Review

DOG1 as an Immunohistochemical Marker of Acinic Cell Carcinoma: A Systematic Review and Meta-Analysis

Vincenzo Fiorentino 1,†, Patrizia Straccia 1,†, Pietro Tralongo 1, Teresa Musarra 1, Francesco Pierconti 1, Maurizio Martini 2, Guido Fadda 2, Esther Diana Rossi 1,‡ and Luigi Maria Larocca 1,3,*,‡

1 Division of Anatomic Pathology and Histology, Fondazione Policlinico Universitario A. Gemelli—IRCCS, Largo Agostino Gemelli, 00168 Rome, Italy
2 Division of Anatomic Pathology and Histology, Università degli Studi di Messina—Piazza Pugliatti, 98122 Messina, Italy
3 Unicamillus, Saint Camillus International University of Health Sciences, International Medical University in Rome, 00131 Rome, Italy
* Correspondence: luigimaria.larocca@unicatt.it; Tel.: +39-06-3015-4433
† These authors contributed equally to this work.
‡ These authors contributed equally to this work.

Abstract: DOG1 is a transmembrane protein originally discovered on gastrointestinal stromal tumors and works as a calcium-activated chloride channel protein. There are a limited number of articles on the potential utility of this antibody in the diagnosis of salivary gland tumors in routine practice. In this study, we aimed to investigate the role of DOG1 as an immunohistochemical marker in patients with salivary acinic cell carcinoma (ACC) through meta-analysis. A literature search was performed of the PubMed, Scopus, and Web of Science databases for English-language studies published from January 2010 to September 2021. The literature search revealed 148 articles, of which 20 were included in the study. The overall rate of DOG1 expression in salivary acinic cell carcinoma was 55% (95% CI = 0.43–0.58). Although ACC is a challenging diagnosis, paying careful attention to the cytomorphological features in conjunction with DOG1 immunostaining can help to reach an accurate diagnosis.

Keywords: acinic cell carcinoma; immunomarkers; DOG1; salivary gland

1. Introduction

Acinic cell carcinoma (ACC) is a low-grade malignant salivary neoplasm. The parotid gland is the predominant site of origin and the median age at diagnosis is 52 years. Possible causes of ACC include previous radiation exposure and familial predisposition. In most cases, this neoplasm has an indolent clinical behavior, and in a minority of cases aggressive behavior; the recurrences and metastases can be seen particularly in the lung and cervical lymph nodes [1]. ACC is histologically defined by serous acinar cell differentiation. However, several cell types and histomorphological growth patterns have been recognized. These include acinar, intercalated ductal, vacuolated, clear-cell, nonspecific glandular, solid-lobular, microcystic, papillary-cystic, and follicular growth patterns [2]. The diagnosis of ACCs is frequently difficult, owing to its radiological [3,4] and cytological similarity to benign tumors and to the normal acinar component of the salivary glands, respectively. The differential diagnosis includes, fundamentally, clear cell carcinoma, mucopidermoid carcinoma, Warthin’s tumor and oncocytoma [5,6]. Both cytomorphic findings and immunohistochemistry have limited value for discriminating ACCs from salivary gland neoplasms with predominantly oncocytic morphology. Discovered on gastrointestinal stromal tumor protein 1 (DOG1), also known as anoctamin-1/ANO1, is a calcium-activated chloride channel protein made up of eight transmembrane segments that was initially identified in gastrointestinal stromal tumors (GISTs) and is used as an immunohistochemical marker for these neoplasms [7].
The DOG1 role in tumor cell biology is supported by several studies that demonstrated that the overexpression of this protein resulted in an increased tumor cell aggressivity, while its downregulation reduced tumoral cell viability [8–15]. In fact, DOG1 seems to interact with various pathways, such as p38/MAPK [15], EGF/EGFR [13], PI3k/AKT [15,16], TGF/SMAD [14] and many others. Some studies have described a correlation between detectable DOG1 immunostaining and poor prognosis or tumor aggressivity among various cancers of the prostate, breast, ovary, esophagus, stomach, pancreas, head and neck, liver and others [9–11,13,14,16–24].

However, DOG1 expression has also been detected in normal tissues: in fact, this protein is expressed in pancreatic centroacinar cells, in a subset of islet cells and also in normal acini of salivary glands [17,25]. The explanation of such findings would lie in the fact that, given its properties of the calcium-activated chloride channel, DOG1 protein could have a secretory role in such cell types and studies in murine models have shown that it is essential for the secretory activity of acini in normal salivary glands [26–29].

On this basis, it is not surprising that DOG1 could be expressed in acinar-derived salivary neoplasms, such as ACC. In fact, a positive DOG1 staining is frequent in ACCs and would support a diagnosis of ACC versus other salivary gland neoplasms. DOG1 positivity can be an admixture of apical membranous, cytoplasmic and complete membranous staining. Interestingly, there are a limited number of studies on the value of DOG1 in salivary glands, particularly focused on ACC [29,30]. Some of these showed different staining patterns, intensity and also an extension of the immunohistochemical reaction. Due to the clonal variability of DOG1 and the limited number of studies, there could be confusion about its role in the diagnosis of ACC. In this study, we aimed to investigate the role of DOG1 as an immunohistochemical marker of salivary ACC through meta-analysis.

2. Materials and Methods

A comprehensive literature search in the online databases of Pubmed, Scopus and Web of Science was conducted by searching papers using the keywords “DOG1” and “salivary acinic cell carcinoma” or “ACC” from January 2010 up to September 2021. To try to expand our search, references of the included articles were also screened to identify additional studies. The language was limited to English only.

