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Case Report

HRAS and *PIK3CA* Mutations in Sinonasal Oncocytoma: Case Report

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Abstract

Purpose: Oncocytomas are benign or malignant neoplasms that can occur in any organ, but in the paranasal sinuses they are rare and are usually malignant. Histologically, they are characterized by the accumulation of defective mitochondria due to mitochondrial dysfunction and an imbalance between mitochondrial biogenesis and mitophagy. Mainly alterations occur in Mitochondrial DNA (mtDNA), without the role of other oncogenes or tumor suppressor genes in the maintenance of the oncocytic phenotype being clearly known. **Methods:** We present a case of a 65-year-old man with a benign sinonasal oncocytoma that presented as a slow-growing sinonasal tumor that affected the lacrimal sac. Complete surgical removal of the tumor was performed without recurrence after four years of follow-up. We performed NGS by analyzing a panel of 131 genes from cancer-related tumors and matching germline DNA (blood sample) to search for somatic mutations and copy number abnormalities. **Results:** NGS revealed two non-synonymous point somatic changes affecting *HRAS* (p.Gln61Arg) in 48% of the tumor reads and *PIK3CA* (p.Glu545Lys) in 36%. *HRAS* is involved in MAPK and AMPK signaling and *PIK3CA* mutation is related to activation of PI3K-AKT-mTOR pathway signaling. Both pathways have been related to mitochondrial biogenesis and mitophagy and they are the most frequently altered in oncocytomas of other locations. We found no significant copy number gains or losses. **Conclusion:** Gene mutations in a case of benign sinonasal oncocytoma are similar to oncocytomas in other locations and may be used to better understand its pathogenesis and promote the development of future therapies.

Keywords: Benign Oncocytoma; Gene Mutations; Next Generation Sequencing; Signaling Pathways; Sinonasal Oncocytoma

Introduction

Oncocytomas are rare neoplasms characterized by epithelial cells with abundant cytoplasmic eosinophilic granules (oncocytes) because of the accumulation of defective mitochondria [1,2]. These tumors are also called oxyphilic, oncocytic, eosinophilic, or, in the case of thyroid tumors, Hürthle cell tumors in the literature.

They can arise in any organ, but they exhibit a particularly high incidence in endocrine organs. Thyroid, parathyroid, adrenal gland, pituitary gland, kidney, salivary gland (mainly parotid), breast and lung are the organs in which oncocytomas are most frequently observed. In the head and neck area, they represent less than 1% of epithelial tumors of the major salivary glands. More infrequently they may arise from the minor salivary glands and from the Schneider respiratory epithelium. This results in oncocytic tumors in the paranasal sinuses, nasal cavity, and nasolacrimal duct [3,4].

The peculiar phenotype of oncocytomas makes them a suitable and appropriate model for studies aiming to better

understand the role of the mitochondria and metabolism in carcinogenesis. A full understanding of the mechanisms involved in the formation and maintenance of the oncocytic phenotype is still needed but the existence of mitochondrial dysfunction and imbalance between mitochondrial biogenesis and mitophagy is known, mainly due to alterations in mitochondrial DNA (mtDNA), and an additional contribution of oncogene/tumor suppressor gene alterations in nuclear DNA (nDNA) [5].

Oncocytomas are typically benign tumors and usually present low proliferation rates but when they arise in minor salivary glands, sinonasal cavity or thyroid they tend to be more locally invasive and have a greater malignant potential [1]. Currently, surgery is the treatment of choice for these neoplasms. Radiotherapy is not indicated because oncocytes are considered radioresistant [6]. In sinonasal oncocytomas, in general, an endoscopic approach is performed, but sometimes open or combined approaches are required to ensure complete removal. Recurrence is rare but can occur due to occult multifocality [3]. Local and/or lymph node recurrences may appear several years after surgery in patients with malignant oncocytoma [7].

We present clinical, histopathological and genetic details of a case of a sinonasal oncocytoma involving the lacrimal sac that was surgically removed using an open approach.

Case Report

A 65-year-old man presented with progressive painless enlargement of the left lacrimal tract accompanied by nasal respiratory failure and ipsilateral purulent rhinorrhea. He had no history of exposure to radiation, metals or wood or asbestos dust. The patient had already been referred to our otorhinolaryngology service 9 years before due to suspicion of a nasal tumor. However, the patient did not come to the consultations until he began with evident clinical changes.

