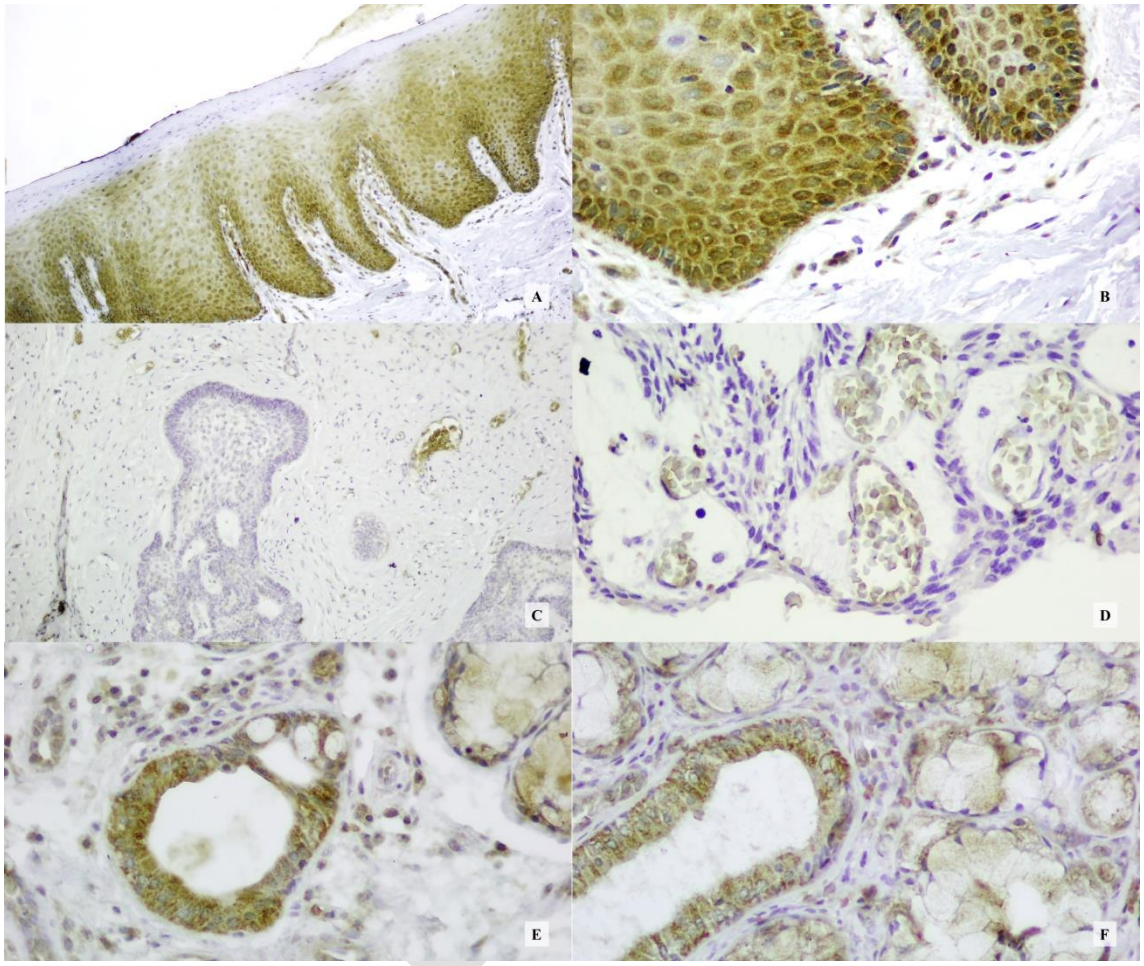

Figure 3. Non-specific immunostaining in wild-type BRAF tissue. **A** (100x) and **B** (400x), Non-specific immunostaining in squamous stratified epithelium of oral mucosa. **C** (100x) and **D** (400x), Non-specific immunostaining in red blood cells. **E** (400x) and **F** (400x), Non-specific immunostaining in salivary gland ducts.

Diagnostic accuracy analysis

Although the BRAF V600E immunostaining was observed in 87% of the ameloblastomas, the mutation was detected in only 72.2% of the samples (n = 39) by Real-Time PCR (Table 2).