2.1. Study Eligibility

For each included study, the following information was extracted independently in a piloted form: author, country, year of publication, total number of ACC cases, age, sex (% male), tumor size (cm), distant metastases, follow up, outcome and DOG1 immunohistochemical expression in salivary ACC.

2.2. Data Extraction

This systematic review was conducted according to PRISMA guidelines (Table 1). Starting from 145 references identified through database searching and 3 additional references identified through other sources, 26 duplicates were removed, and 65 records were excluded with the following reasons: not related to humans (n = 24), not related to acinic cell carcinoma (n = 10), papers not written in English (n = 15), studies published before 2010 (n = 16). Some 57 full-text articles were assessed for eligibility and 28 of them were excluded with the following reasons: some studies had no relevant results (n = 17), and other criteria (e.g., review article, editorials) (n = 11). Finally, 29 studies were included in qualitative synthesis and only 20 references that met the eligibility criteria were retained and included in the current work [29–48].

Data from each eligible study were extracted without modification of original data according to the PICOS: “P”: (population) was constituted by patients with salivary ACC; “I”: (intervention or risk factor) was the ACC group with DOG1 expression, assessed by immunohistochemistry; “C”: “Comparator” was the ACC group without immunohistochemical expression; “S”: “Study design” was the study design of the included studies.
Reporting bias across studies was evaluated by a graphic diagnostic tool named funnel plot according to Egger M. et al. [49]. The x-axis in the present analysis is the DOG1 expression and the y-axis is the standard error. In the absence of publication bias, the points representing the studies have a roughly symmetric funnel shape and are distributed about the average effect across the spectrum of levels of precision (Figure 1).

**Table 1.** Flow diagram of the study selection process.

| Identification | Records identified through database searching (n = 145) | Additional records identified through other sources (n = 3) |
|----------------|--------------------------------------------------------|----------------------------------------------------------|
|                | Records after duplicates (26) removed                  |                                                          |
|                | Records screened (n = 122)                             |                                                          |
|                | Full-text articles assessed for eligibility (n = 57)    |                                                          |
|                | Studies included in qualitative synthesis (n = 29)      |                                                          |
|                | Studies included in quantitative synthesis (meta-analysis) (n = 20) |                                                          |
|                | Records excluded (n = 65) with the following reasons:  |
|                | Not related to humans (24)                             |
|                | Not related to ACC (10)                                |
|                | Not English (15)                                       |
|                | Published < 2010 (16)                                   |
|                | Full-text articles excluded (n = 28), with the following reasons: |
|                | Not relevant results (n = 17)                          |
|                | Others (review article, editorials) (n = 11)            |
bias, the points representing the studies have a roughly symmetric funnel shape and are distributed about the average effect across the spectrum of levels of precision (Figure 1).

**Figure 1.** Symmetric funnel plot consistent with lower likelihood of publication bias. The x-axis indicates DOG1 expression and the y-axis is the standard error.

### 2.3. Data Analysis

We aggregated the results of each study using the meta-analytic software ProMeta 2.0 (Internovi, Cesena, Italy). We employed the random-effect model as a conservative approach to account for different sources of variation among studies (i.e., within-study variance and between-study variance) [50]. Q and I^2 statistics were then conducted to evaluate heterogeneity across studies [51]. Moreover, heterogeneity across study findings was determined using a moderator analysis. Sensitivity analyses were also performed to determine the stability of the study results, computing how the overall rates would change by removing one study at a time. Finally, publication bias analyses were established with two tests: the regression method reported by Egger et al. and the Begg and Mazumdar rank correlation test [49,52]. The absence of publication bias is indicated in both tests by nonsignificant results.

### 3. Results

Based on our criteria, 20 articles that were published between 2010 and 2021 were analyzed and are reported in Table 2.

Table 2. Characteristics of included studies.

| Author Year | Country | ACC Age (y/o) | Sex (% M) | Tumor Size (cm) | Distant Metastasis (n. of Cases) | Follow Up (m) | Outcome | DOG1 Expression (%)
|-------------|---------|---------------|-----------|----------------|-------------------------------|---------------|---------|------------------|
| Chenevert [29] 2012 USA | 28 | 28/28 (100) | | | | | | |
| Raboh [31] 2015 Egypt | 9 | 9/9 (100) | | | | | | |
| Hamamoto [32] 2020 Japan | 8 | 59.8 | 25 | 4.1 | 1 | 93.4 | | 8/8 (100) |

All the analyses were performed using automatic staining.

In our study cohort, the median patient age was 54 years (range: 42–66 y/o), and the median tumor size was 3 cm. At the time of follow-up, the analyses also revealed a median of 36.3 months.

Furthermore, the percentage of male participants was 37.04% with a predominantly female population. The shapes of the funnel plots did not reveal evidence of obvious asymmetry (Figure 1).
Table 2. Characteristics of included studies.

