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BRAF V600E positive papillary thyroid carcinoma (*TERT* and *TP53* mutation coexistence excluded): Correlation of clinicopathological features and the extent of surgical treatment and its complications

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Abstract
Introduction: Papillary thyroid carcinoma (PTC) frequently harbors the *BRAF V600E* mutation. Recent research suggests that aggressive behavior in *BRAF V600E*+ PTC may be due to an undetected mutation in the *TERT* gene. This study aims to observe the clinicopathological features of *BRAF V600*+ PTC and correlate them with surgical treatment complications.
Methods: A retrospective analysis was conducted on the *BRAF V600E*+ PTC cohort from July 2019 to January 2023. The histopathological features and surgical treatment (total thyroidectomy – group A, total thyroidectomy + central block neck dissection – group B) complications were correlated. Patients with *TERT* and *TP53* mutation were excluded. Next-generation sequencing and real-time PCR were used for genetic analysis.
Results: Out of 121 PTCs, 65 cases showed *BRAF V600E* mutation with the following features: intracapsular spread (13.8%), extracapsular spread (27.7%), extrathyroidal spread (15.4%), multifocality (26.2%), angioinvasion (12.3%), and local metastasis (27.7%). The incidence of surgical complications in group A/B was: reversible recurrent laryngeal nerve (RLN) paresis 3.7/7.1%, RLN paresis permanent 0/2.4%, paresthesia 6.8/23.8%, hypocalcemia 36.4/61.9% on day 1 and 27.3/33.3% on day 3, and bleeding 2.3/9.5%. There was no significant difference in clinicopathological features between the *BRAF V600E*+ and *BRAF V600E*– PTC groups. Group B had a significantly higher incidence of hypoacalcaemia on postoperative day 1 ($p = 0.047$).
Conclusion: The *BRAF V600E* mutation will certainly remain important in the preoperative diagnosis of PTC. The more radical surgical procedures currently recommended may be abandoned in the future, particularly elective CLND, which has a higher risk of postoperative complications.

Keywords: *BRAF V600E*; Cancer; Clinicopathologic features; Complications; Mutation; Papillary Thyroid Carcinoma (PTC); Surgery; *TERT*; Thyroid

Highlights:
- Retrospective analysis of 65 *BRAF V600E*+ PTCs (coexistence of TERT and TP53 mutations was excluded).
- In Bethesda III-IV nodules (post-operative diagnosis of PTC), the *BRAF V600E* mutation was found in 25.6%.
- No significant difference in clinicopathologic features between the *BRAF V600E*+ and *BRAF V600E*– PTC groups was found.
- Higher *BRAF V600E* PTC aggressiveness reported in the literature may be due to previously undiagnosed *TERT* mutation.
- More radical surgical procedures currently recommended for *BRAF V600E*+ PTC may be abandoned in the future.

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Introduction

Thyroid cancer (TC) is the most common endocrine malignancy worldwide (Siegel et al., 2018). TC is classified into differentiated (DTC), poorly differentiated (PDTC), medullary (MTC), and anaplastic thyroid carcinomas (ATC). Differentiated thyroid carcinomas comprise papillary thyroid carcinoma (PTC) and follicular carcinoma (FTC), which account for 95% of all TCs. PTC has a good prognosis (5-year survival rate of 98%) when diagnosed early (Miranda-Filho et al., 2021). Although the mortality rate of PTC is low, 15% of cases are locally advanced. If the disease is refractory and does not respond adequately to radioiodine therapy, the 10-year survival rate for PTC patients with distant metastases is less than 50% (Durante et al., 2006).

The *BRAF V600E* mutation is the most common and well-studied genetic alteration in PTC. Its presence is considered a predictive factor for increased aggressiveness of PTC behavior and has become a marker for risk stratification of PTC patients in some cases (Paschke et al., 2017; Xing, 2005; Xing et al., 2015).

The co-existence of *BRAF V600E* and *TERT* mutations is associated with a more aggressive course of PTC than either mutation alone (Yu et al., 2023). The presence of a *TERT* mutation increases the risk of disease persistence, recurrence, local and distant metastasis, and mortality. It is considered an independent predictive factor for poor prognosis in patients with PTC. However, the predictive value of the *BRAF V600E* mutation remains inconclusive (Chen et al., 2021). Recent research has emphasized the importance of distinguishing between *BRAF V600E* positive PTCs with and without concurrent *TERT* mutation. It is possible that the previously reported increased risk of aggressive behavior in *BRAF V600E* PTCs was primarily due to the *TERT*-mutated subset of cases (Melo et al., 2015).