Table 2. Crosstabulation of the index test results by the reference standard results.

	IHC	Accuracy (%)	Sensitivity (95%CI)	Specificity (95%CI)

		Positive	Negative			
Molecular tests*	Positive	39	0	85.18	1.00 (0.90–1.00)	0.46 (0.21–0.73)
	Negative	8	7			

IHC: immunohistochemistry; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value.

*castPCR™ (all cases) and Sanger Sequencing (discordant cases).

Amplification of reference gene detection assay occurred in all samples, confirming the integrity of the *BRAF* gene irrespective of the mutational status.

Sanger Sequencing was carried out in eight cases where there was discordance between the results of Real-Time PCR and IHC. In all instances, the sequencing results were consistent with those obtained from Real-Time PCR. Figure 4 depicts the results of the tests in true positive, true negative, and false positive cases.

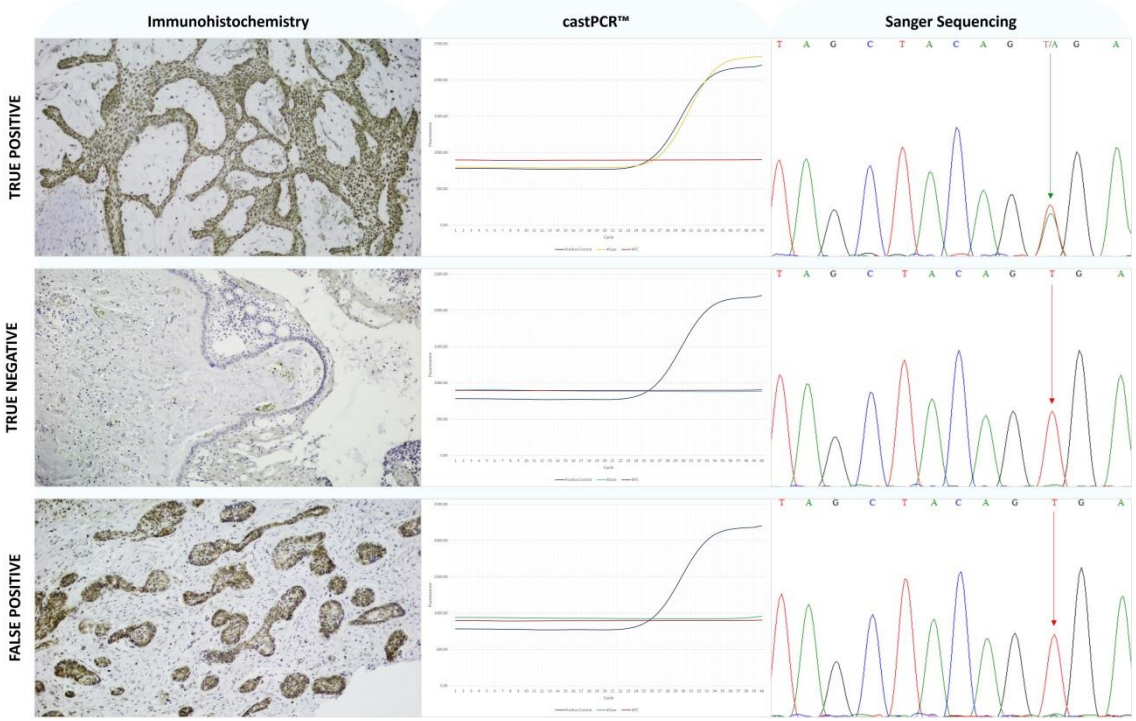

Figure 4. Index test and reference standard results for true-positive, true-negative, and false-positive cases.

The sensitivity and specificity of IHC using specific anti-BRAF V600E antibody (RM8 clone) for the detection of the BRAF V600E mutation in FFPE ameloblastoma samples was 1.00 (95%CI 0.90–1.00) and 0.46 (95%CI 0.21–0.73), respectively, and the diagnostic accuracy was calculated as 85.18%, as shown in Table 2.