| Author                  | Year | Country  | ACC | Age (y/o) | Sex (% M) | Tumor Size (cm) | Distant Metastasis (n. of Cases) | Follow Up (m) | Outcome | DOG1 Expression (%) |
|-------------------------|------|----------|-----|-----------|-----------|-----------------|---------------------------------|---------------|---------|----------------------|
| Chenevert [29]          | 2012 | USA      | 28  | 28/28     | (100)     |                 |                                 |               |         | Alive: 3 Dead: 2 NA: 3 |
| Raboh [31]              | 2015 | Egypt    | 9   |           |           |                 |                                 |               |         | 8/8 (100)            |
| Hamamoto [32]           | 2020 | Japan    | 8   | 59.8      | 25        | 4.1             | 1                              | 93.4          |         | 8/8 (100)            |
| Hsieh [33]              | 2016 | Taiwan   | 28  |           |           |                 |                                 |               |         | Alive: 3 Dead: 2 NA: 3 |
| Hsieh [34]              | 2015 | Taiwan   | 21  | 42        | 50        | 3               | 12                             | 26            |         | 28/28 (100)          |
| Khurram [35]            | 2016 | UK       | 31  | 46        | 47.6      | 2               | 12                             | 26            |         | 31/31 (100)          |
| Khurram [36]            | 2019 | UK       | 15  |           |           |                 | 1                              | 31            |         | 14/15 (93.3)         |
| Naous [37]              | 2017 | USA      | 15  | 49.3      | 33.3      |                 | 1                              | 5/6 (83.3)    |         | 14/15 (93.3)         |
| Owosho [38]             | 2021 | USA      | 6   | 54        | 0         | 2.9             | 4                              | 16            |         | 5/6 (83.3)           |
| Said-Al-Naief [39]      | 2017 | USA      | 14  | 55        | 28.5      |                 | 4                              | 16            | Alive 13 Dead 1     | 11/14 (78.6) |
| Schmitt [30]            | 2014 | USA      | 37  |           |           |                 |                                 |               |         | 32/37 (86.5)        |
| Skaugen [40]            | 2021 | USA      | 11  | 66        | 63.6      |                 | 1                              | 31            | Alive 16 Dead 1     | 9/11 (81.8)  |
| Stevens [41]            | 2015 | USA      | 13  | 63        | 44.4      |                 | 2                              | 13            |         | 13/13 (100)         |
| Thompson [42]           | 2016 | USA      | 25  | 63.2      | 36        | 3.9             | 22                             |               | Alive 5 Dead 1      | 20/25 (80)   |
| Urano [43]              | 2014 | Japan    | 6   | 63        | 50        | 2.4             | 1                              | 10.5          |         | 3/6 (50)             |
| Viviane Mariano [44]    | 2016 | Brazil   | 17  | 46.2      |           |                 |                                 |               |         | 14/17 (82.4)        |
| Hemminger [45]          | 2011 | USA      | 5   |           |           |                 |                                 |               |         | 4/5 (80)             |
| Jung [46]               | 2013 | Korea    | 6   | 44.1      |           | 2.57            | 50                             |               | Alive 6 Dead 0      | 3/6 (50)     |
| Shi [47]                | 2017 | China    | 30  |           |           |                 |                                 |               |         | 29/30 (96.7)        |
| Kuwabara [48]           | 2018 | Japan    | 8   | 55.5      | 12.5      |                 | 2                              |               |         | 8/8 (100)            |

Note: ACC: acinic cell carcinoma; NA: not available; FU: follow-up.
Table 3. DOG1 primary antibody clones, dilutions and manufacturers’ specifications regarding immunohistochemical analyses performed in the included studies.

| Author            | Clone     | Dilution | Manufacturer                        |
|-------------------|-----------|----------|-------------------------------------|
| Chenevert [29]    | Clone 1.1 | 1:50     | Zeta Co, Sierra Madre, CA           |
| Raboh [31]        | Clone 1.1 | NA       | Thermo scientific                  |
| Hamamoto [32]     | SP31      | RTU      | Roche                              |
| Hsieh [33]        | SP31      | RTU      | Roche Ventana                      |
| Hsieh [34]        | SP31      | RTU      | Roche Ventana                      |
| Khurram [35]      | NA        | 1:100    | Leica Microsystems                 |
| Khurram [36]      | Mouse monoclonal | 1:250 | DAKO                              |
| Naous [37]        | SP31      | RTU      | Cell Marque                        |
| Owosho [38]       | SP31      | 1:50     | Thermo Fisher Scientific           |
| Said-Al-Naief [39]| SP31      | RTU      | Ventana                            |
| Schmidt [30]      | SP31      | 1:40     | Cell Marque                        |
| Skaugen [40]      | SP31      | 1:50     | Thermo Fisher Scientific           |
| Stevens [41]      | SP31      | RTU      | Cell Marque                        |
| Thompson [42]     | SP31      | 1:50     | Cell Marque                        |
| Urano [43]        | SP31      | 1:1      | Nichirei                           |
| Viviane Mariano [44]| DOG1.1 | RTU      | Abcam                              |
| Hemminger [45]    | clone K9  | 1:100    | Leica Microsystems                 |
| Jung [46]         | Rabbit polyclonal | 1:200 | Spring Science                     |
| Shi [47]          | SP31      | NA       | MXB                                |
| Kuwabara [48]     | SP31      | 1:50     | Thermo Scientific                  |

Note: NA: not available; RTU: ready to use.

The results indicated that, in a heterogeneous set of 20 studies, the overall rate of DOG1 expression was 55% (95% CI = 0.43–0.58; Q = 3.12; I² = 0.00) with a p value < 0.05. (Table 4). Fifty-five percent is the value measuring the strength of the relationship between two variables (presence and absence of acinar differentiation) and, in our case, there is quite a strong correlation between the expression of DOG1 in salivary neoplasm and its acinar differentiation (i.e., also a correlation with a final diagnosis of ACC).

