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Original Contribution

Central giant cell granuloma: A clinicopathological and immunohistochemical study of macrophages, blood vessels, lymphatic vessels and regulatory proteins

Vinicius Rio Verde Melo-Muniz[a], Fábio Daumas Nunes[b], Maria Cristina Teixeira Cangussu[a], Patrícia Ramos Cury[a], Flávia Caló Aquino Xavier[a,f], Roberto Almeida de Azevedo[c], Águida Cristina Gomes Henriques Leitão[a,f], Ludmila de Faro Valverde[d], Bráulio Carneiro Júnior[e], Jean Nunes dos Santos[a,f,*]

[a] Dentistry and Health Postgraduate Program, School of Dentistry, Federal University of Bahia, Salvador, Bahia, Brazil
[b] Laboratory of Oral and Maxillofacial Pathology, School of Dentistry, University of São Paulo, São Paulo, São Paulo, Brazil
[c] Department of Oral and Maxillofacial Surgery, School of Dentistry, Federal University of Bahia, Salvador, Bahia, Brazil
[d] Gonçalo Moniz Institute, Oswaldo Cruz Foundation (IGM-FIOCRUZ/BA), Salvador, Bahia, Brazil.
[e] Department of Oral and Maxillofacial Surgery, School of Dentistry, Southwest University of Bahia, Jequié, Bahia, Brazil
[f] Laboratory of Oral and Maxillofacial Pathology, School of Dentistry, Federal University of Bahia, Salvador, Bahia, Brazil

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ABSTRACT

Objective: This study seeks to investigate immunohistochemical parameters that could distinguish non-aggressive Central giant cell granuloma (CGCG) from aggressive CGCG, two groups of lesions which differ in their clinical and radiographic features and prognosis.

Material and methods: 12 cases of non-aggressive CGCG and 11 cases of aggressive CGCG were investigated and associated the immunohistochemical expression of macrophages (CD68 and CD163), blood vessels (CD34 and CD105), lymphatic vessels (D2-40) and regulator proteins (p63 and Ki-67). Clinical and radiographic features were also studied.

Results: Associations between all proteins in non-aggressive and aggressive CGCG were not significant ($p > 0.05$). With respect to non-aggressive CGCG, there were no significant correlations, while in aggressive CGCG there was a significant positive correlation between CD68 and CD163 ($p = 0.031$), between CD34 and D2-40 proteins ($p = 0.04$), whereas a significant negative correlation was observed between CD105 and CD68 ($p = 0.040$). However, regardless of aggressiveness of CGCG, there was a significant positive correlation between CD68 and CD163 ($p = 0,04$). Among the clinical and immunohistochemical aspects, only the symptomatology was a significant risk factor for the occurrence of aggressive CGCG (OR = 12.00/$p = 0.016$).

Conclusion: Macrophages and angiogenesis contribute to their maintenance and development of CGCG. In addition, immunohistochemistry used here was not able to differentiate their aggressiveness. However, symptomatology was proved to be a risk factor for the occurrence of aggressive CGCG. It is possible that clinical features, particularly symptomatology, represent the most appropriate parameter to attempt to distinguish GCCG.

1. Introduction

Central giant cell granuloma (CGCG) is a benign osteolytic lesion of the jaws that is composed of osteoclast-like giant cells arranged in a vascular stroma, which can become aggressive [1]. The clinical presentation of CGCG ranges from a slow and asymptomatic growth detected on routine radiographs to more aggressive lesions characterized by painful symptoms and rapid and expansive growth, which can cause root resorption and tooth displacement. The latter are also associated with high rates of recurrence [2-5]. These lesions are generally classified as aggressive and non-aggressive based on clinical and radiographic features. Central giant cell granuloma is classified as

* Corresponding author at: School of Dentistry - Federal University of Bahia, Laboratory of Oral and Maxillofacial Pathology, Faculdade de Odontologia - UFBA, Laboratório de Patologia Cirúrgica Bucal, Avenida Araújo Pinho, 62, Canela, Salvador, Bahia 40110-150, Brazil.
E-mail address: jeanunes@ufba.br (J.N. dos Santos).

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aggressive (A-CGCG) when recurrence occurs after enucleation and curettage, when it is larger than 5.0 cm or when it exhibits at least three of the following five criteria: painful symptoms, rapid growth, root resorption, tooth displacement, cortical bone perforation, and recurrence after surgical enucleation and curettage [6-8]. In contrast, CGCG is classified as non-aggressive (NA-CGCG) when the lesion is smaller than 5.0 cm and asymptomatic, when it displays slow growth, and when no association with teeth or cortical bone perforation and no recurrence are observed.