Considering recent studies that show no increased risk of recurrence and mortality in *BRAF V600E*+ PTC (Li and Kwon, 2020), and the hypothesis that an undetected *TERT* mutation may be a major factor in the described aggressive behavior of *BRAF V600E*+ PTC in a large proportion of cases, it is important to evaluate the risk-benefit ratio before deciding to escalate surgical treatment based solely on the presence of a single *BRAF V600* mutation.

The objective of our study was to observe clinicopathological features associated with higher aggressiveness of *BRAF V600*+ PTC, excluding the presence of *TERT* or *TP53* mutation.

We also aimed to compare surgical outcomes of patients indicated for total thyroidectomy (TT) versus those indicated for TT and elective central cervical lymph node dissection (CLND) with respect to morbidity.

We evaluated the relationship between preoperative cytology and molecular genetic testing in patients with Bethesda III–VI staged PTC.

Materials and methods

The study analyzes a cohort of patients who underwent thyroid surgery between July 2019 and January 2023. Each patient signed an informed consent for molecular genetic testing of thyroid tissue. The project received approval from the ethics committee of the Central Military Hospital in Prague (number 108/16-92/2021). The surgeries were performed by three experienced surgeons at the Department of Otorhinolaryngology and Maxillofacial Surgery of the 3rd Faculty of Medicine, Charles University in Prague. During the surgery, the surgeon visualized the recurrent laryngeal nerve (RLN) using standard methods and verified its functional status through intraoperative neuromonitoring (Medtronic NIM 3.0). Based on European Thyroid Association (ETA) guidelines, elective CLND was considered and subsequently indicated for nodules >1 cm and with preoperative *BRAF V600E* mutation detected (Paschke et al., 2017).

For patients with preoperative Bethesda classification III–VI, a portion of the sample from the suspected tumor lesion was deep-frozen postoperatively. If malignancy was detected in the final histological examination, this sample was sent for additional molecular genetic testing.

The following histopathological features were observed postoperatively: tumor size, multifocality, angioinvasion, intracapsular and extracapsular spread, extrathyroidal spread, presence of local metastases, and extranodal spread. Perioperative and postoperative surgical-related features were monitored, including bleeding, wound infection, and postoperative occurrence of RLN paresis (temporary or permanent), paresthesias, and hypocalcemia on postoperative days 1 and 3. Statistical analysis was performed using the Fischer's exact test with a significance level of $p < 0.05$.

Molecular genetic analysis was performed at the Department of Molecular Endocrinology at the Institute of Endocrinology in Prague. DNA and RNA from fresh-frozen thyroid tissues were extracted using the All Prep DNA/RNA/miRNA Universal Kit and QIA cube Connect Extraction System (Qiagen, Venlo, The Netherlands). Sample concentration and purity were measured using a fluorometer (Quantus, Promega, WI, USA) and a spectrophotometer (QIAxpert, Qiagen). Thyro-ID panel (4bases, Manno, Switzerland) comprising 14 genes (*BRAF, HRAS, KRAS, NRAS, TERT, AKT1, EGFR, TP53, PTEN, PIK3CA, CDKN2A, NOTCH, TSHR*, and *CTNNB1*) associated with thyroid tumorgenesis was used to detect point genetic alterations. Samples that were negative in previous analyses were subjected to the detection of an additional 23 fusion genes, including *RET, NTRK1, NTRK3, ALK, BRAF* and *PPAR*γ genes, by real-time PCR analysis. Selected negative samples were analyzed for the presence of the rare fusion gene using the Fusion Plex Comprehensive Thyroid and Lung panel (ArcherDx, Boulder, CO, USA). MiSeq (Illumina) was used for next-generation sequencing.

Total RNA (2 μg) underwent reverse transcription into cDNA, with the use of random primers, dNTPs, RNase inhibitor, and AMV reverse transcriptase (Promega). The cDNA was diluted five times and amplified using TaqMan Fast Advanced Master Mix (Applied Biosystems) and gene-specific primers and hydrolysis probes that were synthesised based on our design, as detailed in Suppl. Table S1. The ACTB gene served as the reference gene. Each experiment included a positive control and a negative control, in which RNase-free water was used insted of template cDNA. Real-time PCR was performed on a Light Cycler® 480 (Roche) as follows: 50 °C for 2 min, 95 °C for 20 s, followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s.