Table 4. Summary of meta-analytic results.

|    | K  | N   | Overall Rate of Expression (95% CI) | Q     | I²  |
|----|----|-----|-----------------------------------|-------|-----|
| DOG1 | 20 | 333 | 55% (95% CI = 0.43–0.58) | 3.12  | 0.00 |

Note. K: number of studies; N: number of histological cases available for IHC; CI: confidence interval; I²: index for quantifying the degree of heterogeneity; Q: test for heterogeneity; p < 0.001.

These results were highly reliable, as indicated by sensitivity and publication bias analyses (Egger test, −2.09; p 0.04; Begg and Mazumdar test, −0.19; p 0.57). Details of the overall rates were tested through moderator analyses. Table 5 illustrates the cut-off values for DOG1 in the selected studies.
Table 5. Cut-off value for DOG1 in the selected studies.

| Author            | DOG1 Expression (%) | Cut-Off Value                                                                 |
|-------------------|---------------------|-------------------------------------------------------------------------------|
| Chenevert [29]    | 28/28 (100)         | Cases were considered as ‘negative’ if <2% of the tumor expressed DOG1, as ‘focal’ if between 2 and 50%, and as ‘diffuse’ if >50% had staining |
| Raboh [31]        | 9/9 (100)           | The staining intensity was scored as weak 1+, moderate 2+, and strong 3+. The staining of normal serous acini was used as 2+, more intense staining was graded 3+ and less intense as 1+. |
| Hamamoto [32]     | 8/8 (100)           | The tumor cells of the ACC cases generally showed strong DOG1 staining intensity on the apical side, but strong cytoplasmic staining was detected in some ACC cases, especially in areas with a solid pattern. |
| Hsieh [33]        | 28/28 (100)         | Most cases of ACC showed a diffuse (>50% cells) staining, with a mixed apical staining (more frequently observed) and a cytoplasmic staining pattern |
| Hsieh [34]        | 20/21 (95.2)        | Most cases of ACC showed a diffuse (>50% cells) staining, with a mixed apical staining (more frequently observed) and a cytoplasmic staining pattern |
| Khurram [35]      | 31/31 (100)         | Strong apical/luminal DOG1 staining was seen in normal acini, although occasional cells demonstrated lateral and basal expression. Staining was stronger in serous acini compared to mucus and focally intercalated ducts showed positive luminal reactivity. |
| Khurram [36]      | 14/15 (93.3)        | Strong apical/luminal DOG1 staining was seen in normal acini, although occasional cells demonstrated lateral and basal expression. Staining was stronger in serous acini compared to mucus and focally intercalated ducts showed positive luminal reactivity. |
| Naous [37]        | 14/15 (93.3)        | NA                                                                            |
| Owosho [38]       | 5/6 (83.3)          | NA                                                                            |
| Said-Al-Naief [39]| 11/14 (78.6)        | NA                                                                            |
| Schmitt [30]      | 32/37 (86.5)        | Immunostaining was graded as weak (1+), moderate (2+), and intense (3+).      |
| Skaugen [40]      | 9/11 (81.8)         | Staining was semiquantitatively scored for intensity (0, 1+, 2+, 3+) and extent (<1%, 1–25%, 26–50%, 51–75%, 76–100%) |
| Stevens [41]      | 13/13 (100)         | NA                                                                            |
| Thompson [42]     | 20/25 (80)          | Luminal DOG1 staining was considered positive and was assessed as a percentage of the respective (LG vs. HG) component being analyzed |
| Urano [43]        | 3/6 (50)            | NA                                                                            |
| Viviane Mariano [44]| 14/17 (82.4)        | (a) Apical–luminal, (b) mixed membranous and cytoplasmic, (c) cytoplasmic. In the mixed pattern, the membranous component did not exhibit the apical–luminal staining. |
| Hemminger [45]    | 4/5 (80)            | DOG1 immunopositivity was scored quantitatively for the percentage of positive tumor cells staining (%: 0, ≤10, ≤25, ≤50, >50), intensity (0, negative; 1+, weak staining/trace, 2+, moderate staining; 3+, strong staining) and subcellular location (cytoplasmic, membranous and luminal). |
| Jung [46]         | 3/6 (50)            | NA                                                                            |
| Shi [47]          | 29/30 (96.7)        | NA                                                                            |
| Kuwabara [48]     | 8/8 (100)           | DOG1 was expressed in apical-luminal region                                   |

Note. NA: not available.

4. Discussion

ACC is a salivary gland malignancy of ductal origin, representing up to 17% of salivary gland neoplasms [53].

The presence of serous acinar cells is a consistent feature of salivary ACC and the main diagnostic criterion is the histologic architecture of the neoplasm, distinguished into four typical patterns: solid, microcystic, follicular and papillary-cystic. ACC is a common cause of false-negative interpretation due to similarity with the normal parotid acinar cells and
the absence of hallmark features of malignancy such as necrosis, cellular pleomorphism and high mitotic activity [4]. Thus, there is a need to familiarize with the cytological characteristics of ACC and with its differential diagnoses. The key to the accurate cytological diagnosis of ACC lies in the recognition of the neoplastic acinar cells, with numerous bare nuclei in the background and complete absence of ductal epithelial cells [3].

In the present study, we evaluated the IHC staining profile of DOG1 in patients with salivary ACC through a systematic review and meta-analysis. In the present paper, a total of 20 eligible studies with 333 patients were included. In the overall analysis, the observed expression rate for DOG1 was 55%, showing quite a strong correlation between the expression of this marker and a final diagnosis of ACC.

DOG1 (also known as ANO1, TMEM16 A) is a transmembrane anion channel, which mediates Ca^{2+}-dependent Cl- secretion in glands and flat epithelia. Structurally, the DOG1 protein consists of eight transmembrane segments and cytosolic N- and C-termini [54].