DISCUSSION

The recognition of heightened occurrences of the BRAF V600E mutation in ameloblastomas has introduced novel perspectives into the molecular mechanisms that underlie its pathogenesis[16,17]. Preliminary studies have indicated a favorable response to BRAF-targeted therapies in select cases of ameloblastoma, emphasizing the importance of assessing the BRAF mutational status in these tumors[18-21]. Within this scenario, IHC using mutation-specific antibodies, such as VE1 and RM8 clones, is potentially a less expensive and time-consuming alternative for this purpose in both research and healthcare settings[6]. Nevertheless, to the best of the authors' knowledge, this is the first diagnostic accuracy analysis of IHC using anti-BRAF V600E antibody (RM8 clone) in FFPE ameloblastoma samples.

A recent diagnostic accuracy systematic review and meta-analysis conducted by our research team encompassed five studies demonstrating remarkably high concordance between BRAF V600E-specific IHC using the VE1 clone and molecular reference tests in the detection of the mutation in ameloblastomas[6]. In most of the studies (4/5)[9-11,13], both sensitivity and specificity values surpassed 0.90, and no false-positive results were reported for VE1 clone. Contrary to expectations, the present study revealed excellent sensitivity but poor specificity for BRAF V600E-specific IHC using the RM8 clone, with eight false-positive cases.

Likewise, Pereira et al.[22] reported four cases of unicystic ameloblastomas and dentigerous cysts with false-positive results using VE1 clone. In these particular cases, the odontogenic epithelium exhibited unequivocal positive immunostaining in confirmed wild-type BRAF samples. However, special attention should be given to cases with divergent IHC and molecular results, as the occurrence of negative cases in real-time PCR may be attributed to the presence of poorly representative tumor samples. This potential limitation underscores the need to consider sample representativeness regarding tumor tissue, which may impact the accuracy of molecular analyses.

Beyond the occurrence of non-specific staining in neoplastic odontogenic epithelium leading to false-positive results, the present study also observed that IHC

with RM8 clone produced background and non-specific staining patterns in other wild-type BRAF epithelial and mesenchymal structures, such as oral mucosa epithelium, salivary gland ducts, and red blood cells. While the mechanisms behind these non-specific immunostaining patterns are not completely elucidated, it is plausible that the RM8 clone may exhibit cross-reactivity with other immunogens expressed in these structures. However, the significance of the differences between both VE1 and RM8 monoclonal antibodies in diagnostic accuracy remains to be better explored in future studies.

The most prevalent (> 90%) DNA alteration that results in BRAF V600E mutation is a single nucleotide missense mutation (c.1799T>A) and results in the substitution of valine (V) for glutamic acid (E) in the reading of codon 600. However, other described alteration, c.1799_1800TG>AA, can result in the same amino acid change, consequently producing the same BRAF V600E altered protein[23]. Although this protein could be detected by mutation-specific antibodies in IHC, it might be easily missed by the BRAF_476_mu castPCR™ mutation detection assay. To address this potential issue, the present study employed Sanger Sequencing in all samples with discordant results, hereby providing additional confirmation of the false-positive cases identified by IHC using the RM8 clone.

Even when considering neoplasms other than ameloblastomas, data on the diagnostic accuracy of the RM8 clone remains very limited. In a related study, Yakout et al.[24] performed a diagnostic accuracy analysis of IHC using the RM8 clone on a sample of 50 FFPE specimens of melanocytic neoplasms, disclosing suboptimal values for both sensitivity (0.66) and specificity (0.73 to 0.88). This emphasizes the need for further studies involving this monoclonal antibody, as the results can vary substantially across different tumor types, and variations in the index test (IHC technique), such as sample processing, antigen retrieval, primary antibody dilution, immunostaining system, and positivity threshold may also contribute to different accuracy results[6,25].

This diagnostic accuracy study faces some limitations, particularly due to the semi-quantitative nature of the IHC analysis. Determining whether a specific score and cytoplasmic immunostaining pattern indicate positivity requires subjective judgment, and no standardized thresholds for BRAF V600E immunostaining are available in the scientific literature. To minimize the potential bias, we adopted a previously described positivity threshold[15] and two blinded examiners independently assessed the IHC results, with discrepancies resolved through consensus.