Histologically, CGCG is characterized by the non-encapsulated proliferation of spindle-shaped and polygonal mononuclear cells permeated by osteoclast-like multinucleated cells in a vascular stroma, as well as foci of hemorrhage and hemosiderin pigment [9-11]. In view of the similar histological patterns of NA-CGCG and A-CGCG, some studies have attempted to establish immunohistochemical parameters that are able to differentiate these two clinical variants [5,7,8,12-16].

Therefore, this study was designed to contribute to the understanding of immunohistochemical parameters that could distinguish NA-CGCG from A-CGCG, histologically similar lesions with distinct clinical behaviors. For this purpose, we studied markers that identify the presence of macrophages (CD68 and CD163), vascular pattern (CD34, CD105 and D2-40), and expression of regulatory proteins (p63 and Ki-67) in the two clinical variants of CGCG.

2. Material and methods

Twenty-three cases of patients with a histopathological diagnosis of CGCG between 1997 and 2016 were selected from the archives of the surgical pathology laboratories of the Schools of Dentistry of the Federal University of Bahia (FOUFBA) and University of São Paulo (FOUSP). All hematoxylin/eosin-stained histological slides were evaluated by two examiners under a light microscope. All clinical data were obtained of the request forms of the anatomopathological exams. The cases were classified as NA-CGCG and A-CGCG based on the criteria used in previous studies [8,16] and shown in Table 1.

Three-centimeter-thick sections were obtained from the formalin-fixed, paraffin-embedded specimens and mounted on clean and silanized glass slides. The Advance® system (Dako Corporation, Carpinteria, USA) was applied according to the protocol described in Table 2. After antigen retrieval in a water bath, endogenous peroxidase was blocked by immersing the slides in a solution of 3% hydrogen peroxide and distilled water for 10 min (2×), protected from light. The slides were incubated with the primary antibodies diluted in background-reducing solution (Dako), overnight at 4 °C for CD68, CD163, CD34, CD105, D2-40, and Ki-67. For the p63 antibody, the slides were incubated for 1 h. The reaction was developed with 3,3′-diaminobenzidine (Dako) for 5 min in a dark chamber. The tissue samples were counterstained with Harris hematoxylin for 5 min, dehydrated, and mounted.

For immunohistochemical evaluation of the markers, two independent examiners classified the cases according to intensity score and proportion of staining based on criteria used in previous studies [16-18]. The intensity of staining was classified as follows: 0 = no staining; 1 = weak staining; 2 = moderate staining; 3 = intense staining. The proportion of stained cells was defined as follows: 0 = 0 to 10%; 1 = 11 to 40%; 2 = 41 to 75%; 3 = 76 to 100%. The staining index (SI) was then calculated by multiplying the intensity score (0 to 3) by the score obtained for the proportion of staining (0 to 3). Finally, antibody expression was classified according to the SI obtained: negative expression (SI-) for SI = 0; low expression (SI1+) for SI of 1 to 5; high expression (SI2+) for SI ≥ 6. For this purpose, ten consecutive fields were examined under a high-definition light microscope at 40× magnification (Axiostar Plus, Zeiss, Germany, 2008).

Statistical analysis was performed using the Minitab® 14 software (Minitab, Inc., Pennsylvania, USA). Student *t*-test and the Pearson's chi-square test were used to identify associations between the markers, clinical characteristics and type of lesion. Pearson's correlation coefficient was used to evaluate the correlation between markers. Finally, univariate logistic regression analysis was performed to identify possible risk factors for the development of A-CGCG. A level of significance of 95% ($p \leq 0.05$) was adopted.

3. Results

Among the 23 CGCG cases selected, 12 were classified as NA-CGCG and 11 as A-CGCG. The clinical and radiographic characteristics of these cases are described in Table 3. Regarding the immunohistochemical results (Table 4), CD68 staining was positive in 22 cases, with a predominance of cytoplasmic staining in giant cells. Low expression was observed in one case and high expression in 21 cases. For CD163, we observed 19 positive cases with nuclear staining in mononuclear cells, including low expression in 11 cases and high expression in 8. Staining for CD34 was positive in 22 cases, with low expression in 11 and high expression in the other 11 cases. Positive CD105 staining was observed in 8 cases, with low expression in 5 and high expression in 3. D2-40 was positive in 17 cases, including low expression in 6 and high expression in 11. Staining for the p63 and Ki-67 antibodies was detected in some cases but was not sufficient to exhibit a positive SI. Figs. 1 and 2 show the expression of the proteins studied.