Physical examination revealed a 3x3 cm left paranasal tumor completely occupying the left nostril and lacrimal sac with intact skin and displacing the eyeball upwards without affecting ocular mobility.

A facial Computed Tomography (CT) was performed, revealing a large mass that partially occupied the left orbit, the nasolacrimal duct, and the left nostril with an expansive appearance and bone infiltration (Figure 1: a,b,c)

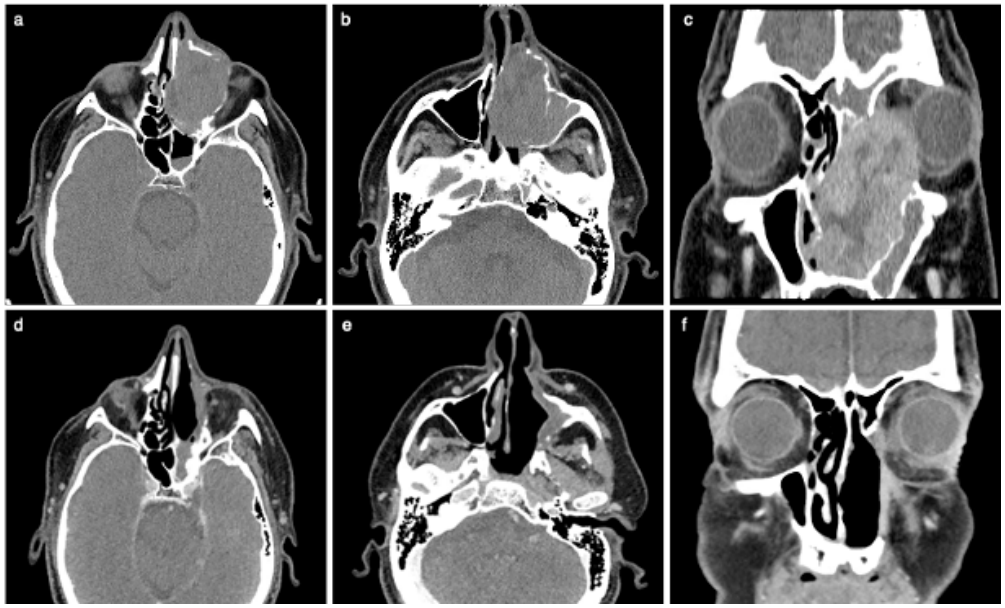

Figure 1: Preoperative (a,b,c) and postoperative (d,e,f) CT images are shown in axial (a,b,d,e) and coronal (c,f) slices of the patient

An endoscopic biopsy of the nasal tumor was performed. Pathological examination showed an encapsulated lesion with nests of cells with extensive granular and eosinophilic cytoplasm, with occasional glandular lights, but no trace of atypia or lymphovascular or perineural invasion (Figure 2). Immunohistochemical staining showed that cells retained myoepithelial lining with calponin, CK34β12, and p63. Variable positivity was observed with CD10 and CK8. CK7 was focal positive. Neuroendocrine differentiation markers synaptophysin and chromogranin were negative. CDK20, CDX2, S100 and C117 were also negative, and the Ki 67 index was 5%. Also, RCC ("Renal Cell Carcinoma Marker") was negative. The most relevant immunohistochemical results are shown in Figure 3.