Strong DOG1-positive staining ruled out most of the benign entities in the oncocytic salivary gland neoplasm group. In contrast to the weak membranous apical-luminal staining in benign salivary gland acini, DOG1 staining in ACC was moderate to strong, diffuse, membranous and often cytoplasmic [30]. A similar pattern of DOG1 staining was also noted by the study of Chênevert et al. [29], while Hemminger et al. observed a pure luminal pattern of staining [45].

In the overall analysis, we observed that DOG1 expression in salivary tissues is mainly localized in salivary acini, where DOG1 shows a variably intense apical membranous staining and progressively decreases in mucinous acini and intercalated ducts, becoming completely absent more proximally [6]. In our analysis, DOG1 showed a heterogenous positivity in ACCs and the distribution of staining and intensity were moderate to high. Regarding subcellular localization, the majority of the studies included in our work showed diffuse granular cytoplasmic staining in addition to apical-luminal staining and complete membranous staining in some foci. Only Chenevert et al. [29] found a slightly different subcellular localization: in fact, while DOG1 was expressed in some ACCs in their series, it was mostly apical luminal with scattered foci of complete membranous and cytoplasmic staining. Therefore, our findings indicate that DOG1 staining is of pivotal importance in the diagnosis of ACC together with routine hematoxylin and eosin staining that in most cases allows orientation of the diagnosis. Typically, ACCs show readily recognizable serous acinar differentiation on a routine hematoxylin and eosin-stained section, but when this cell type is less prominent, several stainings could help in the diagnosis, such as Periodic Acid-Schiff (PAS) in combination with diastase (PAS-D), mucicarmine, iron stain and in some instances anti-amylase immunostain. However, the sensitivity of these stainings for acinar differentiation is very low. Thus, DOG1 staining offers a sensitive and robust marker to support the diagnosis of ACC. The positivity of DOG1, in fact, is essential to establish the acinar nature of a salivary neoplasm and may represent an ‘exaggerated acinar’ phenotype in ACC, different from neoplasms where DOG1 overexpression is due to gene amplification [29] and can be related to an increased tumor aggressiveness [9–11,13,14,16–24]. Therefore, DOG 1 is a helpful marker in the diagnostic process of ACCs and its strong positivity can support the diagnosis of these neoplasms. Moreover, the negativity of such markers in our meta-analysis is limited to a minority of cases, many of them represented by poorly differentiated neoplasms; poor differentiation, in fact, entails a partial loss of the acinar phenotype with consequent possible reduction or loss of DDOG1 expression. Based on this finding, DOG1 expression, being prevalent in well-differentiated ACCs, could be interpreted as an index of lower aggressiveness of such neoplasms.

In our experience, we diagnosed 22 ACCs between 2005 and 2021; in this cohort, the ratio male:female was 3:19 (male percentage: 13.63%), the mean patient age was 53.3 years (range: 18–95 y/o), and the mean tumor size was 1.85 cm (range: 0.8–4.5 cm). DOG1 immunostaining, available for only five cases, was performed using Clone SP31 (dilution 1:50; Thermo Scientific, Cheshire, UK) and the I-view 2′-diaminobenzamide (DAB) detection kit (Ventana systems, Tucson, AZ, USA) on a Ventana Benchmark XT automated staining
system (Ventana Medical Systems, Inc, Tucson, AZ, USA). Of these five cases, n. 2 ACCs resulted negative, while among the positive ones n. 2 showed focal positivity and only one case showed a strong expression of DOG1 (Figure 2B).

Interestingly, both of the two cases that showed DOG1 negativity in our series were poorly differentiated neoplasms, confirming the findings of the studies included in our meta-analysis. However, our series is really small and not representative; studies on larger cohorts are needed to investigate DOG1 expression in poorly differentiated ACCs.

5. Conclusions

Although ACC is a challenging diagnostic entity, paying careful attention to the cytomorphological features of the neoplasm in conjunction with DOG1 immunostaining can help to reach an accurate diagnosis.

Author Contributions: Conceptualization: P.S., V.F., P.T., T.M. and E.D.R. methodology: P.S., V.F., P.T., M.M. and F.P. software: P.S. validation: P.S., V.F., P.T. and L.M.L. formal analysis: P.S., V.F., P.T. and G.F. investigation: P.S., V.F., P.T., T.M., F.P. and M.M. data curation: V.F., E.D.R. and L.M.L. writing—original draft preparation: V.F., P.T., P.S. and E.D.R. writing—review and editing: V.F., P.T., G.F. and L.M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are all available in this manuscript and in the references.

Conflicts of Interest: The authors declare no conflict of interest.
References

1. Al-Zaheer, N.; Obeid, A.; Al-Salam, S.; Al-Kayyali, B.S. Acinic cell carcinoma of the salivary glands: A literature review. Hematol. Oncol. Stem Cell Ther. 2009, 2, 259–264. [CrossRef]

2. Skalova, A.; Michal, M.; Simpson, R.H.W. Newly described salivary gland tumors. Mod. Pathol. 2017, 30 (Suppl. S1), S27–S43. [CrossRef] [PubMed]

3. Nagel, H.; Laskawki, R.; Büter, J.J.; Schröder, M.; Chilla, R.; Droese, M. Cytologic Diagnosis of Acinic-Cell Carcinoma of Salivary Glands. Diagn. Cytopathol. 1997, 16, 402–412. [CrossRef]

4. Alphs, H.H.; Eisele, D.W.; Westra, D.H. The role of fine needle aspiration in the evaluation of parotid masses. Curr. Opin. Otolaryngol. Head Neck Surg. 2006, 14, 62–66. [CrossRef]

5. Godwin, J.T.; Foot, F.W., Jr.; Frazell, E.L. Acinic cell adenocarcinoma of the parotid gland: Report of twenty-seven cases. Am. J. Pathol. 1954, 30, 465–477.