Comparison of the SI of CD68, CD163, CD34, CD105, D2-40, p63 and Ki-67 between NA-CGCG and A-CGCG revealed no statistically significant difference ($p > 0.05$, chi-squared test) (Table 4). Spearman's correlation coefficient revealed a significant positive correlation between the expression of CD68 and CD163 ($p = 0.031$) in A-CGCG and in CGCG regardless of the clinical variant. There was also a significant positive correlation between the expression of CD34 and D2-40 in A-CGCG ($p = 0.04$). A significant negative correlation was found between the expression of CD105 and CD68 in A-CGCG ($p = 0.04$) and between CD68 and CD163 in CGCG regardless of the clinical variant ($p = 0.04$). The other correlations were not statistically significant ($p > 0.05$) (Table 5).

Only lesion-associated symptoms was a significant risk factor for the occurrence of A-CGCG (OR = 12.0, $p = 0.016$). The variables CD68, CD163, CD34, CD105, D2-40, p63, Ki-67, sex (female), age (≤30 year), tooth association, localization (mandible) and disease duration

Table 1
Clinical and radiographic features of non-aggressive and aggressive central giant cell granulomas.

Criteria	NA-CGCG	A-CGCG
Primary	< 50 mm in diameter	≥50 mm in diameter
	Absence of recurrence after enucleation and curettage	Recurrence after enucleation and curettage
Secondary	Slow growth (> 6 months)	Rapid growth (< 6 months)
	Absence of tooth resorption/displacement	Presence of tooth resorption/displacement
	Absence of cortical bone perforation	Presence of cortical bone perforation
	Absence of lesion-associated symptoms	Presence of lesion-associated symptoms

NA-CGCG = non-aggressive central giant cell granuloma; A-CGCG = aggressive central giant cell granuloma. This table is based on the criteria of previous studies [8,16] and to be classified as A-CGCG, the lesions must meet one primary criterion or at least three secondary criteria.

Table 2
Specificity of the immunohistochemical markers.

Antibody	Manufacturer	Clone	Positive control	Antigen retrieval	Dilution
CD68	Dako	PGM1	Mucocele	EDTA pH 8.0 (95 °C), 20 min	1:100
CD163	Cell Marque	GHI/61	Mucocele	EDTA pH 8.0 (95 °C), 20 min	1:25
CD34	Dako	QBEND 10	Mucocele	Trypsin 1% (37 °C), 30 min	1:100
CD105	Dako	SN6h	Breast cancer	Citrate pH 8.0 (95 °C), 20 min	1:30
D2-40	Dako	D2-40	OK	Citrate pH 6.0 (95 °C), 20 min	1:150
p63	Biosystems	7JUL	SCC	Citrate pH 6.0 (95 °C), 20 min	1:10
Ki-67	Cell Marque	SP6	SCC	Citrate pH 6.0 (95 °C), 20 min	1:100

Table 3
Clinical data of the cases of the central giant cell granulomas.

		CGCG (n = 23)	NA-CGCG (n = 12)	A-CGCG (n = 11)	p value
Sex	Female	17 (74%)	9 (75%)	8 (73%)	0.72[b]
	Male	6 (26%)	3 (25%)	3 (27%)	
Age (years)	NA	1	1	–	0.246[c]
	Mean	33.55	37.91	29.18	
	SD	± 17.12	± 17.78	± 16.44	
	Range	10–72	16–72	10–67	
Tooth association (tooth resorption/ displacement)	NA	8 (35%)	6 (50%)	2 (18%)	0.047[a,b]
	Yes	9 (39%)	2 (17%)	7 (64%)	
	No	6 (26%)	4 (33%)	2 (18%)	
Size (mm)	NA	4	–	4	0.00[a,c]
	Mean	35.95	23.17	57.86	
	SD	± 9.69	± 10.88	± 6.99	
	Range	3–70	3–45	50–70	
Disease duration (months)	NA	7	4	3	0.817[c]
	Mean	13.31	14.50	12.63	
	SD	± 15.87	± 18.70	± 12.41	
	Range	3–60	3–60	4–36	
Symptoms	NA	1 (4%)	1 (8%)	–	0.03[a,b]
	Yes	10 (44%)	2 (17%)	8 (73%)	
	No	12 (52%)	9 (75%)	3 (27%)	
Localization	Maxilla	5 (22%)	3 (25%)	2 (18%)	0.692[b]
	Mandible	18 (78%)	9 (75%)	9 (82%)	
Radiographic appearance	NA	2	1	1	0.02[a,b]
	Radiolucent	17 (81%)	11 (100%)	6 (60%)	
	Radiopaque/ mixed	4 (19%)	0 (0%)	4 (40%)	