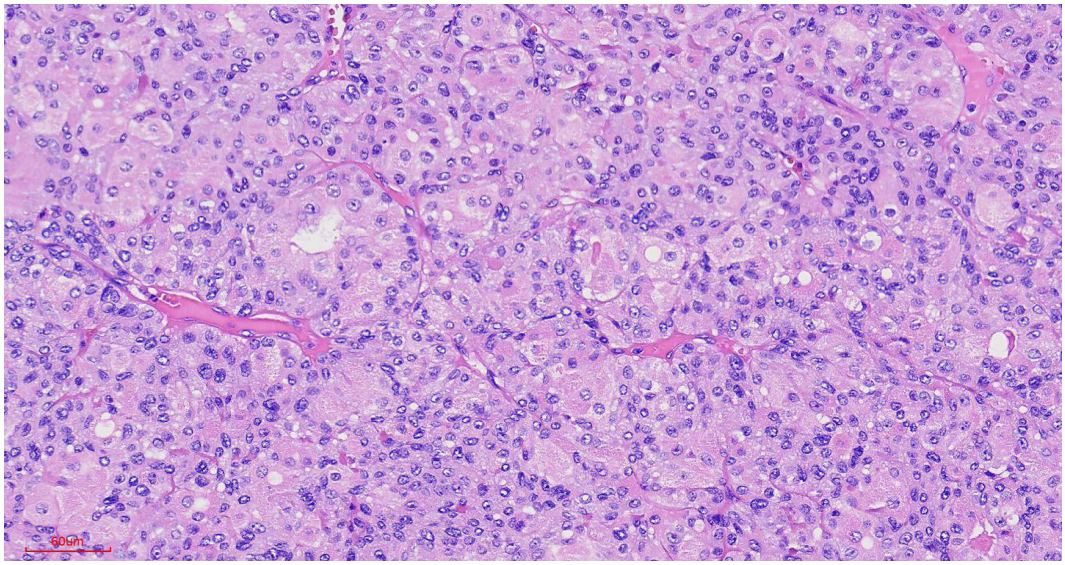

Figure 2: A representative histological image of the oncocytoma with hematoxylin and eosin staining is shown. Magnification 20

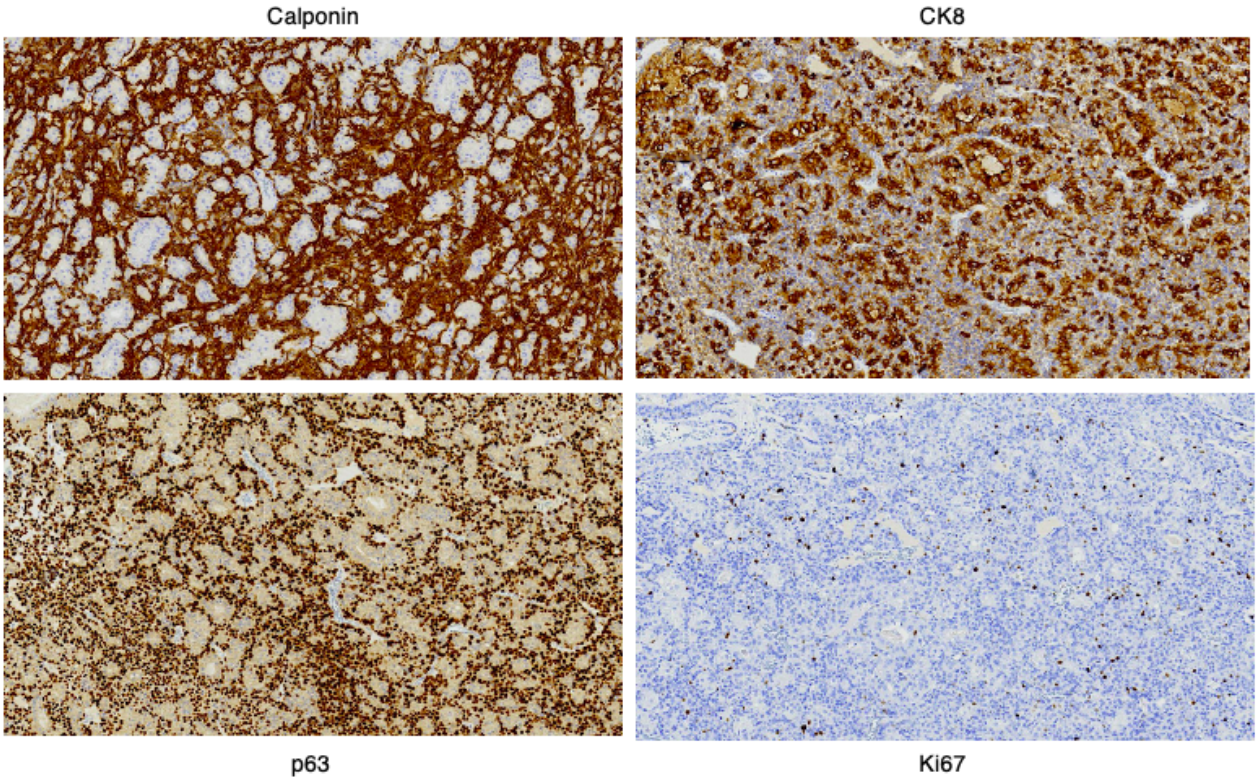

Figure 3: The most representative immunohistochemical stainings of the lesion are shown. Magnification 10

With all this, the final diagnosis was an epithelial neoplasm with a benign oncocytoma pattern. The differential diagnosis with more aggressive tumors such as melanoma, carcinoma or adenocarcinoma was not supported by the results.