6. Spiro, H.; Huvos, A.G.; Strong, E.W. Acinic cell carcinoma of salivary origin: A Clinicopathologic Study of 67 Cases. Cancer 1978, 41, 924–935. [PubMed]

7. Miettinen, M.; Wang, Z.F.; Lasota, J. DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: A study of 1840 cases. Am. J. Surg. Pathol. 2009, 33, 1401–1408. [CrossRef]

8. Britschgi, A.; Bill, A.; Brinkhaus, H.; Rothwell, C.; Clay, I.; Duss, S.; Rehban, M.; Raman, P.; Guy, C.T.; Wetzel, K.; et al. Calcium-activated chloride channel ANO1 promotes breast cancer progression by activating EGFR and CAMK signaling. Proc. Natl. Acad. Sci. USA 2013, 110, E1026–E1034. [CrossRef]

9. Crottes, D.; Lin, Y.T.; Peters, C.J.; Gilchrist, J.M.; Wiita, A.P.; Jan, Y.N.; Jan, L.Y. TMEM16A controls EGF-induced calcium signaling implicated in pancreatic cancer prognosis. Proc. Natl. Acad. Sci. USA 2019, 116, 13026–13035. [CrossRef]

10. Duvvuri, U.; Shiwasrki, D.J.; Xiao, D.; Bertrand, C.; Huang, J.; Edinger, R.S.; Rock, J.R.; Harle, B.D.; Henson, B.J.; Kunzelmann, K.; et al. TMEM16A induces MAPK and contributes directly to tumorigenesis and cancer progression. Cancer Res. 2012, 72, 3270–3281. [CrossRef]

11. Godse, N.R.; Khan, N.; Yochum, Z.A.; Gomez-Casal, R.; Kemp, C.; Shiwasrki, D.J.; Seethala, R.S.; Seshadri, M.; Burns, T.F.; et al. TMEM16A/ANO1 inhibits apoptosis by downregulation of Bim expression. Clin. Cancer Res. 2017, 23, 7324–7332. [CrossRef] [PubMed]

12. Hu, C.; Zhang, R.; Jiang, D. TMEM16A as a potential biomarker in the diagnosis and prognosis of lung cancer. Arch. Iran. Med. 2019, 22, 32–38. [PubMed]

13. Wang, H.; Yao, F.; Luo, S.; Ma, K.; Liu, M.; Bai, L.; Chen, S.; Song, C.; Wang, T.; Du, Q.; et al. A mutual activation loop between the Ca(2+)-activated chloride channel TMEM16A and EGFR/STAT3 signaling promotes breast cancer tumorigenesis. Cancer Lett. 2019, 455, 48–59. [CrossRef] [PubMed]

14. Yu, Y.; Cao, J.; Wu, W.; Zhu, Q.; Tang, Y.; Zhu, C.; Dai, J.; Li, Z.; Wang, J.; Xue, L.; et al. Genome-wide copy number variation analysis identified ANO1 as a novel oncogene and prognostic biomarker in esophageal squamous cell cancer. Carcinogenesis 2019, 40, 1198–1208. [CrossRef]

15. Zhang, C.; Liu, J.; Han, Z.; Cui, X.; Peng, D.; Xing, Y. Inhibition of TMEM16A suppresses growth and induces apoptosis in hepatocellular carcinoma. Int. J. Clin. Oncol. 2020, 25, 1145–1154. [CrossRef] [PubMed]

16. Liu, Z.; Zhang, S.; Hou, F.; Zhang, C.; Gao, J.; Wang, K. Inhibition of Ca(2+)-activated chloride channel ANO1 suppresses ovarian cancer through inactivating PI3K/Akt signaling. Int. J. Cancer. 2019, 144, 2215–2226. [CrossRef]

17. Ardeleanu, C.; Arsene, D.; Hinescu, M.; Andrei, F.; Gutu, D.; Luca, L.; Popescu, L.M. Pancreatic expression of DOG1: A novel gastrointestinal stromal tumor (GIST) biomarker. Appl. Immunohistochem. Mol. Morphol. 2009, 17, 413–418. [CrossRef]

18. Li, Y.; Zhang, J.; Hong, S. ANO1 as a marker of oral squamous cell carcinoma and silencing ANO1 suppresses migration of human SCC-25 cells. Med. Oral Patol. Oral Cir. Bucal 2014, 19, e313–e319. [CrossRef]

19. Liu, F.; Cao, Q.H.; Lu, D.J.; Luo, B.; Lu, X.F.; Luo, R.C.; Wang, X.G. TMEM16A overexpression contributes to tumor invasion and poor prognosis of human gastric cancer through TGF-beta signaling. Oncotarget 2015, 6, 11585–11599. [CrossRef]

20. Liu, W.; Lu, M.; Liu, B.; Huang, K. Wang. Inhibition of Ca(2+)-activated Cl(-) channel ANO1/TMEM16A expression suppresses tumor growth and invasiveness in human prostate carcinoma. Cancer Lett. 2012, 326, 41–51. [CrossRef]

21. Sahin, S.; Ekinci, O.; Seckin, S.; Dursun, A. The diagnostic and prognostic utility of DOG1 expression on gastrointestinal stromal tumors. Turk. Patoloji Derg. 2017, 33, 1–8. [PubMed]