CGCG = central giant cell granuloma; NA-CGCG = non-aggressive central giant cell granuloma; A-CGCG = aggressive central giant cell granuloma; NA = information not available; SD = standard deviation.
[a] Statistical significance.
[b] Pearson's chi-square test.
[c] Student t-test.

(≤6 months) were not significant risk factors (p > 0.05). The variables p63 and Ki-67 did not enter the logistic regression model (Table 6).

4. Discussion

Despite the wide variation in the proportion between A-CGCG and NA-CGCG [3,8,13,15,19], almost 50% of the present cases were classified as aggressive and were significantly larger than the non-aggressive variant, similar to the findings of other studies [5,6,8,20]. The lesions were more frequent in the mandible of young women, also in agreement with other authors [3,5,6,8,12,21,22]. The peak age incidence was in the fourth decade of life, in contrast to other studies in which the disease was more common among patients in the second or third decade of life [3-5,8,11,22].

Aggressive lesions were more significantly associated with teeth (tooth resorption/displacement), similar to the finding of other studies [5,8]. In addition, the mean duration of CGCG until treatment was shorter in the aggressive variant compared to the non-aggressive variant. Other authors also reported a shorter disease duration or rapid growth of the former [5,6,8]. There was a significant difference in the radiographic appearance of the lesions, with the aggressive variant exhibiting a mixed appearance in 40% of cases. This mixed appearance might correspond to multilocular radiolucencies as described by WHO[1].

Table 4
Comparison of staining index between aggressive and non-aggressive central giant cell granuloma.

		NA-CGCG (n = 12)	A-CGCG (n = 11)	p value[a]
CD68	SI1 +	0	1	0.76
	SI2 +	11	10	
CD163	SI1 +	6	5	0.43
	SI2 +	3	5	
CD34	SI1 +	6	5	1.00
	SI2 +	6	5	
CD105	SI1 +	2	3	0.63
	SI2 +	1	2	
D2-40	SI1 +	2	4	0.37
	SI2 +	5	6	
p63	SI1 +	–	–	–
	SI2 +	–	–	
Ki-67	SI1 +	–	–	–
	SI2 +	–	–	

NA-CGCG = non-aggressive central giant cell granuloma; A-CGCG = aggressive central giant cell granuloma; SI = staining index.
[a] Pearson's chi-square test.

We also observed that NA-CGCG cases had significantly fewer lesion-associated symptoms than patients with A-CGCG. This finding is expected since aggressive lesions are supposed to cause greater destruction of gnathic bones. Patients with lesion-associated symptoms had a 12-fold higher risk of developing A-CGCG. Similar results have been reported in previous studies [5,8,12,20], although these findings were not based on a logistic regression model. Investigation of a larger number of cases should permit to establish a clearer distinction between the clinical variants of CGCG.

Protein CD68 was highly expressed in slightly over 90% of cases, while CD163, detected in 83% of cases, showed high expression in only 35%. In addition, we found a positive correlation between CD68 and CD163 in A-CGCG and in CGCG regardless of the clinical variant. These results suggest that the macrophages identified play an important role in the development and/or maintenance of these lesions and that the distinction between macrophage type (M1 and M2) does not seem to influence the clinical behavior of CGCG.

To our knowledge, only Kahn et al. [15] evaluated the expression of CD163 in CGCG and found immunopositivity for this marker in giant cells, which was not observed in the present study. These divergent results might be explained by the use of different antibody clones. In addition, the high expression of CD163 was considered a predictor of the occurrence of NA-CGCG in other studies [23,24]. In contrast, despite the lack of statistical significance in the logistic regression model used, we found that the higher expression of CD163 may increase the risk of developing A-CGCG.