Based on these findings, an open craniofacial approach was performed with complete resection of the tumor on control CT (Figure 1: d,e,f). The patient is currently alive and free of recurrence after 4 years of follow-up.

To further understand the molecular etiology of this lesion and because of its rarity, we performed Next-Generation Sequencing (NGS) analyzing a panel of 131 cancer-related genes in the tumor and matched germline DNA (blood sample) to search for somatic mutations and copy number abnormalities. NGS revealed two non-synonymous point somatic changes affecting *HRAS* (p.Gln61Arg) in 48% of the tumor reads and *PIK3CA* (p.Glu545Lys) in 36% of the reads. The sequence variants were confirmed by PCR sequencing and shown in Figure 4. We found no significant copy number gains or losses.

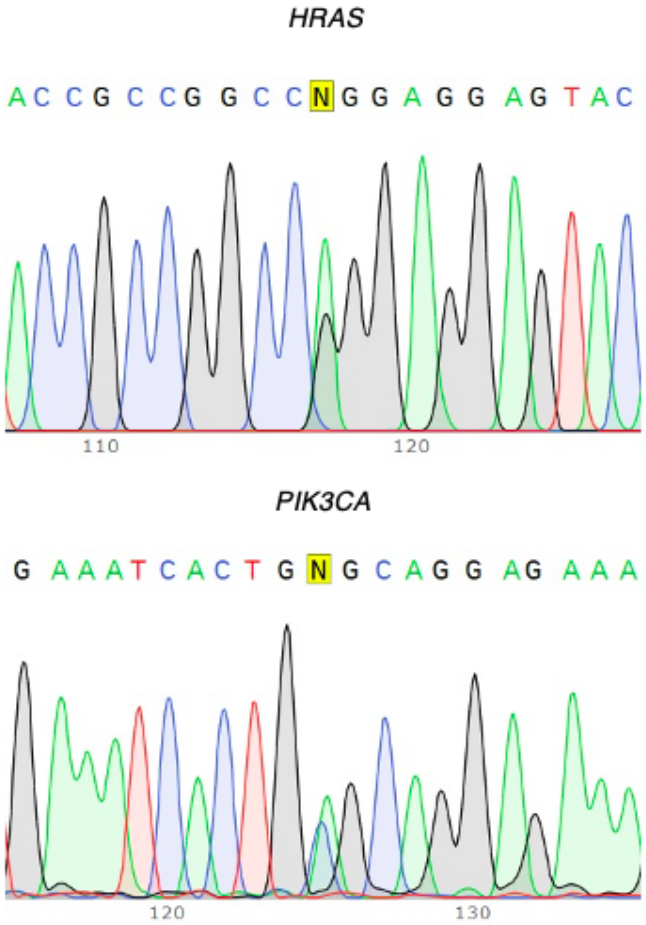

Figure 4: *HRAS* and *PIK3CA* point mutations confirmed by Sanger

Material and Methods

The primary tumor and peripheral blood samples were obtained from the otorhinolaryngology department of the Hospital Universitario Central de Asturias (Oviedo, Spain). HE and IHC stainings of the tumor sample were performed by the Department of Pathologic Anatomy of the Central Hospital of Asturias on an automatic staining workstation (Dako Autostainer Plus; DakoCytomation, Glostrup, Denmark) as part of routine diagnostics. The study and histopathological description of the sample was carried out by the pathologist B.V. Tumor DNA was extracted from fresh frozen tissue using the Qiagen tissue extraction kit (Qiagen GmbH, Hilden, Germany). Germline DNA from peripheral blood was obtained using the Roche High Pure Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). NGS using the SureSelect QXT Target Enrichment Kit for Illumina Multiplexed Sequencing (Agilent Technologies, Santa Clara, CA, USA) was performed as described previously using a panel covering all exons of 131 cancer-related genes (Appendix S1; Sánchez-Fernández et al., 2021 plus *KMT2B, KMT2C, KMT2D, OTX1, OTX2, PRDM2, PRDM9, PRDM14, SETD1A, SETD1B, SETD2* and *TP53)*. Samples were sequenced in a MiSeq system (Illumina Inc.) at the sequencing service of IMOMA (Oviedo, Spain). The coverage of the sequencing was at a minimum of 150X. For bioinformatic analysis, the software Genome One, certified with UNE-EN ISO 13485:2016 and IVD/CE-marking (DREAMgenics) was applied. After variant calling, sequence variants with a minor allele frequency >5% in the normal population were filtered out and only non-synonymous changes with an impact on the sequence of the protein were considered. Only those variants with an allelic frequency >10% of the total reads in the tumor sample were considered relevant. HRAS and PIK3CA point mutations were confirmed by Sanger sequencing with the following primers: *HRAS* FW AGAGGCTGGCTGTGTGAACT, *HRAS* RV TGGTGTTGTTGATGGCAAAC, *PIK3CA* FW CATCTGTGAATCCAGAGGGGAA, *PIK3CA* RV GCTGAGATCAGCCAAATTCAGT-3. Products amplified were sent for sequencing to Macrogen Inc., Korea.