22. Zeng, X.; Pan, D.; Wu, H.; Chen, H.; Yuan, W.; Zhou, J.; Shen, Z.; Chen, S. Transcriptional activation of ANO1 promotes gastric cancer progression. Biochem. Biophys. Res. Commun. 2019, 512, 131–136. [CrossRef]

23. Lee, C.H.; Liang, C.W.; Espinosa, I. The utility of discovered on gastrointestinal stromal tumor 1 (DOG1) antibody in surgical pathology-the GIST of it. Adv. Anat. Pathol. 2010, 17, 222–232. [CrossRef] [PubMed]

24. Carles, A.; Millon, R.; Cromer, A.; Ganguli, G.; Lemaire, F.; Young, J.; Wasylyk, C.; Muller, D.; Schultz, I.; Rabouel, Y.; et al. Head and neck squamous cell carcinoma transcriptome analysis by comprehensive validated differential display. Oncogene 2006, 25, 1821–1831. [CrossRef] [PubMed]

25. Bergmann, F.; Andrilis, M.; Hartwig, W.; Penzel, R.; Gaida, M.M.; Herpel, E.; Schirmacher, P.; Mechtlersheimer, G. Discovered on gastrointestinal stromal tumor 1 (DOG1) is expressed in pancreatic centroacinar cells and in solid-pseudopapillary neoplasms—novel evidence for a histogenetic relationship. Hum. Pathol. 2011, 42, 817–823. [CrossRef]
26. Almeida, J.; Tian, Y.; Aldehni, F.; Ousingsawat, J.; Kongsuphol, P.; Rock, J.R.; Harfe, B.D.; Schreiber, R.; Kunzelmann, K. TMEM16 proteins produce volume-regulated chloride currents that are reduced in mice lacking TMEM16A. *J. Biol. Chem.* 2009, 284, 28571–28578. [CrossRef]

27. Kunzelmann, K.; Kongsuphol, P.; Aldehni, F.; Tian, Y.; Ousingsawat, J.; Warth, R.; Schreiber, R. Bestrophin and TMEM16-Ca^{2+}-activated Cl^{-} channels with different functions. *Cell Calcium* 2009, 46, 233–241. [CrossRef]

28. Ousingsawat, J.; Martins, J.R.; Schreiber, R.; Rock, J.R.; Harfe, B.D.; Kunzelmann, K. Loss of TMEM16A causes a defect in epithelial Ca^{2+}-dependent chloride transport. *J. Biol. Chem.* 2009, 284, 28698–28703. [CrossRef]

29. Cheever, J.; Duvvuri, U.; Chiosea, S.; Dacic, S.; Cieply, K.; Kim, J.; Shiwarski, D.; Seethala, R.R. DOG1: A novel marker of salivary acinar and intercalated duct differentiation. *Mod. Pathol.* 2012, 25, 919–929. [CrossRef]

30. Schmitt, A.C. DOG1, p63, and S100 protein: A novel immunohistochemical panel in the differential diagnosis of oncocytic salivary gland neoplasms in fine-needle aspiration cell blocks. *J. Am. Soc. Cytopathol.* 2014, 3, 303–308. [CrossRef]

31. Hamamoto, Y.; Harada, H.; Kohara, M.; Honma, K.; Nakatsuka, S.I.; Morii, E. Usefulness of immunohistochemistry to distinguish between secretory carcinoma and acinic cell carcinoma in the salivary gland. *Med. Mol. Morphol.* 2021, 54, 23–30. [CrossRef] [PubMed]

32. Hamamoto, Y.; Harada, H.; Kohara, M.; Honma, K.; Nakatsuka, S.I.; Morii, E. Usefulness of immunohistochemistry to distinguish between secretory carcinoma and acinic cell carcinoma in the salivary gland. *Med. Mol. Morphol.* 2021, 54, 23–30. [CrossRef] [PubMed]

33. Hsieh, M.S.; Chou, Y.H.; Yeh, A.J.; Chang, Y.L. Papillary-cystic pattern is characteristic in mammary analogue secretory carcinomas of salivary gland. *Hum. Pathol.* 2016, 47, 137–142. [CrossRef] [PubMed]

34. Hsieh, M.S.; Jeng, Y.M.; Jhuang, Y.L.; Chou, Y.H.; Lin, C.Y. Carbonic anhydrase VI: A novel marker for salivary serous acinar differentiation and its application to discriminate acinic cell carcinoma from mammary analogue secretory carcinoma of the salivary gland. *Histopathology* 2016, 68, 641–647. [CrossRef] [PubMed]

35. Khurram, S.A.; Sultan-Khan, J.; Atkey, N.; Speight, P.M. Cyto genetic and immunohistochemical characterization of mammary analogue secretory carcinoma of salivary glands. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* 2016, 122, 731–742. [CrossRef]

36. Khurram, S.A.; Speight, P.M. Characterisation of DOG-1 Expression in Salivary Gland Tumours and Comparison with Myoepithelial Markers. *Head Neck Pathol.* 2019, 13, 140–148. [CrossRef]

37. Naous, R.; Zhang, S.; Valente, A.; Stemmer, M.; Khurana, K.K. Utility of Immunohistochemistry and ETV6 (12p13) Gene Rearrangement in Identifying Secretory Carcinoma of Salivary Gland among Previously Diagnosed Cases of Acinic Cell Carcinoma. *Pathol. Res. Int.* 2017, 2017, 1497023. [CrossRef] [PubMed]