Based on the results of this study, we hypothesize that, unlike

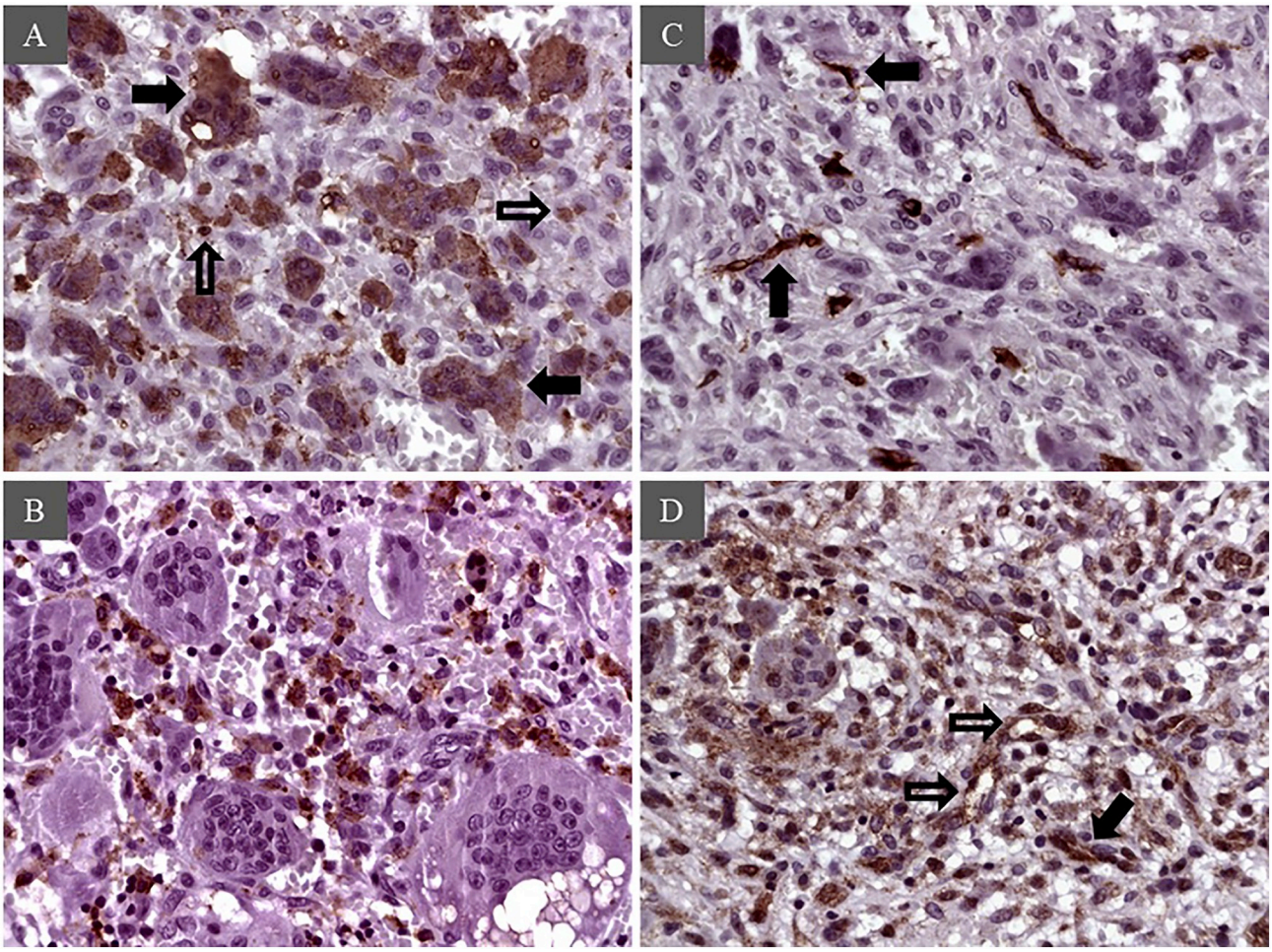

Fig. 1. Immunohistochemical expression in central giant cell granuloma. (A) Cytoplasmic expression of CD68 in giant cells (CGs) - full arrow - and few mononuclear cells (MCs) with CD-68 positive nuclei - empty arrow. (B) Note the absence of CD163 expression in CGs and predominantly nuclear expression in MCs. (C) Collapsed microvessels with CD34 immunopositivity amidst GCs - full arrow. (D) Observe immunopositivity for CD105 in collapsed - full arrow - or non-collapsed microvessels - empty arrow.