Results

Between July 2019 and January 2023, a total of 918 thyroid surgeries were performed. Preoperatively, fine needle aspiration cytology (FNAC) was performed in 543 cases and evaluated according to the Bethesda System for Reporting Thyroid Cytopathology (Cibas and Ali, 2017). Nodules clas-

sified as Bethesda III, IV, V, and VI were described in 21.4%, 30.2%, 81.6%, and 96% of cases as malignant, respectively. Table 1 provides the number of cases and percentage of benign and malignant tumors due to FNAC.

Table 1. Relation of FNAC to definitive benign/malignant diagnosis – number of cases (percentages)

	Benign (%)	Malignant (%)	Total
Bethesda I	32 (86.5)	5 (13.5)	37
Bethesda II	219 (92.4)	18 (7.3)	237
Bethesda III	92 (78.6)	25 (21.4)	117
Bethesda IV	44 (69.8)	19 (30.2)	63
Bethesda V	9 (18.4)	40 (81.6)	49
Bethesda VI	2 (4)	38 (96)	40
SUMA	398 (73.3)	145 (26.7)	543

Genetic examination was performed postoperatively on 121 samples from the Bethesda III–VI group with histologically proven TC. PTC was diagnosed in 103 cases, with the following mutation incidence: *BRAF V600E* (65×), *RAS* (6×), *BRAF V600E + TERT* (4×), *BRAF V600E + TP53* (1×), *TP53* alone (1×), *RET* fusion genes (5×), *NTRK* fusion genes (5×), and other rare alterations (11×). No genetic alterations were detected in 5 PTCs.

The prevalence of the *BRAF V600E* mutation in the PTC group was 62.5%. The mutation was present in 28% of Bethesda III cases, 22% of Bethesda IV cases, 65% of Bethesda V cases, and 76% of Bethesda VI cases.

There was no statistically significant difference in the incidence of histopathological features associated with aggressive behavior between *BRAF V600E* mutation-positive TCs and *BRAF V600E* negative TCs without *TERT* and *TP53* mutations. Table 2 shows the frequency of each feature and its statistical dependence.

Table 2. The occurrence of risk prognostic factors due to the *BRAF V600E* mutation

	BRAF V600E-positive PTCs (%)	*BRAF V600E*-negative PTCs (%)	*p*
	n = 65	*n* = 32	
Intracapsular spread	9 (13.8)	9 (28.1)	0.079
Extracapsular spread	18 (27.7)	10 (31.3)	0.446
Extrathyroidal spread	10 (15.4)	6 (18.8)	0.440
Multifocality	17 (26.2)	9 (28.1)	0.510
Angioinvasion	8 (12.3)	6 (18.8)	0.288
Local metastasis	18 (27.7)	4 (12.5)	0.074
Extranodal spread	2 (3.1)	1 (3.1)	0.704

Note: PTC – papillary thyroid carcinoma.

Table 3. The occurrence of risk prognostic factors due to the *BRAF V600E + TERT + TP53* mutation

	BRAF V600E + TERT positive PTCs (%)	*BRAF V600E + TP53* positive PTCs (%)
	n = 4	*n* = 1
Intracapsular spread	0	1 (100)
Extracapsular spread	4 (100)	0
Extrathyroidal spread	3 (75)	0
Multifocality	0	0
Angioinvasion	1 (25)	0
Local metastasis	1 (25)	0
Extranodal spread	1 (25)	0

Note: PTC – papillary thyroid carcinoma.

The frequency of clinicopathologic features in PTC with the combination of *BRAF V600E, TERT*, and *TP53* mutations is shown in Table 3 to complete the overall picture. However, due to the small number of cases, no statistical correlation of the frequency of clinicopathologic features is made.

A total of 65 *BRAF V600E*-positive PTCs were operated on, 44 without CLND (group A) and 21 with CLND (group B). In group A, 37 TTs and 7 hemithyroidectomies were performed, with a total of 81 exposed RLNs. Surgery was performed on 9 men with a mean age of 42.6 ± 12.4 years and 35 women with a mean age of 50.1 ± 14.0 years. In group B, 21 TTs were performed, with 42 RLNs exposed. Six men and fifteen women underwent surgery, with the men having a mean age of 46.8 ± 16.7 years and the women having a mean age of 37.0 ± 10.1 years.