CONCLUSION

IHC using BRAF V600E-specific antibody (RM8 clone) showed extremely high sensitivity, but suboptimal (<0.50) specificity when compared with Real-Time PCR in the detection of BRAF V600E mutation in FPPE ameloblastoma samples. Therefore, its feasibility for routine clinical application is still considerably restricted due to the likelihood of false-positive results.

AUTHOR'S CONTRIBUTIONS

The authors AVMB, FACA, and MVC contributed to the study conception and design. AVMB, AMIB, and RJGSL contributed to data acquisition; AGBN, HAMS, SJF and MTCM contributed to data analysis and interpretation; The first draft of the manuscript was written by AVMB. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

Funding: The authors declare that this study was supported by grants from the Research Support Foundation of the State of Pernambuco, Brazil (FACEPE, Fundação de Amparo a Ciência e Tecnologia do Estado de Pernambuco) and from the Coordination for the Improvement of Higher Education Personnel (CAPES, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: This study was approved by the Ethics Committee of the University of Pernambuco (#4.309.512; CAAE: 35920620.7.0000.5192) and all procedures

performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

REFERENCES

1. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417(6892):949-54. https://doi.org/10.1038/nature00766

2. Cantwell-Dorris ER, O'Leary JJ, Sheils OM. BRAFV600E: implications for carcinogenesis and molecular therapy. Mol Cancer Ther. 2011;10(3):385-94. https://doi.org/10.1158/1535-7163.MCT-10-0799

3. World Health Organization. Head and neck tumours. Lyon: International Agency for Research on Cancer; 2022.

4. Martins-de-Barros AV, Silva CCG, Gonçalves KKN, Almeida RAC, Silva EDO, Araújo FAC, et al. Does BRAF V600E mutation affect recurrence rate of ameloblastomas? Systematic review and meta-analysis. J Oral Pathol Med. 2023;52(8):701-9. https://doi.org/10.1111/jop.13458

5. Effiom OA, Ogundana OM, Akinshipo AO, Akintoye SO. Ameloblastoma: current etiopathological concepts and management. Oral Dis. 2018;24(3):307-16. https://doi.org/10.1111/odi.12646

6. Martins-de-Barros AV, Anjos RS, Silva CCG, Silva EDO, Araújo FAC, Carvalho MV. Diagnostic accuracy of immunohistochemistry compared with molecular tests for detection of BRAF V600E mutation in ameloblastomas: systematic review and meta-analysis. J Oral Pathol Med. 2022;51(3):223-30. https://doi.org/10.1111/jop.13278

7. Loo E, Khalili P, Beuhler K, Siddiqi I, Vasef MA. BRAF V600E mutation across multiple tumor types: correlation between DNA-based sequencing and mutation-specific immunohistochemistry. Appl Immunohistochem Mol Morphol. 2018;26(10):709-13. https://doi.org/10.1097/pai.0000000000000516

8. Capper D, Preusser M, Schittenhelm J. Characterization of immunohistochemical staining patterns of an antibody specific for BRAF V600E protein in primary and metastatic brain tumors. Clin Neuropathol. 2011;30:254-5.

9. Brown NA, Rolland D, McHugh JB, Weigelin HC, Zhao L, Lim MS, et al. Activating FGFR2-RAS-BRAF mutations in ameloblastoma. Clin Cancer Res. 2014;20(21):5517-26. https://doi.org/10.1158/1078-0432.ccr-14-1069

10. Kurppa KJ, Catón J, Morgan PR, Ristimäki A, Ruhin B, Kellokoski J, et al. High frequency of BRAF V600E mutations in ameloblastoma. J Pathol. 2014;232(5):492-8. https://doi.org/10.1002/path.4317

11. Heikinheimo K, Huhtala JM, Thiel A, Kurppa KJ, Heikinheimo H, Kovac M, et al. The mutational profile of unicystic ameloblastoma. J Dent Res. 2019;98(1):54-60. https://doi.org/10.1177/0022034518798810