CD105, the vascular proteins CD34 and D2-40 strongly contribute to the development of CGCG, probably because of the later stage of the lesions. However, we found no significant differences in the expression of the vascular markers between NA-CGCG and A-CGCG, corroborating the findings of previous studies [8,12]. Some authors [7,8,14] report a higher degree of vascularization in A-CGCG compared to the non-aggressive variant, which is consistent with the clinical course of these lesions. However, Dewsnup et al. [7] and Susarla et al. [14] evaluated the expression of CD34 in NA-CGCG and A-CGCG in the same sample consisting of an approximately four times larger number of A-CGCG cases than NA-CGCG cases, a fact that might have influenced their results. Nevertheless, Susarla et al. [14] concluded that a percentage of CD34 staining higher than 2.5% would be predictive of A-CGCG, diverging from the minimum value of 10% used in this study for the definition of a CD34-immunopositive case.

Our results also demonstrated a negative correlation between the expression of CD105 and CD68 in A-CGCG. This finding can be explained by the fact that the increasingly larger number of newly formed vessels, associated with tissue repair, would culminate in a decline in the number of M1 macrophages, which are more related to early proinflammatory processes. There was no other significant correlation between the vascular proteins and macrophage markers evaluated in this study, although the latter are known to stimulate angiogenesis in different types of tumors [25,26]. This stimulation would occur at the expense of VEGF production by macrophages [25].

Although macrophages can contribute to lymphangiogenesis [27], we found no correlation between the macrophage markers and D2-40 despite the positive correlation of the latter with CD34. These results suggest that a larger number of blood vessels would be accompanied by lymphatic vessels in aggressive lesions; however, our results disagree with those reported by Falci et al. [28] who found no significant correlation between D2-40 and CD34 in CGCG. Additionally, these authors observed lower expression of D2-40 when compared to CD105. Our results showed the opposite, i.e., higher expression of D2-40 compared to CD105. Further studies are needed to establish the role of lymphatic vessels in CGCG.

The p63 protein encodes two isoforms: TAp63, which is involved in the process of apoptosis, and Np63, which is important for cell proliferation [29-31] but can also act as an oncogene [29,32,33]. In the present study, only few mononuclear cells, especially mitotic cells, exhibited immunopositivity for p63, reinforcing that this protein does not seem to participate in cell proliferation in CGCG, in line with other studies [34-36], but different from the findings obtained for giant cell tumors [34-36], a histologically similar lesion.

The expression of Ki-67 was classified as negative in all cases since only few mononuclear cells exhibited immunopositivity for this marker, as also observed in other studies [12,37-40]. This lack of expression of Ki-67 does not seem to affect the aggressive clinical behavior of CGCG. Thus, more studies are needed to understand what actually contributes to the aggressiveness of these lesions.

Despite the lack of a correlation between the markers studied, macrophages and angiogenesis contribute to the development and