Discussion

Sinonasal oncocytomas are very rare and the majority are malignant tumors [7,8]. However, this case does not meet the histological criteria for malignancy necessary to be considered as such [3], so it was considered benign. In this patient, an open approach was performed to ensure removal of the tumor in the lacrimal sac.

The cytology of oncocytomas is very similar regardless of the site of origin. The oncocytic phenotype is determined by the presence of cells with accumulation of defective mitochondria. To achieve this phenotype, there must be mitochondrial dysfunction

that prevents proper energy production, an imbalance between mitochondrial biogenesis and mitophagy that predisposes to the accumulation of damaged mitochondria, and the added contribution of oncogenes/tumor suppressor genes [2,9].

Oncocytic tumors have been shown to harbor disruptive mutations mainly in mtDNA genes encoding mainly respiratory chain subunits, but also in nDNA genes necessary for the maintenance, replication, and expression of mtDNA-encoded genes, structural components and biogenesis factors for the mitochondrial ribosome, proteins of the mitochondrial fusion and fission machinery, and proteins involved in mitophagy [5].

The mtDNA genes that encode the OXPHOS subunits, responsible for mitochondrial oxidative phosphorylation, are frequently mutated in oncocytic tumors of different tissues. The genes encoding respiratory complex I subunits in mtDNA (NADH-ubiquinone [ND] genes) are by far the most susceptible to mutation [5,10]. The large mtDNA deletion (4977 bp, commonly known as "Common Deletion" - CD), which comprises approximately one third of the total mtDNA, is another frequent alteration detected in oncocytomas of the parathyroid, thyroid and kidney [5].

The mitochondrial dysfunction can lead to cells undergoing a compensatory mechanism of mitochondrial overproduction as a way of replenishing the energy deficit. There is an overexpression of genes related to mitochondrial biogenesis that could partially explain the abnormal accumulation of mitochondria. *PGC1* (Peroxixoma Proliferator-Activated Receptor Γ Coactivator-1), *NRF* (Nuclear Respiratory Factor), *TFAM* (Mitochondrial Transcription Factor A), *MRPL49* (Mitochondrial Ribosomal Protein L49), *PMPCB* (Mitochondrial Peptidase Processing Beta Subunit), *DAP3*, *ERR α, TK2* (Mitochondrial Thymidine Kinase 2), and *THG1L* (TRNA-like Histidine Guanylyl transferase 1, also known as IHG-1: Induced by High Glucose 1) are some of the genes related to the mitochondrial biogenesis of oncocytic tumors [5].

In addition, oncocytomas may show a decreased mitophagy capacity that would favor the accumulation of non-functional mitochondria. Decreased expresión of genes reguladores de la autophagia such as *ATG7* (autophagy related protein 7) [11], *PARK2* [9,12] and *BECLIN1* (an autophagy regulator) [9] have been described in oncocytic tumors.