The incidence of unilateral RLN paresis was 3.7% in group A, with no cases of permanent paresis. In group B, the incidence of unilateral RLN paresis was 7.1%, with 4.8% of cases resulting in permanent paresis (calculated per exposed nerve). Bilateral RLN paresis did not occur in the described cohort. The mean calcium levels on postoperative days 1 and 3 in group A were 2.13 ± 0.14 and 2.16 ± 0.16 mmol/l. The mean calcemia values on the postoperative days 1 and 3 in group B were 2.08 ± 0.15 and 2.14 ± 0.13 mmol/l. There was no statistically significant difference in the incidence of temporary and permanent RLN paresis between these groups. However, the incidence of hypocalcemia on postoperative day 1 was significantly higher in group B ($p = 0.047$). The incidence of postoperative paresthesia was more frequent in group B, however, the differences only approached statistical significance ($p = 0.065$). The difference in postoperative complications between group A and the group with PTCs negative for *BRAF V600E, TERT*, and *TP53* mutations was not statistically significant. Table 4 presents the number of cases and the percentage incidence of surgical complications in group A, group B, and the group with *BRAF V600E, TERT*, and *TP53* negative PTCs.

Table 4. The occurrence of surgical complications due to the *BRAF V600E* mutation and the extent of surgery

	BRAF V600E-positive (group A, %)	*BRAF V600E*-positive (group B, %)	*p* (group A vs. group B)	*BRAF V600E*-negative (%)	*p* (*BRAF V600E*-positive vs. *BRAF V600E*-negative)
RLN paresis	3/81 (3.7)	3/42 (7.1)	0.333	3/77 (3.9)	0.745
RLN paresis permanent	0/81	1/42 (2.4)	0.341	0/77	1
Paresthesias	3/44 (6.8)	5/21 (23.8)	0.065	4/45 (8.9)	0.406
Hypocalcemia 1. day	16/44 (36.4)	13/21 (61.9)	**0.047**	13/45 (28.9)	0.070
Hypocalcemia 3. day	12/44 (27.3)	7/21 (33.3)	0.411	7/45 (15.6)	0.075
Bleeding	1/44 (2.3)	2/21 (9.5)	0.242	0/42	0.220
Infection	0/44	0/21	/	1/42 (2.4)	0.393

Discussion

The 2023 Bethesda system for reporting thyroid cytopathology presents the risk of malignancy as follows: 13% for category I (non-diagnostic sample), 4% for category II (benign), 22% for category III (atypia of uncertain significance), 30% for category IV (follicular neoplasia), 74% for category V (suspected malignancy), and 97% for category VI (malignant) (Ali et al., 2023). Our results indicate a similar risk of malignancy for categories I, III, IV, V, and VI. However, there is a slightly higher risk of malignancy for category II (7.3%) in the present cohort. Therefore, patients with Bethesda III and IV nodes of uncertain malignant potential, which carry a risk of malignancy ranging from 22–30%, may benefit from preoperative molecular genetic analysis of the FNAC specimen. This analysis can identify tumors at high risk of malignancy, which can help minimize the number of diagnostic and two-stage procedures, such as totalization for malignancy (Carty et al., 2020). When genetic mutations are detected in the preoperative period, the risk of malignancy is reported to be between 59–100% for Bethesda categories III and IV (Steward et al., 2019). ETA recommends considering genetic testing (if possible) for thyroid nodules in Bethesda categories III and IV, recurrent non-diagnostic specimens (category I), and TIRADS4 ultrasound evaluations (Paschke et al., 2017; Tessler et al., 2017).

When discussing extrathyroidal spread, it is important to distinguish between microscopic and visually apparent spread outside the thyroid boundaries. However, it is worth noting that in cases where separate microscopic extrathyroidal invasion is detected on histological examination, there is no difference in recurrence or survival compared to patients without extrathyroidal spread (Haugen et al., 2016). Macroscopic spread beyond the thyroid tissue is associated with higher recurrence rates (29–52%) and decreased survival (85%) (Jin et al., 2015; Park et al., 2018). In a meta-analysis of 23,303 cases studying the clinicopathological features in PTC, extrathyroidal spread, whether microscopic or macroscopic, was demonstrated in 52.6% of *BRAF V600E* positive cases and in 38% of *BRAF V600E* negative cases (Zhang et al., 2016). In our study, we observed a lower incidence of extrathyroidal spread in both *BRAF V600E* positive and negative cases (15.4% and 18.8%).