12. Oh K, Cho SD, Yoon HJ, Lee JI, Ahn SH, Hong SD. High prevalence of BRAF V600E mutations in Korean patients with ameloblastoma: clinicopathological significance and correlation with epithelial-mesenchymal transition. J Oral Pathol Med. 2019;48(5):413-20. https://doi.org/10.1111/jop.12851

13. Marcelino BMRS, Parise GK, Canto AM, Sassi LM, Sarmento DJS, Costa ALF, et al. Comparison of immunohistochemistry and DNA sequencing for BRAF V600E mutation detection in mandibular ameloblastomas. Appl Immunohistochem Mol Morphol. 2021;29(5):390-3. https://doi.org/10.1097/PAI.0000000000000904

14. Cohen JF, Korevaar DA, Altman DG, Bruns DE, Gatsonis CA, Hooft L, et al. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. BMJ Open. 2016;6(11):e012799. https://doi.org/10.1136/bmjopen-2016-012799

15. Fregnani ER, Perez DEC, Almeida OP, Fonseca FP, Soares FA, Castro-Junior G, et al. BRAF-V600E expression correlates with ameloblastoma aggressiveness. Histopathology. 2017;70(3):473-84. https://doi.org/10.1111/his.13095

16. Sweeney RT, McClary AC, Myers BR, Biscocho J, Neahring L, Kwei KA, et al. Identification of recurrent SMO and BRAF mutations in ameloblastomas. Nat Genet. 2014;46(7):722-5. https://doi.org/10.1038/ng.2986

17. Martins-de-Barros AV, Barros AMI, Lazo RJGS, Barbosa Neto AG, Araújo FAC, Carvalho MV. BRAF V600E mutation detected in cell-free DNA from conventional ameloblastomas fluid aspirate. Oral Dis. 2024;30(6):3962-5. https://doi.org/10.1111/odi.14855

18. Kaye FJ, Ivey AM, Drane WE, Mendenhall WM, Allan RW. Clinical and radiographic response with combined BRAF-targeted therapy in stage 4 ameloblastoma. J Natl Cancer Inst. 2014;107(1):378. https://doi.org/10.1093/jnci/dju378

19. Tan S, Pollack JR, Kaplan MJ, Colevas AD, West RB. BRAF inhibitor treatment of primary BRAF-mutant ameloblastoma with pathologic assessment of response. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(1):e5-7. https://doi.org/10.1016/j.oooo.2015.12.016

20. Fernandes GS, Girardi DM, Bernardes JPG, Fonseca FP, Fregnani ER. Clinical benefit and radiological response with BRAF inhibitor in a patient with recurrent ameloblastoma harboring V600E mutation. BMC Cancer. 2018;18(1):887. https://doi.org/10.1186/s12885-018-4802-y

21. Hirschhorn A, Campino GA, Vered M, Greenberg G, Yacobi R, Yahalom R, et al. Upfront rational therapy in BRAF V600E mutated pediatric ameloblastoma promotes ad integrum mandibular regeneration. J Tissue Eng Regen Med. 2021;15(12):1155-61. https://doi.org/10.1002/term.3254

22. Pereira NB, Pereira KMA, Coura BP, Diniz MG, Castro WH, Gomes CC, et al. BRAFV600E mutation in the diagnosis of unicystic ameloblastoma. J Oral Pathol Med. 2016;45(10):780-5. https://doi.org/10.1111/jop.12443

23. Roskoski Jr R. Targeting oncogenic Raf protein-serine/threonine kinases in human cancers. Pharmacol Res. 2018;135:239-58. https://doi.org/10.1016/j.phrs.2018.08.013

24. Yakout NM, Abdallah DM, Abdelmonsif DA, Kholosy HM, Talaat IM, Elsakka O. BRAFV600E mutational status assessment in cutaneous melanocytic neoplasms in a group of the Egyptian population. Cancer Cell Int. 2023;23(1):17. https://doi.org/10.1186/s12935-023-02858-1

25. Chang JYF, Lu PH, Tseng CH, Wang YP, Lee JJ, Chiang CP. Factors affecting the accuracy of anti-BRAF V600E immunohistochemistry results in ameloblastomas. J Oral Pathol Med. 2023;52(4):342-50. https://doi.org/10.1111/jop.13399