38. Owosho, A.; Tyler, D.; Adesina, O.; Odujoko, O.; Summersgill, K. NR4A3 (NOR-1) Immunostaining Shows Better Performance than DOG1 Immunostaining in Acinic Cell Carcinoma of Salivary Gland: A Preliminary Study. *J. Oral Maxillofac. Res.* 2021, 12, e4. [CrossRef]

39. Said-Al-Naief, N.; Carlos, R.; Vance, G.H.; Miller, C.; Edwards, P.C. Combined DOG1 and Mammaglobin Immunohistochemistry Is Comparable to ETV6-break apart Analysis for Differentiating Between Papillary Cystic Variants of Acinic Cell Carcinoma and Mammary Analogue Secretory Carcinoma. *Int. J. Surg. Pathol.* 2017, 25, 127–140. [CrossRef]

40. Skagen, J.M.; Seethala, R.R.; Chiosea, S.I.; Landau, M.S. Evaluation of NR4A3 immunohistochemistry (IHC) and fluorescence in situ hybridization and comparison with DOG1 IHC for FNA diagnosis of acinic cell carcinoma. *Cancer Cytopathol.* 2021, 129, 104–113. [CrossRef] [PubMed]

41. Stevens, T.M.; Kovalovsky, A.O.; Velosa, C.; Shi, Q.; Dai, Q.; Owen, R.P.; Bell, W.C.; Wei, S.; Althof, P.A.; Sanmann, J.N.; et al. Mammary analogue secretory carcinoma, low-grade salivary duct carcinoma, and mimickers: A comparative study. *Mod. Pathol.* 2015, 28, 1084–1100. [CrossRef] [PubMed]

42. Thompson, L.D.; Aslam, M.N.; Stall, J.N.; Udager, A.M.; Chiosea, S.; McHugh, J.B. Clinicopathologic and Immunophenotypic Characterization of 25 Cases of Acinic Cell Carcinoma with High-Grade Transformation. *Head Neck Pathol.* 2016, 10, 152–160. [CrossRef] [PubMed]

43. Urano, M.; Nago, T.; Miyabe, S.; Ishibashi, K.; Higuchi, K.; Kuroda, M. Characterization of mammary analogue secretory carcinoma of the salivary gland: Discrimination from its mimics by the presence of the ETV6-NTRK3 translocation and novel surrogate markers. *Hum. Pathol.* 2015, 46, 94–103. [CrossRef] [PubMed]

44. Mariano, F.V.; Gomez, C.A.C.; Nascimento, J.D.S.D.; dos Santos, H.T.; Egal, E.S.; Montalli, V.A.M.; Vargas, P.A.; de Almeida, O.P.; Altemani, A. Lysozyme Expression Can Be Useful to Distinguish Mammary Analog Secretory Carcinoma from Acinic Cell Carcinoma of Salivary Glands. *Head Neck Pathol.* 2016, 10, 429–436. [CrossRef]

45. Hemminger, J.; Iwenofu, O.H. Discovered on gastrointestinal stromal tumours 1 (DOG1) expression in non-gastrointestinal stromal tumour (GIST) neoplasms. *Histopathology* 2012, 61, 170–177. [CrossRef]

46. Jung, M.J.; Song, J.S.; Kim, S.Y.; Nam, S.Y.; Roh, J.-L.; Choi, S.-H.; Kim, S.-B.; Cho, K.-J. Finding and Characterizing Mammary Analogue Secretory Carcinoma of the Salivary Gland. *Korean J. Pathol.* 2013, 47, 36–43. [CrossRef]

47. Shi, K.; Xin-Quan, L. Pathological features of mammary analogue secretory carcinoma of the salivary gland. *Int. J. Clin. Exp. Pathol.* 2017, 10, 7460–7465.

48. Kuwabara, H.; Yamamoto, K.; Terada, T. Hemorrhage of MRI and Immunohistochemical Panels Distinguish Secretory Carcinoma from Acinic Cell Carcinoma. *Laryngoscope Investig.* *Otolarygol.* 2018, 3, 268–274. [CrossRef]

49. Egger, M.; Davey Smith, G.; Schneider, M.; Minder, C. Bias in meta-analysis detected by a simple, graphical test. *BMJ (Clin. Res. Ed.)* 1997, 315, 629–634. [CrossRef]
50. Huedo-Medina, T.B.; Sanchez-Meca, J.; Marin-Martinez, F.; Botella, J. Assessing heterogeneity in meta-analysis: Q statistic or I² index? *Psychol. Methods* **2006**, *11*, 193–206. [CrossRef]

51. Higgins, J.P.; Thompson, S.G.; Deeks, J.J.; Altman, D.G. Measuring inconsistency in meta-analyses. *BMJ* **2003**, *327*, 557–560. [CrossRef] [PubMed]

52. Begg, C.B.; Mazumdar, M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* **1994**, *50*, 1088–1101. [CrossRef] [PubMed]

53. Ellis, G.L.; Auclair, P.L. Tumors of the salivary glands. In *AFIP Atlas of Tumor Pathology, 4th Series, Fascicle 9*; ARP Press: Silver Spring, MD, USA, 2008; pp. 204–225.

54. Yang, Y.D.; Cho, H.; Koo, J.Y.; Tak, M.H.; Cho, Y.; Shim, W.S.; Park, S.P.; Lee, J.; Lee, B.; Kim, B.M.; et al. TMEM16A confers receptor-activated calcium-dependent chloride conductance. *Nature* **2008**, *455*, 1210–1215. [CrossRef] [PubMed]