Multifocality of TCs has been reported in 18–87% and 20–36% of PTCs (Feng et al., 2020; Iacobone et al., 2014). It is considered a significant risk factor for increased persistence and recurrence of the disease (Joseph et al., 2018; Wang et al., 2017). Additionally, an increase in extrathyroidal spread, lymphovascular invasion, and lymph node metastasis was observed with multifocality and increasing numbers of PTC foci (Tam et al., 2016). The study found that multifocality was present in 35.9% of *BRAF V600E*+ PTC and 29.6% of *BRAF V600E*– PTC (Zhang et al., 2016). In the presented cohort, multifocality was observed in 26.2% of *BRAF V600E*+ and 28.1% of *BRAF V600E*– PTC.

Angioinvasion is considered a negative prognostic factor in DTC and should be distinguished from lymphangioinvasion, which has no prognostic significance (Cipriani, 2019). In PTC, angioinvasion is described in 5–30% of cases (Furlan et al., 2004; Wreesmann et al., 2015). An analysis of several studies found a significant association between the presence of the *BRAF V600E* mutation and angioinvasion (18.1%) (Zhang et al., 2016). In our cohort, we observed angioinvasion in 12.3% of *BRAF V600E* positive PTCs.

The presence of metastases in local lymph nodes is a predictive factor for the course of the disease. In patients with DTC, a low recurrence rate (less than 5%) was observed when clinically negative but histologically confirmed lymph node metastases (cN0, pN1) were present, provided that the number of affected nodes was less than 5 and the lesion size was less than 0.2 cm. Higher recurrence rates (over 20%) were found in clinically and histologically evident affected lymph nodes, particularly when the lesions were larger than 3 cm and/or the number of nodes was more than 5 (Randolph et al., 2012). Additionally, the size of a metastatic lesion is thought to be directly proportional to the probability of extranodal spread, and the rate of cancer recurrence is higher in the presence of extranodal spread than without it (Du et al., 2016; Rowe et al., 2018). A meta-analysis of 11,359 cases of *BRAF V600E*+ PTCs reported that 46.5% of cases had local metastasis (Zhang et al., 2016). In our cohort, 27.7% of patients showed local metastasis to the lymph nodes and 3.1% had extranodal spread.

Recent studies have linked *BRAF V600E, TERT*, and *RET/PTC* mutations to aggressive behavior in thyroid cancers (Bulanova Pekova et al., 2023; Zhao et al., 2020). These mutations have also been associated with a statistically significant higher incidence of extrathyroidal spread, angioinvasion, and lymph node metastasis compared to TCs with other mutations (*RAS, PAX8-PPARs*) and TCs without mutations (Kim et al., 2012; Krasner et al., 2019; Liu et al., 2013). ETA considers molecular profiles with the presence of late mutations such as *TERT, TP53*, and *PIK3CA*, or coexistence with early mutations such as *BRAF V600E* or *RAS* to pose the highest risk (Durante et al., 2023; Yip et al., 2021). It has been reported that approximately 48% of radioiodine-refractory TCs are positive for either the *TERT/TP53* mutation alone or in combination with other mutations (*BRAF, RAS*, or *RET* fusion genes) (Nannini et al., 2023). Recently, there has been increasing discussion that undetected *TERT* (or other) mutations may be the main factor

in the aggressive behavior of *BRAF V600E*+ PTCs previously identified and still described (Melo et al., 2015). This hypothesis is supported by more recent works. The authors of a large meta-analysis disagree with the original findings of increased recurrence rates of *BRAF V600E*+ PTC. According to their results, the rate of PTC recurrence does not depend on the *BRAF V600E* mutation (Li and Kwon, 2020). The original studies investigating the relationship between PTC mortality and *BRAF V600E* mutation did not find a statistically significant difference in survival for this group of patients (Li and Kwon, 2020; Melo et al., 2015). When considering the different stages of PTC disease (according to ATA – American Thyroid Association, TNM classification) and predicting the development of persistent disease, the presence of *BRAF V600E* mutation alone was not found to be significant. In contrast, the presence of *TERT* alone or in combination with the *BRAF V600E* mutation correlated well with disease staging and persistence (Mukhtar et al., 2023).