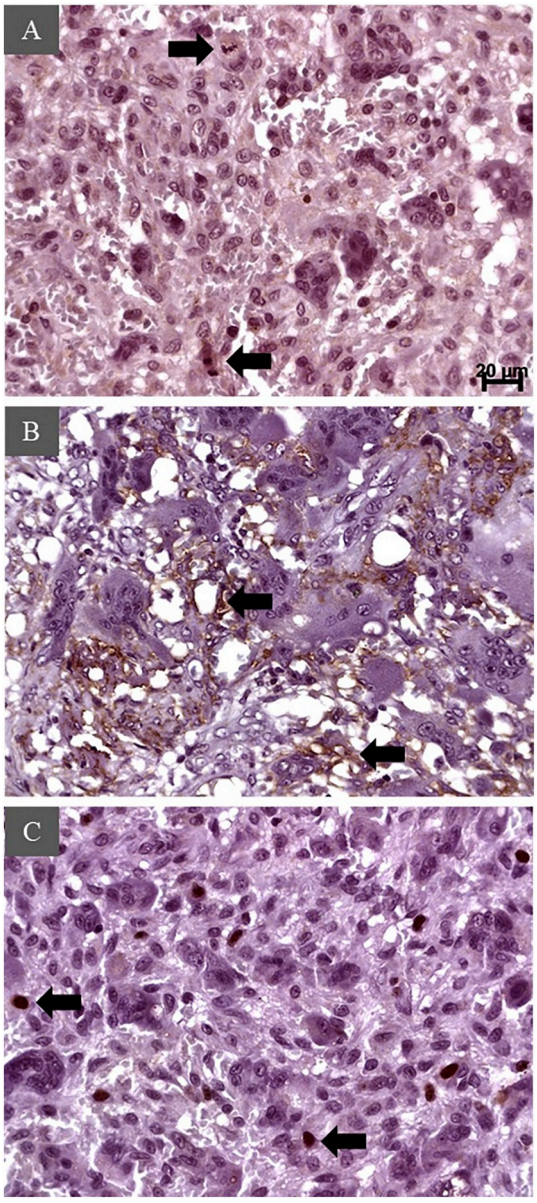

Fig. 2. Immuohistochemical expression in central giant cell granuloma. (A) Note the mild nuclear expression of p63 in some mononuclear cells (MCs), especially mitotic cells - full arrow. (B) Moderate expression of D2-40 in non-collapsed microvessels - full arrow. (C) Presence of some Ki-67-positive MCs scattered in the tumor stroma - full arrow.

Table 5
Pearson's correlation coefficient correlations between the staining indices of the markers in non-aggressive and aggressive central giant cell granuloma.

Correlation	NA-CGCG		A-CGCG		CGCG	
	R^2	p value	R^2	p value	R^2	p value
CD68 × CD163	0.388	0.213	0.484	0.031*	0.40	0.05*
CD34 × D2-40	0.357	0.254	0.481	0.04*	0.32	0.13
CD105 × CD68	0.257	0.445	−0.625	0.04*	−0.10	0.64

CGCG = central giant cell granuloma; NA-CGCG = non-aggressive central giant cell granuloma; A-CGCG = aggressive central giant cell granuloma.
* Statistically significant.

Table 6
Univariate logistic regression between antibodies and central giant cell granuloma.

Antibodies	Coefficient	OR	Lower limit	Upper limit	p value
CD68	1.48017	0.91	0.05	16.54	0.949
CD163	0.900616	2.50	0.43	14.61	0.309
CD34	0.836660	0.83	0.16	4.3	0.827
CD105	1.30809	2.22	0.17	28.86	0.542
D2-40	0.842332	1.68	0.32	8.76	0.538
p63[a]	–	–	–	–	–
Ki-67[a]	–	–	–	–	–
Sex (female)	0.950146	0.89	0.14	5.72	0.901
Age (≤30 years)	0.988826	5.40	0.78	37.51	0.088
Tooth association	1.18019	7.00	0.69	70.75	0.099
Localization (mandible)	1.02740	1.50	0.20	11.24	0.693
Size (≥50 mm)[a]	–	–	–	–	–
Associated symptoms	1.03413	12.00	1.58	91.09	0.016**
Radiographic appearance (radiopaque or mixed)[a]	–	–	–	–	–
Disease duration (≤6 months)	1.03279	2.78	0.37	21.03	0.323

OR = odds ratio.
[a] Variables that did not enter the logistic regression model.
** Statistical significance.

maintenance of CGCG and lymphangiogenesis also appears to influence this process. Additionally, immunoexpression of the markers used in this study was unable to differentiate the aggressive from the non-aggressive variant, although the presence of lesion-associated symptoms must be considered a risk factor for the occurrence of A-CGCG.

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Author contributions

Vinícius Muniz - Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing (Original Draft), and Visualization; Fábio Daumas Nunes – Methodology, Writing (Review & Editing), Visualization; Maria Cristina Teixeira Cangussu - Data Curation, Methodology, Formal Analysis, Writing (Review & Editing); Patrícia Ramos Cury – Formal analysis, Investigation, Methodology, Resources, Writing (Review & Editing) and Visualization; Flávia Caló Aquino Xavier – Methodology, Validation, Investigation, Writing (Review & Editing), Visualization; Roberto Almeida de Azevedo - Methodology, Software, Validation, Formal analysis and Writing (Review & Editing); Águida Cristina Gomes Henriques Leitão - Validation, Investigation, Writing (Review & Editing) and Visualization; Ludmila de Faro Valverde - Software, Validation, Investigation, Data Curation and Writing (Review & Editing); Bráulio Carneiro Júnior – Methodology, Software, Formal Analysis, and Writing (Review & Editing); Jean Nunes dos Santos - Conceptualization, Methodology, Investigation, Writing (Review & Editing), Supervision, Project administration and Funding acquisition.

Declaration of competing interest

None.

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