It is hypothesized that depending on the severity of the mtDNA mutation, cells may or may not progress to a more aggressive stage [2,9]. In this way, severe mtDNA mutations would give rise to a major bioenergetic crisis that gives rise to the activation of AMPK (AMP-Activated Protein Kinase), Golgi alteration and alteration of the function of lysosomes, essential for the degradation of the autophagic load leading the oncocytoma to a dead end, preventing its progression to a more aggressive stage [2]. Conversely, cells affected by mild mitochondrial dysfunction could cause tumor proliferation and transformation [2,9,13]. Based on this, it becomes interesting to investigate whether these mechanisms can be induced in the context of aggressive oncocytomas. In fact, respiratory chain inhibitors such as metformin, as well as autophagy regulators such as chloroquine, have been studied as adjuvants [14,15].

Mutations in oncogenes and tumor suppressor genes have also been described, although their role in the oncocytic phenotype is the least known. From the translational point of view, it is worth understanding whether oncogenes, tumor suppressor genes and modifier genes may be responsible for the oncocytic phenotype per se rather than being involved in accumulation of severe mtDNA mutations, but it is not yet known. RAS/RAF/MAPK and PI3K/AKT/MTOR pathway are most frequently affected in oncocytomas of different locations [16,17]. Depending on the location of the oncocytic tumor, mutations of several genes of these pathways (for example, receptor tyrosine kinase mutations, *PI3K, TSC1/2, RHEB, MTOR, NRAS, HRAS, KRAS, BRAF)* of several controllers of members of these pathways (for example, *PTEN* and *NF1*) and of epigenetic regulators have been described [16-21]. In addition, mutations in the DNA damage/repair pathway were identified. These included mutations in the tumor suppressor *TP53* and ATM [17,22]. T*P53, PTEN* and *TERT* promoter mutations have been associated with malignancy and increased aggressiveness in renal and thyroid oncocytomas [2,23,24].

To date, this is the first study to analyze point mutations and copy number alterations in the nDNA of sinonasal oncocytoma. We identified a somatic *HRAS* (p.Gln61Arg) activating point mutation, a specific variant previously reported in pituitary oncocytoma [25], but also associated with many other cancers. The *HRAS* mutation has also been described in 2% of Hürthle tumors [17]. *HRAS* is involved in MAPK (Mitogen-Activated Protein Kinase) and AMPK signaling. AMPK is a known regulator of mitochondrial biogenesis and autophagy, so its relationship with the *HRAS* mutation should be assessed in other studies. In addition, we identified the somatic mutation of *PIK3CA* (p.Glu545Lys) that is related to activation of the PI3K-AKT-mTOR pathway signaling. mTOR (mammalian Target of Rapamycin) is an important regulator of energy production in mitochondria, but it is also an inhibitor of mitophagy [26,27] which, as we already mentioned, are two key processes in the oncocytic phenotype. Our results are in agreement with those reported in oncocytic thyroid, renal and pituitary tumors, where the most frequent variant found in *PIK3CA* coincides with the one found in this case [17,18,28]. We did not find mutations in *PTEN* or *TP53* that have been related to progression to

a malignant phenotype in renal and thyroid oncocytomas [9,29]. It is worth studying the status of these genes in sinus-nasal malignant oncocytoma to determine if the association of those affected with *PTEN* and *TP53* with more aggressive subtypes of oncocytoma is restricted to the context of renal or thyroid oncocytoma or can be extended to other organs in which oncocytic neoplasms occur.

Conclusion

Mutations in the RAS/RAF/MAPK and PI3K/AKT/MTOR pathways are among the most frequently altered pathways in oncocytomas of different locations. This report shows for the first time that genetic mutations in a case of sinonasal oncocytoma are similar to oncocytomas elsewhere and can be used to better understand its pathogenesis and promote the development of future therapies.

Author Contributions: Conceptualization, J.L.L. and M.A.H.; Data curation, P.S.-F., C.R. and M.A.H.; Formal analysis, P.S.-F., C.R., M.C.C., J.L.L. and M.A.H.; Funding acquisition, J.L.L. and M.A.H.; Investigation, C.R., V.N.C., L.S.-F. and B.V.; Methodology, C.R., V.N.C., L.S.-F. and B.V.; Project administration, M.A.H.; Resources, P.S.-F., M.C.C. and J.L.L.; Software, C.R.; Supervision, J.L.L. and M.A.H.; Validation, C.R., V.N.C. and L.S.-F.; Visualization, P.S.-F. and C.R.; Writing–original draft, P.S.-F., M.C.C and C.R.; Writing–review & editing, V.N.C., L.S.-F., B.V., M.A.H and J.L.L. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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