In certain cases, genetic testing results can be used as an additional factor to personalize the scope of surgical treatment. The detection of genetic changes and a thorough understanding of their clinical significance during the preoperative period are important factors in deciding the radicality of surgical treatment, especially in two patient groups: (1) For patients with Bethesda III and Bethesda IV (20–25% of FNAC results), where cytological examination provides only an indicative result and surgery is often necessary for definitive histological examination. (2) For patients with confirmed TC without clinically present lymph node metastases where the risks and benefits of elective CLND are always weighed against the morbidity of treatment and prognosis of the disease (Durante et al., 2023; Filetti et al., 2019; Haugen et al., 2016; Paschke et al., 2017).

Our results indicate a higher incidence of postoperative paresthesias and hypocalcemia on postoperative day 1 in cN0 patients in group B (TT+CLND) compared to group A (TT). The relatively higher incidence of temporary and permanent RLN paresis in group B was not considered statistically significant, nor was complicating bleeding. Similarly, studies involving large numbers of patients have described an increased incidence of surgical complications in elective CLNDs and, at the same time, some authors have reported no difference in overall survival and recurrence rates in DTC (Calò et al., 2017; Dobrinja et al., 2017; Giordano et al., 2017; Unlu et al., 2021).

If the presence of other risk mutations is excluded, the question is whether patients with cN0 *BRAF V600E* PTC really benefit from elective CLND and the associated higher risk of surgical complications. Our results suggest that CLND may be unnecessary in these patients, even though PTC is not proven to be more aggressive. However, it is important to note that our study was limited by the relatively small number of patients operated on at a single institution. In the future, it will be interesting to observe whether postoperative hypocalcemia in patients is a temporary or permanent condition in the long term.

Conclusion

The incidence of thyroid cancer was 7.3% in patients with Bethesda II nodules, while patients with Bethesda III and IV nodules had an incidence of 21.4–30.2%. Bethesda III–IV classified nodules with a postoperative diagnosis of PTC were found to have the *BRAF V600E* mutation in 25.6% of cases. Therefore, molecular genetic testing in the preoperative period appears to be an important complementary factor in determining the strategy and extent of treatment.

There was no significant difference in the incidence of clinicopathological features associated with aggressive PTC behavior between the *BRAF V600E*+ and *BRAF V600E*– mutationgroups.

Patients who underwent TT + elective CLND (*BRAF V600E*+ PTC, cN0) had a significantly higher incidence of hypocalcemia on postoperative day 1 and the incidence of paresthesias approached the threshold of statistical significance. However, the incidence of temporary and permanent RLN paresis and bleeding complications was not statistically significant.

As the genetic analysis of TC rapidly evolves and new genetic alterations are discovered, the original data need to be revised to account for the possible influence of previously undetected but now known aggressive mutations on outcomes. Newly obtained data may lead to the refinement of already established prognostic models and recommendations that determine the extent of surgical treatment. According to our results, more radical surgical procedures currently recommended for *BRAF V600E*+ PTC should be abandoned in the future, especially elective CLND, which has a higher risk of postoperative complications, provided the coexistence of *TERT* and *TP53* mutations is excluded.

Even if the prognostic value of *BRAF V600E* will not be confirmed, the detection of this mutation will continue to be essential for preoperative diagnostic and/or therapeutic purposes.

Authors' contribution

All authors contributed to the conception and design of the study. All authors have read and agreed to the published version of the manuscript and gave their consent for publication. Conceptualization, *J.H., B.P.B., J.S.* and *J.A.*; validation, *J.H., R.H., V.K.* and *B.B.*; investigation, *J.A., J.R., J.H., P.H., J.S., V.K.* and *B.P.B.*; resources, *J.H., J.R., R.H., T.H.* and *J.A.*; data curation, *R.H., J.S., P.H.* and *J.H.*; writing-original draft preparation, *J.H., B.P.B, T.H.* and *J.A.*; writing review and editing, *J.H., R.H., J.R., J.S.* and *J.A.*; visualization, *J.H., B.P.B., T.H.* and *J.S.*; supervision, *J.H., B.B., V.K., B.P.B., P.H., R.H.* and *J.A.*; project administration, *J.H., R.H., B.P.B.* and *J.A.*; funding acquisition, *J.H., B.B.* and *J.A.*

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Ethical aspects and conflict of interest

The authors declare no conflicts of interest related to the topic, preparation, or publication of this study. They also state that no pharmaceutical company provided support for the preparation or publication of this work.

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